

Greater Insula White Matter Fiber Connectivity in Women Recovered from Anorexia Nervosa

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Anorexia nervosa is a severe psychiatric disorder associated with reduced drive to eat. Altered taste-reward circuit white matter fiber organization in anorexia nervosa after recovery could indicate a biological marker that alters the normal motivation to eat. Women recovered from restricting-type anorexia (Recovered AN, $n=24$, age= 30.3 ± 8.1 years) and healthy controls ($n=24$, age= 27.4 ± 6.3 years) underwent diffusion weighted imaging of the brain. Probabilistic tractography analyses calculated brain white matter connectivity (streamlines) as an estimate of fiber connections in taste-reward-related white matter tracts, and microstructural integrity (fractional anisotropy, FA) was assessed using tract-based spatial statistics. Recovered AN showed significantly (range $P < 0.05$ – 0.001 , Bonferroni corrected) greater white matter connectivity between bilateral insula regions and ventral striatum, left insula and middle orbitofrontal cortex (OFC), and right insula projecting to gyrus rectus and medial OFC. Duration of illness predicted connectivity of tracts projecting from the insula to ventral striatum and OFC. Microstructural integrity was lower in Recovered AN in most insula white matter tracts, as was whole-brain FA in parts of the anterior corona radiata, external capsule, and cerebellum ($P < 0.05$, family-wise error-corrected). This study indicates higher structural white matter connectivity, an estimate of fibers connections, in anorexia after recovery in tracts that connect taste-reward processing regions. Greater connectivity together with less-fiber integrity could indicate altered neural activity between those regions, which could interfere with normal food-reward circuit function. Correlations between connectivity and illness duration suggest that connectivity could be a marker for illness severity. Whether greater connectivity can predict prognosis of the disorder requires further study.

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INTRODUCTION

Anorexia nervosa is a severe psychiatric illness (American Psychiatric Association, 2013) with high relapse rate and mortality, and unknown etiology. It is characterized by food restriction leading to a significantly low body weight and intense fear of becoming fat although underweight. Anorexia nervosa has its onset typically during adolescence and is associated with high anxiety, harm avoidance, and sensitivity to punishment (American Psychiatric Association, 2013; Jappe *et al*, 2011; Kaye *et al*, 2009).

The ability to resist or not have a normal drive to eat could involve alterations in central reward pathways that direct food intake. The brain reward system integrates more basic metabolic hunger signals with taste stimulation and higher

order cognitive-emotional processes to regulate food approach and consumption (Kelley, 2004). The insula, which is activated by taste stimulation (Rolls *et al*, 2010), has a central role in this circuitry and is also involved with somatosensory integration, cognitive processes, and interoception (Deen *et al*, 2011). The insula projects directly to the ventral striatum, a basal ganglia structure that is important for dopamine-mediated reward learning (Hinton *et al*, 2004; O'Doherty *et al*, 2003), as well as to the orbitofrontal cortex (OFC), which fine-tunes striatal dopamine activation and the motivation to approach food (Rolls *et al*, 2010). Other structures that are part of the larger taste-reward system are midbrain, amygdala, and hypothalamus (Rolls *et al*, 2010). However, the proximity of insula, OFC, and ventral striatum makes the circuitry between these closely related structures a very good target to study their interconnectedness and whether there are alterations in the tracts connecting those structures. Altered white matter fiber organization could change the neural connectivity between those regions and alter taste and food-reward processing in anorexia nervosa.

In fact, insula, ventral striatum, and OFC have been repeatedly implicated in functional taste-reward and structural brain-imaging studies in anorexia nervosa including

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after recovery (Cowdrey *et al*, 2011; Frank *et al*, 2012, 2013a, b; Lipsman *et al*, 2015; Oberndorfer *et al*, 2013a, 2013c). For instance, studies found lower insula response to repeated (Oberndorfer *et al*, 2013c) and heightened response to random taste application (Cowdrey *et al*, 2011) in women recovered from anorexia nervosa, and our group found higher insula, ventral striatum, and OFC response in anorexia nervosa during a taste-reward learning paradigm compared with healthy controls (Frank *et al*, 2012).

Contributing to neural connectivity and brain function may be brain gray and white matter macro- and micro-structure (Dukart and Bertolino, 2014). Past studies often found widespread reduced gray matter and white matter volumes in anorexia nervosa (Van den Eynde *et al*, 2012). Such reduced brain volumes or cortical thickness typically recover with illness remission though, suggesting state-related factors (King *et al*, 2015). Importantly, findings during the acute state could be effects of acute dehydration, starvation or excessive exercise (Streitburger *et al*, 2012), and controlling for those factors may help to identify brain structures that drive illness behavior. To avoid those confounds, we previously studied a short-term nutritionally highly controlled sample of individuals with eating disorders and found larger right insula and left OFC gray matter volumes in adults and adolescents with anorexia nervosa and anorexia after recovery (Frank *et al*, 2013a, b). Especially, the result in the recovered anorexia nervosa group suggested that these regions could be particularly involved in the pathophysiology of anorexia nervosa.

Brain white matter has been consistently shown to be altered in psychiatric disease, although we know little yet about specific alterations that can be mapped to the various disorders (Lindquist, 2013). Most commonly studied has been the so-called white matter microstructural integrity as an indicator of fiber quality. In contrast, white matter connectivity strength as a measure of numbers of fibers or fiber path organization has only recently started to be investigated. Diffusion weighted imaging (DWI) maps water diffusivity along axons and can give information about white matter fiber integrity and organization (Le Bihan, 2003). White matter fiber connectivity is measured by so-called 'streamlines' along fiber paths and they are thought to provide estimates of number of connections, although this measure is also affected by development and organization of those fibers such as whether fibers grew more or less straight or have more or less branching (Jones *et al*, 2013). White matter connectivity could provide important insight into developmental aspects of brain structure affecting neural function, but to date no white matter structural connectivity studies have been reported in anorexia nervosa or other eating disorders. The more commonly assessed fractional anisotropy (FA) measures fiber integrity and is a scalar value that describes the degree of diffusion anisotropy along axons and is calculated from the three eigenvalues of the diffusion tensors (Jones and Leemans, 2011). FA is related to axon diameter, packing density of fibers, membrane permeability and myelination and is thought to reflect axon integrity (Jones *et al*, 2013). Four studies have suggested reduced FA in ill anorexia nervosa in the fornix, with evidence of lower FA in fronto-occipital tracts and cerebellum compared with controls (Frank *et al*, 2013b; Kazlouski *et al*, 2011; Nagahara *et al*, 2014; Via *et al*, 2014). After recovery from anorexia

nervosa, small studies (*n* between 9 and 12 subjects) found differing results with reduced FA in the posterior thalamic radiation (Frieling *et al*, 2012) or no differences compared with controls (Yau *et al*, 2013), and clarification is needed.

In the present study, we aimed to test whether white matter fiber organization would be altered in anorexia nervosa after recovery compared with controls between taste-reward regions, specifically in tracts between insula and OFC and ventral striatum using probabilistic tractography. This could indicate altered neural connectivity and network function, affecting taste and food-reward processing. We chose the insula seeds as proposed by Deen *et al* because of the functional segregation of these regions into antero-dorsal (cognitive control and decision making), antero-ventral (interoception, emotion, and salience processing), and posterior (somatosensory functions) subsections. We hypothesized that malnutrition during the ill state might impair white matter integrity and that with recovery there could be a compensatory process to repair such affected white matter tracts. This could especially be the case in tracts originating from the anteroventral insula as this region has been previously implicated in anorexia nervosa in taste reward paradigms (Frank *et al*, 2012). However, we did not want to exclude other insula seed regions, as the insula also contributes to processing of interoception, somatosensory inputs and emotions, an interplay of a complex network important in anorexia nervosa pathophysiology. Importantly, altered white matter tract organization could become a marker for relapse risk or recovery (Wang *et al*, 2015). In addition, we wanted to test across the whole brain whether white matter integrity is reduced in recovered anorexia nervosa to clarify the previous conflicting results (Frieling *et al*, 2012; Yau *et al*, 2013).

SUBJECTS AND METHODS

Participants

Twenty-four women recovered from restricting-type anorexia nervosa (Recovered AN) and 24 healthy control women were recruited through local advertisements in the Denver/Metro area. The Colorado Multiple Institutional Review Board approved all research procedures and study subjects gave written informed consent. Participants were administered the structured clinical interview for DSM-IV diagnoses (American Psychiatric Association, 2000) (doctoral level interviewer). Controls had a lifetime history of healthy body weight (90–110% of ideal body weight since menarche), no eating or weight concerns, no lifetime major medical or psychiatric illness. Recovered AN had a history of Recovered AN nervosa and met DSM-IV criteria for anorexia nervosa (American Psychiatric Association, 2000), but had for at least 1 year normal weight for height, menstrual cycle, exercise behavior, and food intake. Bingeing and/or purging during the course of anorexia were exclusion criteria for the Recovered AN.

Self-Assessments

Study participants completed as described previously (Frank *et al*, 2013a) Eating Disorder Inventory-3, Temperament and Character Inventory, Spielberger State and Trait Anxiety

Inventory, Beck Depression Inventory, and Revised Sensitivity to Punishment and Reward Questionnaire.

MRI Acquisition for Diffusion-Weighted Imaging

To determine whether there were volumetric brain differences between groups, structural brain images were acquired and analyzed using the VBM8 toolbox in SPM8 as described previously (Frank *et al*, 2013a).

DWI for DTI mapping included 25 DWI diffusion directions and one T2-weighted ($b=0$) baseline image. Each image included 45 slices acquired in anterior–posterior commissure orientation (128×128 matrix, TR/TE = 16000/82.6 ms, field of view = 26 cm, b -value = 1000, ASSET, slice thickness/gap = 2.6/0 mm). Brain imaging occurred during the first 10 days of the menstrual cycle (follicular phase).

Diffusion Image Preprocessing

Diffusion images were processed using FSL's Diffusion Toolbox 4.1.3 (FDT, Oxford Centre for Functional MRI of the Brain, <http://www.fmrib.ox.ac.uk/fsl>). Images were corrected for eddy current distortions and head motion.

Taste Reward-Circuit White Matter Connectivity Analysis: For each subject, probabilistic fiber tractography was computed using PROBTRACKX2 to generate estimates of the most likely connectivity distribution between seed (dorsal anterior, ventral anterior, and posterior insula, drawn by hand on template image as defined by (Deen *et al*, 2010, 2011)) and ipsilateral target regions (ventral striatum, medial, middle and inferior OFC, and orbitofrontal gyrus rectus) (Behrens *et al*, 2007), using default parameters: 5000 sample tracts per seed voxel, 0.2 curvature threshold, step length of 0.5 and maximum number of steps 2000 (see Figure 1). This segmentation of the insula was chosen for its functional separation of the insula into somatosensory,

cognitive, and interceptive/emotion. Connection strength was calculated by computing the mean probability of streamlines for each seed-target combination and normalizing and rescaling the values to correct for size of seed and target regions as well as path length (Eickhoff *et al*, 2010). In addition, using *fslstats*, mean connection per insula voxels was extracted for each seed-target combination.

Taste Reward-Circuit White Matter Integrity Analysis: FA was extracted from reconstructed tracts between seed and target regions. Images were thresholded to include images whose voxels had > 5% probability of being connected to the seed masks (LeWinn *et al*, 2014). Mean values for FA, mean (MD), axial (AD), and radial diffusivity (RD) of the tracts were extracted using *fslstats*.

Whole-Brain WM Integrity Analysis: Voxelwise maps were generated for FA (rotationally invariant measure of anisotropy), MD (average of tensor eigenvalues), AD (largest eigenvalue, diffusion parallel to axonal fibers), and RD (mean of second and third eigenvalues, perpendicular diffusion to axonal fibers) (Jones and Leemans, 2011). Voxelwise analysis was carried out using FSL's Tract Based Spatial Statistics (Smith *et al*, 2006). Subjects' raw FA data were non-linearly registered to the FMRI58_FA template and averaged to create a mean FA image for all subjects. FA skeletonization created a mean FA skeleton representing the center of tracts common to all participants. A threshold of $FA \geq 0.2$ restricted the skeleton to white matter and excluded areas with high individual variability. Each participant's normalized FA image was projected onto this common skeleton and the resulting skeletonized data were analyzed using voxelwise cross-subject statistics (Smith *et al*, 2006). FA registration and projection vectors were used to generate MD, AD, and RD skeletons.

Statistical Analyses

The extracted values for tract-based connectivity strength, mean connections originating per voxel and tract-based FA were analyzed using MANOVAs, and in the model, results were corrected for medication use, anxiety, and mood disorder diagnoses as well as multiple comparisons (Bonferroni).

Voxelwise whole-brain comparisons of FA, MD, AD, and RD skeletons were carried out using FSL's randomize toolbox (Nichols and Holmes, 2002) and a nonparametric 5000-permutation algorithm. Significant clusters were identified using threshold-free cluster enhancement (Smith and Nichols, 2009) corrected for age, comorbid conditions and medication use, total intracranial volume, and multiple comparisons (family-wise error, $P < 0.05$, 25 voxel cluster threshold).

Demographic, behavioral, and extracted brain data were analyzed using SPSS 23.0 (IBM-SPSS, Chicago, IL).

Linear regression analyses to test behavior–brain relationships were applied for age, body mass index, Harm Avoidance, Depression, Reward and Punishment Sensitivity, State and Trait Anxiety. In addition, for Recovered AN we tested whether duration of illness or recovery predicted brain results. Significant correlations were corrected using the false-discovery rate using the method proposed by Benjamini and Hochberg (1995).

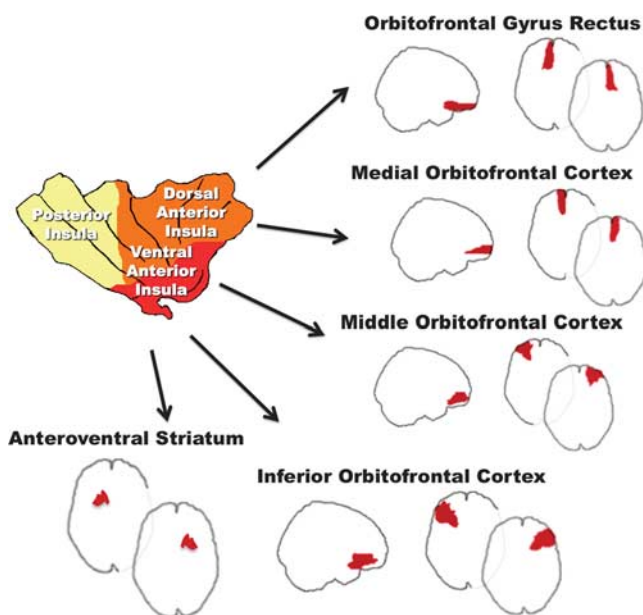


Figure 1 Insula seed regions and ROI targets.

RESULTS

Demographic and Behavioral Data

Recovered AN and controls were similar in age, education, and body mass index (BMI, kg/m²) (Table 1). Recovered AN scored higher on Depression, Drive for Thinness, Bulimia, Body Dissatisfaction, Punishment Sensitivity, Harm Avoidance, and State and Trait Anxiety.

Taste Reward Circuit Connectivity Analysis

Total streamlines were greater in the Recovered AN compared with controls (Figure 2; Supplementary Table 2).

Connectivity strength was significantly greater in Recovered AN compared with controls (MANOVA Wilks-Lambda = 0.050, $P < 0.001$, $\eta_p^2 = 0.950$) in several tracts between insula seeds and their targets including from all three left insula regions to left ventral striatum and left middle OFC targets, and from all three right-sided insula subregions to right gyrus rectus and medial OFC. Right anterior dorsal and posterior insula showed stronger connectivity to the ventral striatum in Recovered AN.

Mean connections per insula voxels were also greater in Recovered AN compared with controls in those insula subregions (MANOVA Wilks-Lambda = 0.044, $P < 0.001$, $\eta_p^2 = 0.956$), except for the left ventral anterior insula seed to left inferior OFC target where Recovered AN had less mean connections per insula voxels compared with controls.

To test whether there is a methodological reason for increased connectivity in the Recovered AN group, we analyzed pathways that did not connect to the insula, including the right middle OFC seed to the ipsilateral ventral striatum target, the left medial OFC seed to the ipsilateral ventral striatum target, and the left inferior OFC seed to the left prefrontal cortex, Brodmann Area 10 target. The fiber path probabilistic connectivity between these seed-target combinations did not differ between groups, suggesting that no general confound accounted for the group differences.

Taste-Reward Circuit FA Analysis

Recovered AN showed significantly reduced mean FA values in several reconstructed tracts between insula subregions and ventral striatum and OFC regions (MANOVA Wilks-Lambda = 0.150, $P < 0.007$, $\eta_p^2 = 0.850$) (Figure 3). Wilks-Lambda for MD, AD, and RD MANOVA was not significant.

Whole-brain FA analysis

FA was reduced in recovered AN compared with controls in anterior corona radiata, external capsule, and cerebellum including the corticopontine tract, corpus callosum, anterior thalamic radiation, inferior, and middle cerebellar peduncle as well as inferior fronto-occipital and uncinate fasciculus (family-wise error, $P < 0.05$, 25 voxel cluster threshold; MRI

Table 1 Demographic and Behavioral Variables for Control and Recovered Anorexia Nervosa Women

	Healthy control women, n = 24		Recovered anorexia nervosa women, n = 24		t	P-value
	Mean	SD	Mean	SD		
Age (years)	27.42	6.28	30.25	8.13	-1.35	0.183
Education (years)	16.63	2.06	16.88	2.73	-0.38	0.723
Body mass index (kg/m ²)	21.64	1.26	20.83	2.37	1.48	0.146
Harm avoidance	9.58	3.99	15.54	6.47	-3.84	<0.001
Depression	1.13	0.95	4.50	4.21	-3.83	0.001
Drive for thinness	2.63	3.41	8.46	6.45	-3.92	<0.001
Body dissatisfaction	4.38	4.25	10.46	2.53	-3.26	0.002
Bulimia	0.79	1.22	2.33	8.08	-2.69	0.011
Punishment sensitivity	4.04	1.85	6.63	4.14	-2.79	0.009
Reward sensitivity	4.42	2.84	5.83	3.25	-1.61	0.115
State anxiety	32.67	11.79	44.96	9.41	-3.99	<0.001
Trait anxiety	33.92	11.35	43.58	6.90	-3.57	0.001
Illness low body mass index (kg/m ²)	—	—	15.33	1.14	—	—
Age illness onset (Years)	—	—	16.63	2.43	—	—
Illness Duration (years)	—	—	5.90	5.21	—	—
Years in recovery	—	—	7.90	6.01	—	—
	N	%	N	%		
Anti-depressant use	0	0	6	25.0	—	—
Major depression	0	0	3	12.5	—	—
Anxiety disorder	0	0	4	16.6	—	—
Major depression and anxiety disorder	0	0	2	8.3	—	—

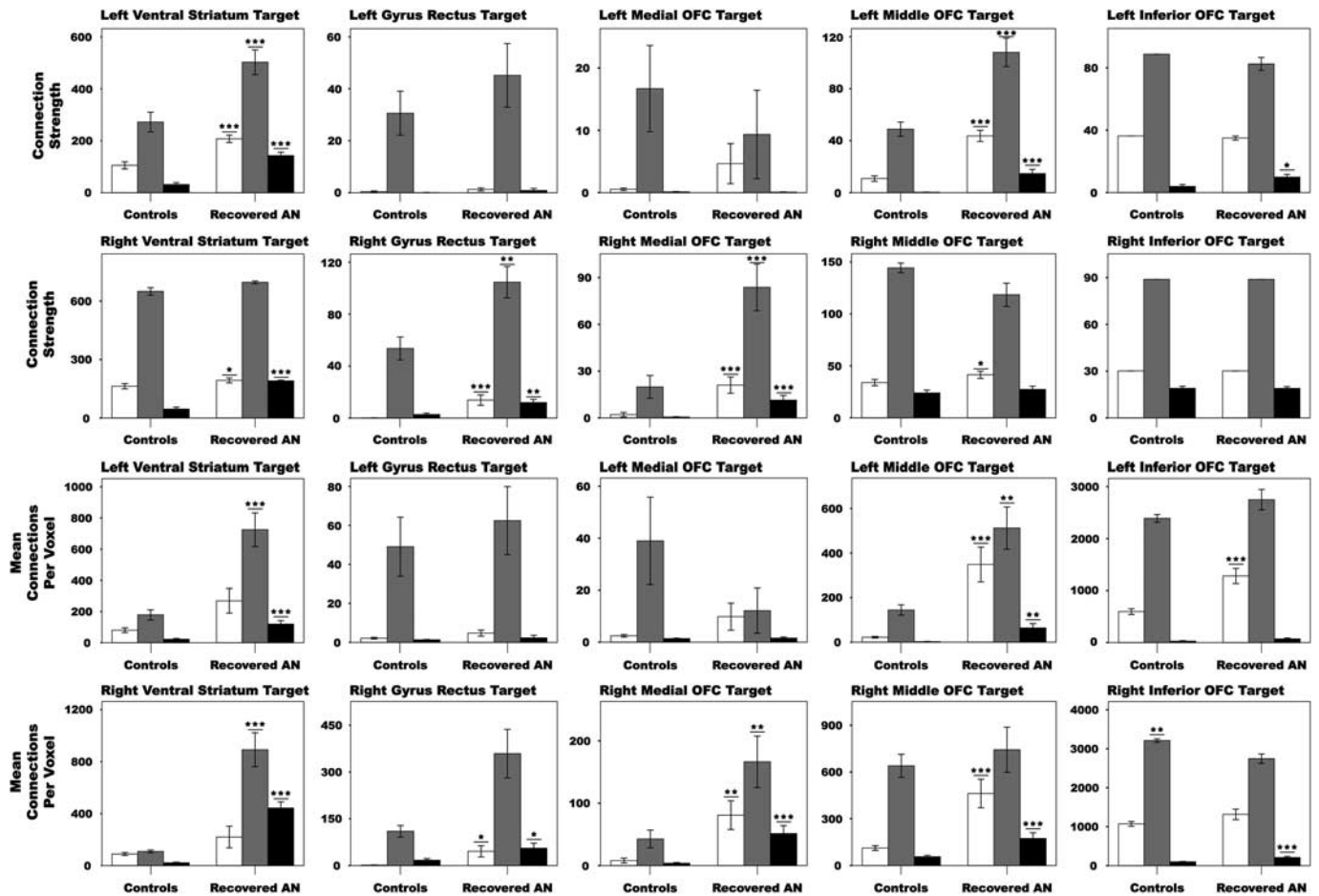


Figure 2 Connectivity strength from insula seeds to ROI targets; OFC = orbitofrontal cortex (upper two rows) and mean number of connections per insula voxels (lower two rows); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. □ dorsal anterior insula, ■ ventral anterior insula, ■ posterior insula.

Atlas of Human White Matter (Mori *et al*, 2005)) (Figure 4, Supplementary Table 1).

Total gray matter, white matter, and cerebral spinal fluid did not differ between groups. Total intracranial volume was also similar between groups (Supplementary Table 2).

Demographic and Behavioral Correlation Results

Controls. There were no significant correlations between connectivity and demographic or behavioral measures after FDR correction. Tract-based FA from anterior ventral insula to ipsilateral gyrus rectus was significantly negatively correlated with state anxiety (left, $r = -0.646$, $R^2 = 0.417$, $P < 0.024$; right, $r = -0.667$, $R^2 = 0.444$, $P < 0.001$; all FDR corrected) and trait anxiety (left, $r = -0.664$, $R^2 = 0.441$, $P < 0.001$; right, $r = -0.693$, $R^2 = 0.481$, $P < 0.001$; all FDR corrected).

Recovered AN. Illness duration was significantly positively correlated with insula voxel connectivity between left ventral anterior insula and striatum ($r = 0.528$, $R^2 = 0.279$, $P < 0.032$, FDR-corrected) as well as between right posterior insula and gyrus rectus ($r = 0.608$, $R^2 = 0.370$; $P < 0.008$, FDR-corrected) after FDR correction (Supplementary Figure 1). Lifetime low BMI during the illness or change of BMI from illness to

recovery state did not correlate with any imaging measure. Tract-based FA showed no significant correlations with any behavioral measure.

DISCUSSION

This study has three novel findings. Anorexia nervosa after recovery is associated with greater structural white matter fiber connections between insula, ventral striatum, and OFC, but white matter integrity of those tracts is reduced. This may indicate altered neural connectivity and activation within taste-reward pathways and could impair the normal cortical-subcortical reward circuit function that drives food intake. Duration of past illness predicted the estimate of fibers projecting from the insula to the OFC and ventral striatum. This may indicate a remodeling process in response to effects from the illness on white matter structure or a premorbid condition. In addition, white matter integrity was reduced across large frontal regions as well as in the cerebellum in this group of adult women recovered from anorexia nervosa, which could result in less-efficient information processing. Those alterations could interfere with the normal neural circuitry of food-reward processing, and could become markers for illness severity or prognosis.

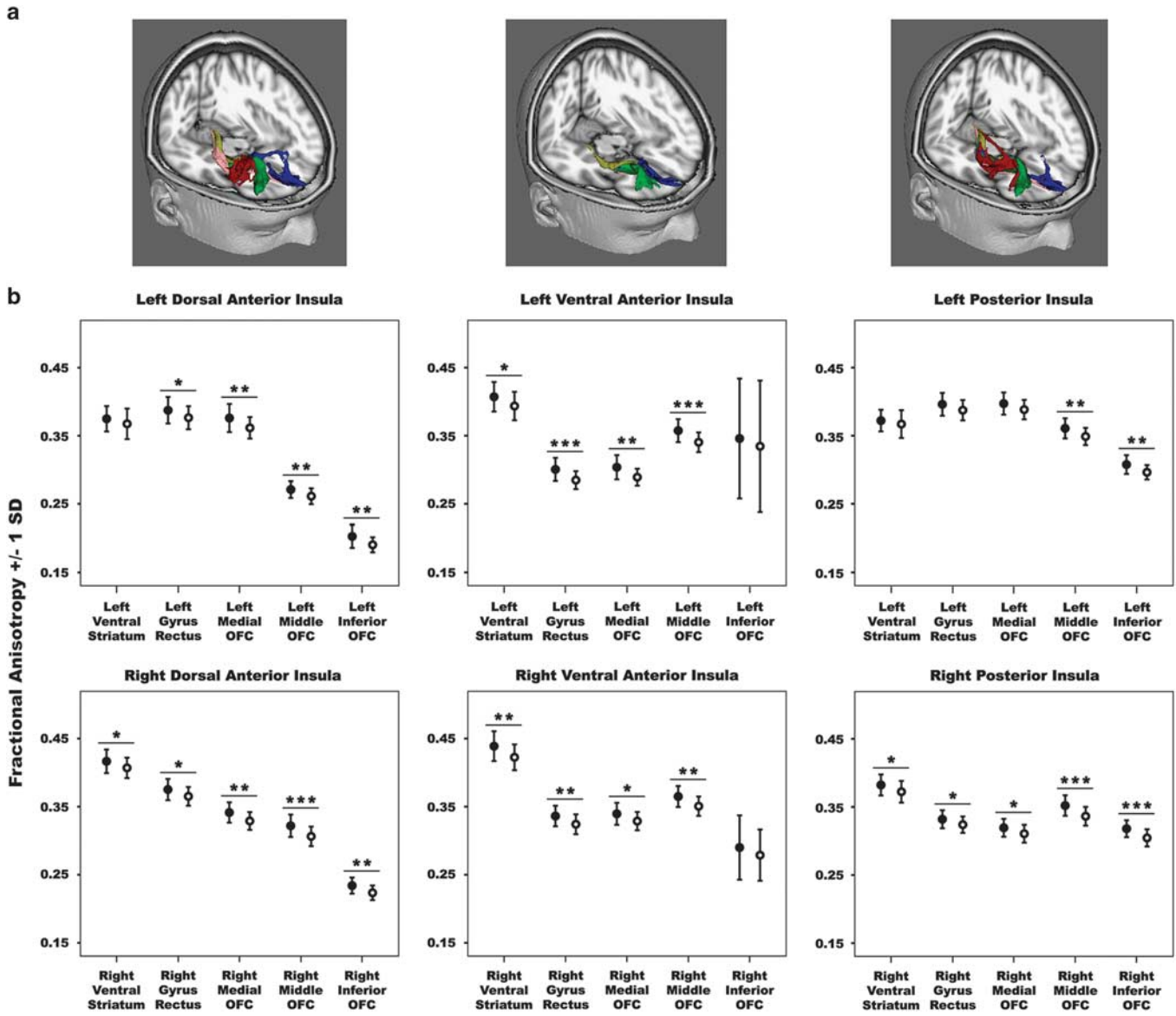


Figure 3 (a) Reconstructed fiber tracts for dorsal anterior insula, ventral anterior insula, and posterior insula seeds and inferior OFC (red), medial OFC (blue), orbitofrontal gyrus rectus (pink), middle OFC (green), and ventral striatum (yellow) inclusion masks. For visualization purposes only the right-sided connections are shown. (b) Mean fractional anisotropy values for reconstructed tracts from insula seeds to ROI inclusion masks; black-filled circle = controls; empty circle = Recovered AN; OFC = orbitofrontal cortex; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

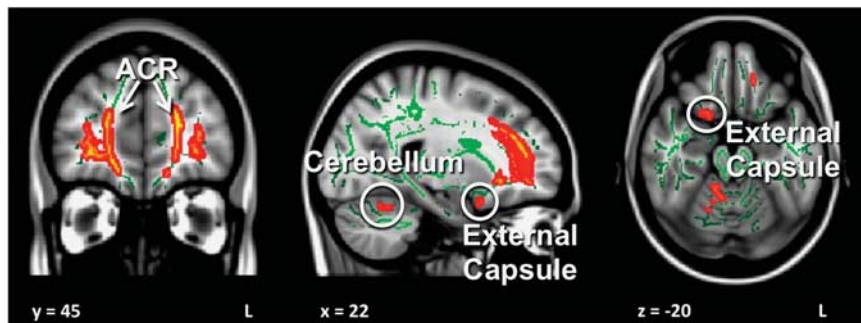


Figure 4 White matter clusters showing significantly lower fractional anisotropy (red–yellow, $P < 0.05$ FWE-corrected) in Recovered AN in comparison with controls from whole-brain TBSS analysis. Green color depicts the common white matter skeleton across all subjects.

Unique to this study is the probabilistic tractography analysis that measures white matter fiber connections in taste-reward pathways between insula, OFC, and ventral striatum in anorexia nervosa. Those regions have frequently been implicated in the pathophysiology of the disorder (Cowdrey *et al*, 2011; Frank *et al*, 2013a; Oberndorfer *et al*, 2013c). The insula not only contains the primary taste cortex, but also processes social-emotional, cognitive, and sensorimotor information (Klein *et al*, 2013). There is a gradient from less to more granular cortex from anterior to posterior insula (von Economo and Koskinas, 1925) and functionally, the insula has been separated into antero-dorsal (cognitive control and decision making), antero-ventral (interoception, emotion, and salience processing), and posterior (somatosensory functions) subsections (Deen *et al*, 2011; Klein *et al*, 2013). The complexity of insula functions continues to emerge (Castro *et al*, 2014), and especially, taste and interoception are processed across the previously proposed insula regions (Kurth *et al*, 2010). Thus, we included the entire insula in this analysis, but separated by the subregions as suggested by Deen *et al* (2011). Here, we specifically investigated white matter fiber tracts originating from the insula and projecting to OFC, and ventral striatum because altered cross-talk between those structures could alter taste-reward processing and dopamine-mediated food approach in anorexia nervosa (Rolls *et al*, 2010). Another reason for the focus on this circumscribed area was that investigating white matter fiber connectivity in longer fiber paths across the brain is associated with more confounding factors and studying a system that is in close physical proximity promised to yield the most reliable and valid results (Jones and Leemans, 2011).

Across both groups the antero-ventral insula had the strongest connections to ventral striatum and OFC, followed by the dorsal-ventral and then posterior insula. This was largely consistent with basic science research, although the literature in this area is limited (Fudge *et al*, 2005; Haber and Behrens, 2014). Thus, although the gustatory cortex is somewhat more centrally located in the insula (Kurth *et al*, 2010), the dominance of fibers to OFC and ventral striatum originate from the ventral anterior insula. The anterior insula receives limbic input including from the amygdala and it has been suggested that the anterior insula is in an 'ideal position to assess the hedonic value of sensory stimuli, either actual or anticipated' (Fudge *et al*, 2005) as it integrates information from the viscera with emotional response. This may suggest that the anterior insula has a key role in the early value determination of taste stimuli, even before downstream reward circuits get activated to trigger food approach or avoidance.

The connectivity analysis of the larger fiber organization between those regions indicated significantly higher connectivity in Recovered AN, especially in insula tracts projecting to the bilateral ventral striatum, right gyrus rectus and medial OFC as well as left middle OFC. In contrast, the tract-based FA analysis showed that most tracts in Recovered AN had lower FA compared with controls, though the patterns of FA across tracts studied were similar between groups. For instance, there was a gradual decrease in FA for tracts originating from the right dorsal anterior insula to the OFC, from its medial to the more lateral and inferior aspects, suggesting that fiber integrity is reduced in Recovered AN,

but that the balance of FA across those pathways within groups is comparable. The higher total number of streamlines in Recovered AN indicates a widespread alteration of white matter connectivity in this group.

One interpretation of the greater measured white matter connectivity in Recovered AN is that this is due to more connections or white matter fiber tracts. However, higher connectivity estimates could also be due to thicker, less curved or less-branched connecting fibers (Jones *et al*, 2013; Jones and Leemans, 2011). To obtain further insight, we studied connectivity as measured by streamlines per voxel originating from each insula subsection. This analysis mirrored the prior connectivity analysis, with more streamlines per insula voxels and suggesting that indeed more connections originate from the insula and connect with ventral striatum and OFC.

The mechanism that might increase white matter connectivity and how this could affect behavior is yet uncertain; however, these results may point to an important new understanding of brain white matter pathophysiology in anorexia nervosa. Duration of illness, not recovery, predicted connectivity estimates per insula voxels between right posterior insula and gyrus rectus and for left antero-ventral insula connectivity to left ventral striatum after correction for multiple comparisons. Anorexia nervosa is associated with malnutrition and excessive exercise, stress and elevated glucocorticoids (Kaye *et al*, 1987), factors that decrease neurogenesis but stimulate myelin-producing cells, altering neuron composition and function (Chetty *et al*, 2014). It is possible that white matter tracts need to be rebuilt during recovery, after a prolonged underweight state and presumed resulting damage (Fuller, 1983); however, there may be an overshoot in number of fibers together with reduced fiber quality or integrity. This is an important direction to test in future research including animal models. Another possibility is that these individuals had more connections prior to illness onset and connectivity could be a premorbid feature of anorexia nervosa. Insula gray and white matter development occurs far into young adulthood and overlaps with the typical age of onset of anorexia nervosa. For instance, insula gray matter volume peaks around age 18 years followed by decrease in volume and there is a dynamic interplay with white matter development (Shaw *et al*, 2008). White matter fiber density from insula to temporal lobe and parietal lobe (including the right insula to right medial orbitofrontal cortex) decreased with age in a study in individuals between ages 12 and 30 years, but the same group showed increase in fiber density with age between the insula and the temporal cortex (Dennis *et al*, 2014). It is therefore possible that altered white matter development and increased fiber density or connectivity is a premorbid trait, or that environmental effects during those years of brain development contribute to higher connectivity before any effects from the illness take place. This will require further study in subsequent studies. Furthermore, it will be important to test whether greater connectivity in Recovered AN is a marker for illness severity, will predict relapse or is a marker for successful recovery. A recent study in obesity found reduced white matter tract streamline-based connectivity within reward circuits, which might indicate that food restriction and over-nutrition may have opposite effects on white matter connectivity (Marques-Iturria *et al*, 2015); however, one might also argue that in

obesity lower connectivity could be a trait that drives high food intake. This will require longitudinal studies.

Although the connectivity of most white matter fiber tracts was increased in the Recovered AN group compared with controls, mean connectivity per voxel was decreased in Recovered AN in the tract connecting the ventral anterior insula with the right inferior OFC. Whether this is an outlier or whether this difference between groups will hold in future studies remains to be seen. However, the connectivity measures between those two regions for the mean connectivity measure as well as the per voxel connectivity on the left side are very close across both groups and it is more likely that this is an outlier finding.

The results from the whole-brain FA analysis in a substantially larger sample compared with the previous studies (Frieling *et al*, 2012; Yau *et al*, 2013) suggest large areas of reduced integrity in Recovered AN in the anterior corona radiata, external capsule, and cerebellum, tracts that have been associated with taste processing. For instance, the corona radiata, a collection of fiber bundles that extend from the internal capsule to cerebral cortex (Schmahmann *et al*, 2008) basal ganglia, and spinal cord, has been associated with altered taste perception when lesioned (Onoda *et al*, 2012), and lesions in the anterior corpus callosum were associated with altered taste signal transmission (Fabri *et al*, 2011). The external capsule lies between putamen medially and claustrum laterally, connecting ventral and medial prefrontal cortices with limbic regions via fibers from the uncinate fasciculus and inferior fronto-occipital fasciculus (Schmahmann *et al*, 2008), and white matter FA in these pathways predicted in the past brain reward activation (Olson *et al*, 2009). And at last, the cerebellum has bidirectional projections to the hypothalamus, an integral brain structure in hunger regulation (Zhu and Wang, 2008) and altered white matter functionality in these pathways may drive decreased appetite and weight loss (Mahler *et al*, 1993). Taken together, all these white matter tracts are associated with taste, reward, and feeding drive processing, and reduced integrity in Recovered AN. This may suggest inefficient taste-reward pathway functionality that is present even when individuals with anorexia nervosa are recovered and at normal body weight but typically still present with higher scores on measures of eating disorder pathology as well as elevated sensitivity to salient stimuli as in our study.

The combination of increased connectivity and decreased FA is particularly striking. FA is the scalar composite of AD, MD and RD, thus studying diffusion across the various directions along paths. The connectivity is a probability measure for how many fibers may connect a seed with a target region, without emphasis on RD. Thus, the connectivity measure tests the number of connections that go from one point to another, whereas the FA value is thought to represent the structural integrity of those fibers. Previously higher connectivity has been described in, for instance, Alzheimer's, a condition that is by the same time associated with reduced FA (Wang *et al*, 2015). That association could strengthen the hypothesis that there are developmental processes ongoing in anorexia nervosa after recovery that could be related to injury from the underweight state.

Limitations: Probabilistic tractography connectivity analyses do not provide an absolute fiber count, but how probable it is that the connection strength or number of

axons exceeds a certain value (Jones *et al*, 2013). Still, this method does provide results comparable to direct white matter neuron tracing (Gao *et al*, 2013) and suggests that our results are valid. The literature on structural connectivity is small, and greater white matter connectivity in anorexia nervosa has not been described before. Greater connectivity in the context of brain reorganization has been described though (Wang *et al*, 2015), which could support our hypothesis of compensatory fiber growth. To limit confounding factors we have investigated anatomically well-defined and short white matter tracts as suggested previously (Jones and Leemans, 2011). Commonly, FA is described as a marker for white matter integrity. However, FA can be altered due to a variety of reasons (Jones *et al*, 2013; Jones and Leemans, 2011). Larger axon diameter and lower packing density of fibers, both of which mean fewer barriers to diffusion in a given space, can lead to lower FA, as can increased membrane permeability and reduced myelination (Jones *et al*, 2013). Thus, the results cannot give a definite mechanistic explanation for altered white matter tract FA. Future studies will need to further explore whether those alterations are a result from underweight or even premorbid traits. A limitation of this study is the cross-sectional design and we are currently studying individuals with anorexia nervosa during recovery and hope that this will help better describe potential underlying mechanisms. Although our results indicate sufficient effect size (η_p^2 range = 0.85–0.95), our sample size is modest and needs replication. We included Recovered AN individuals some of which who were taking medication and/or had mood or anxiety disorder diagnoses and although we controlled for these variables, we cannot rule out the impact these factors on the results.

In summary, this study provides novel insight into the pathophysiology of anorexia nervosa. It indicates higher structural white matter fiber connectivity, suggesting higher fiber counts, within the reward circuitry in Recovered AN. The correlation of duration of illness with white matter connectivity further suggests a direct impact of the illness on white matter organization. Those results in conjunction with reduced white matter integrity in those pathways could indicate white matter tract remodeling after impairments during the underweight state or a premorbid condition that could contribute to illness onset and severity. Altered structural white matter fiber connectivity could cause altered neural connectivity and interfere with normal food-reward processing. Whether altered connectivity after recovery from anorexia nervosa is a marker for illness severity or sustained recovery, or whether it is a premorbid trait requires further study.

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REFERENCES

- American Psychiatric Association (2000). *Diagnostic and Statistical Manual of Mental Disorders - Text Revision (DSM-IV-TR)*. Handbook of Psychiatric Measures. 4th edn. American Psychiatric Association: Washington, DC.
- American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5(TM))*. 5th edn. American Psychiatric Publishing: Arlington, VA.
- Behrens TE, Berg HJ, Jbabdi S, Rushworth MF, Woolrich MW (2007). Probabilistic diffusion tractography with multiple fibre orientations: what can we gain? *Neuroimage* **34**: 144–155.
- Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* **57**: 289–300.
- Castro DC, Chesterman NS, Wu MKH, Berridge KC (2014). *Two Cortical Hedonic Hotspots: Orbitofrontal and Insular Sites of Sucrose 'Liking' Enhancement*. Society for Neuroscience: Washington, DC, USA.
- Chetty S, Friedman AR, Taravosh-Lahn K, Kirby ED, Mirescu C, Guo F et al (2014). Stress and glucocorticoids promote oligodendrogenesis in the adult hippocampus. *Mol Psychiatry* **19**: 1275–1283.
- Cowdrey FA, Park RJ, Harmer CJ, McCabe C (2011). Increased neural processing of rewarding and aversive food stimuli in recovered anorexia nervosa. *Biol Psychiatry* **70**: 736–743.
- Deen B, Pitskel NB, Pelphrey KA (2011). Three systems of insular functional connectivity identified with cluster analysis. *Cereb Cortex* **21**: 1498–1506.
- Dennis EL, Jahanshad N, McMahon KL, de Zubicaray GI, Martin NG, Hickie IB et al (2014). Development of insula connectivity between ages 12 and 30 revealed by high angular resolution diffusion imaging. *Hum Brain Mapp* **35**: 1790–1800.
- Dukart J, Bertolino A (2014). When structure affects function - the need for partial volume effect correction in functional and resting state magnetic resonance imaging studies. *PLoS One* **9**: e114227.
- Eickhoff SB, Jbabdi S, Caspers S, Laird AR, Fox PT, Zilles K et al (2010). Anatomical and functional connectivity of cytoarchitectonic areas within the human parietal operculum. *J Neurosci* **30**: 6409–6421.
- Fabri M, Polonara G, Mascioli G, Salvolini U, Manzoni T (2011). Topographical organization of human corpus callosum: an fMRI mapping study. *Brain Res* **1370**: 99–111.
- Frank GK, Reynolds JR, Shott ME, Jappe L, Yang TT, Tregellas JR et al (2012). Anorexia nervosa and obesity are associated with opposite brain reward response. *Neuropsychopharmacology* **37**: 2031–2046.
- Frank GK, Shott ME, Hagman JO, Mittal VA (2013a). Alterations in brain structures related to taste reward circuitry in ill and recovered anorexia nervosa and in bulimia nervosa. *Am J Psychiatry* **170**: 1152–1160.
- Frank GK, Shott ME, Hagman JO, Yang TT (2013b). Localized brain volume and white matter integrity alterations in adolescent anorexia nervosa. *J Am Acad Child Adolesc Psychiatry* **52**: e1065.
- Frieling H, Fischer J, Wilhelm J, Engelhorn T, Bleich S, Hillemeier T et al (2012). Microstructural abnormalities of the posterior thalamic radiation and the mediodorsal thalamic nuclei in females with anorexia nervosa—a voxel based diffusion tensor imaging (DTI) study. *J Psychiatr Res* **46**: 1237–1242.
- Fudge JL, Breitbart MA, Danish M, Pannoni V (2005). Insular and gustatory inputs to the caudal ventral striatum in primates. *J Comp Neurol* **490**: 101–118.
- Fuller G (1983). *Myelination in Undernourished Rat Brain*. The University of Texas: Houston, TX, USA.
- Gao Y, Choe AS, Stepniewska I, Li X, Avison MJ, Anderson AW (2013). Validation of DTI tractography-based measures of primary motor area connectivity in the squirrel monkey brain. *PLoS One* **8**: e75065.
- Haber SN, Behrens TE (2014). The neural network underlying incentive-based learning: implications for interpreting circuit disruptions in psychiatric disorders. *Neuron* **83**: 1019–1039.
- Hinton EC, Parkinson JA, Holland AJ, Arana FS, Roberts AC, Owen AM (2004). Neural contributions to the motivational control of appetite in humans. *Eur J Neurosci* **20**: 1411–1418.
- Jappe LM, Frank GK, Shott ME, Rollin MD, Pryor T, Hagman JO et al (2011). Heightened sensitivity to reward and punishment in anorexia nervosa. *Int J Eat Disord* **44**: 317–324.
- Jones DK, Knosche TR, Turner R (2013). White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI. *Neuroimage* **73**: 239–254.
- Jones DK, Leemans A (2011). Diffusion tensor imaging. *Methods Mol Biol* **711**: 127–144.
- Kaye WH, Fudge JL, Paulus M (2009). New insights into symptoms and neurocircuit function of anorexia nervosa. *Nat Rev Neurosci* **10**: 573–584.
- Kaye WH, Gwirtsman HE, George DT, Ebert MH, Jimerson DC, Tomai TP et al (1987). Elevated cerebrospinal fluid levels of immunoreactive corticotropin-releasing hormone in anorexia nervosa: relation to state of nutrition, adrenal function, and intensity of depression. *J Clin Endocrinol Metab* **64**: 203–208.
- Kazlouski D, Rollin MD, Tregellas J, Shott ME, Jappe LM, Hagman JO et al (2011). Altered fimbria-fornix white matter integrity in anorexia nervosa predicts harm avoidance. *Psychiatry Res* **192**: 109–116.
- Kelley AE (2004). Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neurosci Biobehav Rev* **27**: 765–776.
- King JA, Geisler D, Ritschel F, Boehm I, Seidel M, Roschinski B et al (2015). Global cortical thinning in acute anorexia nervosa normalizes following long-term weight restoration. *Biol Psychiatry* **77**: 624–632.
- Klein TA, Ullsperger M, Danielmeier C (2013). Error awareness and the insula: links to neurological and psychiatric diseases. *Front Hum Neurosci* **7**: 14.
- Kurth F, Zilles K, Fox PT, Laird AR, Eickhoff SB (2010). A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. *Brain Struct Funct* **214**: 519–534.
- Le Bihan D (2003). Looking into the functional architecture of the brain with diffusion MRI. *Nat Rev Neurosci* **4**: 469–480.
- LeWinn KZ, Connolly CG, Wu J, Drahos M, Hoefft F, Ho TC et al (2014). White matter correlates of adolescent depression: structural evidence for frontolimbic disconnectivity. *J Am Acad Child Adolesc Psychiatry* **53**: 909 e891–897.
- Lindquist DM (2013). White matter abnormalities in psychiatric disorders. *J Pediatric Neuroradiol* **2**: 109–118.
- Lipsman N, Woodside DB, Lozano AM (2015). Neurocircuitry of limbic dysfunction in anorexia nervosa. *Cortex* **62C**: 109–118.
- Mahler P, Guastavino JM, Jacquart G, Strazielle C (1993). An unexpected role of the cerebellum: involvement in nutritional organization. *Physiol Behav* **54**: 1063–1067.
- Marques-Iturrria I, Scholtens LH, Garolera M, Pueyo R, Garcia-Garcia I, Gonzalez-Tartiere P et al (2015). Affected connectivity

- organization of the reward system structure in obesity. *Neuroimage* **111**: 100–106.
- Mori S, Wakana S, van Zijl P, Nagae-Poetscher L (2005). *MRI Atlas of Human White Matter*. Elsevier: Amsterdam, The Netherlands.
- Nagahara Y, Nakamae T, Nishizawa S, Mizuhara Y, Moritoki Y, Wada Y *et al* (2014). A tract-based spatial statistics study in anorexia nervosa: abnormality in the fornix and the cerebellum. *Prog Neuropsychopharmacol Biol Psychiatry* **51**: 72–77.
- Nichols TE, Holmes AP (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp* **15**: 1–25.
- O'Doherty JP, Dayan P, Friston K, Critchley H, Dolan RJ (2003). Temporal difference models and reward-related learning in the human brain. *Neuron* **38**: 329–337.
- Oberndorfer T, Simmons A, McCurdy D, Strigo I, Matthews S, Yang T *et al* (2013a). Greater anterior insula activation during anticipation of food images in women recovered from anorexia nervosa versus controls. *Psychiatry Res* **214**: 132–141.
- Oberndorfer TA, Frank GK, Simmons AN, Wagner A, McCurdy D, Fudge JL *et al* (2013c). Altered insula response to sweet taste processing after recovery from anorexia and bulimia nervosa. *Am J Psychiatry* **170**: 1143–1151.
- Olson EA, Collins PF, Hooper CJ, Muetzel R, Lim KO, Luciana M (2009). White matter integrity predicts delay discounting behavior in 9- to 23-year-olds: a diffusion tensor imaging study. *J Cogn Neurosci* **21**: 1406–1421.
- Onoda K, Ikeda M, Sekine H, Ogawa H (2012). Clinical study of central taste disorders and discussion of the central gustatory pathway. *J Neurol* **259**: 261–266.
- Rolls ET, Critchley HD, Verhagen JV, Kadohisa M (2010). The representation of information about taste and odor in the orbitofrontal cortex. *Chemosens Percept* **3**: 16–33.
- Schmahmann JD, Smith EE, Eichler FS, Filley CM (2008). Cerebral white matter: neuroanatomy, clinical neurology, and neuro-behavioral correlates. *Ann N Y Acad Sci* **1142**: 266–309.
- Shaw P, Kabani NJ, Lerch JP, Eckstrand K, Lenroot R, Gogtay N *et al* (2008). Neurodevelopmental trajectories of the human cerebral cortex. *J Neurosci* **28**: 3586–3594.
- Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE *et al* (2006). Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage* **31**: 1487–1505.
- Smith SM, Nichols TE (2009). Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* **44**: 83–98.
- Streitburger DP, Moller HE, Tittgemeyer M, Hund-Georgiadis M, Schroeter ML, Mueller K (2012). Investigating structural brain changes of dehydration using voxel-based morphometry. *PLoS One* **7**: e44195.
- Van den Eynde F, Suda M, Broadbent H, Guillaume S, Van den Eynde M, Steiger H *et al* (2012). Structural magnetic resonance imaging in eating disorders: a systematic review of voxel-based morphometry studies. *Eur Eat Disord Rev* **20**: 94–105.
- Via E, Zalesky A, Sanchez I, Forcano L, Harrison BJ, Pujol J *et al* (2014). Disruption of brain white matter microstructure in women with anorexia nervosa. *J Psychiatry Neurosci* **39**: 130135.
- von Economo C, Koskinas G (1925). *Die Cytoarchitektonik der Hirnrinde des Erwachsenen Menschen: Textband und Atlas mit 112 Mikrophotographischen Tafeln*. Springer: Vienna, Austria.
- Wang Z, Xia M, Dai Z, Liang X, Song H, He Y *et al* (2015). Differentially disrupted functional connectivity of the subregions of the inferior parietal lobule in Alzheimer's disease. *Brain Struct Funct* **220**: 745–762.
- Yau WY, Bischoff-Grethe A, Theilmann RJ, Torres L, Wagner A, Kaye WH *et al* (2013). Alterations in white matter microstructure in women recovered from anorexia nervosa. *Int J Eat Disord* **46**: 701–708.
- Zhu JN, Wang JJ (2008). The cerebellum in feeding control: possible function and mechanism. *Cell Mol Neurobiol* **28**: 469–478.

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