Supplementary figures captions

sf1: Average connection strength maps for different insular territories along the trajectory of connectivity variability

In both hemispheres, Laplacian eigenmaps identified one main trajectory of continuous connectivity variation along the rostro-caudal axis. One extreme was located in the anterior insula, in a territory encompassing the anterior short gyrus and the ventral anterior insula around the limen; the other extreme was located in the dorsal and caudal extent of the posterior long gyrus. In the representation of the Laplacian eigenmaps, these are therefore the two insular fields featuring the most different connectivity patterns between each other.

In order to describe the target regions for different insular territories, in the main text we used a map matching each territory on the cortical surface with the insular voxel from which it received the highest amount of samples (See Fig. 4 in the main text). We complement here that information by describing for each insular territories, sampled in 10 bins of equal size along the recovered trajectory of connectivity variability, the regions in the brain where at least 90 samples (corresponding to $e^{4.5}$ in the picture) were found during tractography. More precisely, in order to build these maps we measured in each subject the distance along the trajectory of connectivity variation recovered by the Laplacian eigenmaps from the point with the minimum abscissa, we normalized the distance in the range 0..1, and we mapped these values in the corresponding insular voxels, according to the procedure detailed in the main text (see in the main text Materials and Methods: Laplacian eigenmaps of the insula; see also Fig. 2 in the main text). Then we grouped the calculated voxels' values in ten deciles. Each decile represents therefore a single seed territory in the insula, for which the results obtained from probabilistic tractography were considered. Within each of these decile seed region, the connectivity maps derived from probabilistic tractography of each voxel were summed together, and the results were taken in the standard MNI space using the transformation matrix derived by combining the transformation matrices from diffusion-weighted to anatomical (T1-

weighted) space, and from anatomical space to standard MNI space (See in main text Materials and Methods: Image acquisition and preprocessing). A trilinear interpolation was used for the transformation of the connectivity maps of each decile seed region into MNI space. We calculated the median of the resulting connectivity maps across subjects, and excluded all the voxel with very low connection strength (< 90 samples). Finally, the thresholded average connectivity maps were mapped in Caret (`http://www.nitrc.org/projects/caret/`) onto the Population-Average, Landmark- and Surface-based atlas of human cerebral cortex (PALS (Van Essen and Dierker, 2007; Van Essen, 2005) available at `http://sumsdb.wustl.edu/sums/humanpalsmore.do`). The volumes in MNI space, as well as the Caret `.metric` files are available upon request to the corresponding author.

We stress the fact that the decision to group the seed region in ten subregions had no particular anatomical meaning: we do not believe that the insula contains ten distinct subregions. We resorted to describe the sum of the projections from the deciles of the trajectory of connectivity variability since a much smaller number (e.g. 3) might, unlike our data, suggest the presence of distinct clusters, while a much larger number of bins (e.g. 100) would make unfeasible the qualitative comparison across different insular territories which we aimed here.

Samples drawn from the connectivity distribution of the most anterior territories of the insula (**d1**) were found mostly in the limbic and paralimbic regions, as well as in anterior territories on the inferior frontal gyrus. The central and lateral orbitofrontal gyri, the frontal pole, as well as the the most anterior part of the superior temporal lobe, including the operculum, were found to be mostly connected with this insular territory. Consistent projections were also found from this part of the insula to the amygdaloid complex, however further analyses (See main text: Topographical and cytoarchitectonic specificity of connectivity patterns across insular territories) showed that for the amygdala, most of the samples were sent mostly from the anterior most ventral part of the insula. In the inferior frontal gyrus (IFG), *pars orbitalis* was found to be mostly connected with this insular territory, and many samples were also found in *pars triangularis*; on the other side *pars opercularis* was weakly labelled in comparison more posterior insular regions. Almost no

samples from this insular territory was found in posterior brain regions, and virtually none in the left hemisphere. In the right hemisphere samples were also recorded in extrastriate occipital regions (See Main text: A comment on the claustrum), and small but strong projection were observed the most anterior part of the inferior parietal lobule and in the superior parietal lobule.

Following the trajectory of connectivity variation (**d2**, **d3**), more caudal insular territories located approximately in the middle short gyrus and in the ventral insula caudal to the limen were found to have a similar pattern of connectivity with respect to the most rostral region; however the number of samples in the frontal pole, in the orbitofrontal cortex and in the limbic lobe gradually decreases, while more consistent projections are found to *pars opercularis* and *pars triangularis* of the IFG.

More caudal insular territories (**d4-d6**) located in the posterior short gyrus and in the ventral part of the posterior long gyrus, as well as inside the central insular sulcus (CIS), are characterized by connections with the premotor cortex, predominantly with its the dorsolateral and ventrolateral extent, as well as by large clusters of samples located in the dorsal part of the primary somatosensory cortex and in the right posterior parietal cortex. Moderate to strong projections were also found to extrastriate regions of the occipital lobe (See Main text: A comment on the claustrum). Projections to the IFG progressively decrease, except for the left *pars orbitalis*, while in the temporal lobe, target regions are represented mainly by the posterior part of the STL, including STS, as well as by the middle temporal gyrus (MTG), especially in the left hemisphere.

The next insular locations (**d7-d9**) on the recovered trajectory of connectivity variability are represented by insular territories intermingled with the previous ones (especially those in d5-d6), mostly located in the putative posterior dysgranular insular cortex, and extending to more dorsal parts of the posterior insular gyri. The dorsal premotor cortex is not reached by samples from this insular region, and very few targets are found also in the ventral premotor as well as in the adjacent pars opercularis and triangularis of the IFG. Also samples from this region are found in the same extrastriate occipital regions (putative BA18 and BA19) as in the previous insular location (See Main text: A comment on the claustrum), and especially in the right hemisphere. In the parietal lobe more consistent

bilateral projections to the superior parietal and to the anterior part of the inferior parietal lobule are appreciable, and clusters of samples are present also in SII, while in the temporal lobe samples are mostly confined to the the posterior extent of STG, STS, MTG. Finally, the last location on the trajectory of connectivity variability (**d10**) is confined to the most dorsal and caudal extent of the anterior long gyrus. Samples from this region are sent to the right *pars opercularis* and left *pars triangularis* of the IFG, as well as to the adjacent part of putative BA10 and BA46. Clusters of samples are also found in the dorsal premotor area, in SI, SII and in the posterior parietal lobule. In the temporal lobe, the main target is represented by the posterior STS/MTG, while some clusters of samples are present also in more anterior regions of the STG.

sf2: Laplacian eigenmaps of one macaque subject's insular connectivity

In macaques, a consistent relationship between the gradual transition in connectivity pattern and in cytoarchitecture was found by tracer injection studies (Chikama et al., 1997; Fudge et al., 2005; Mesulam and Mufson, 1985) (See also Main Text: Introduction and Discussion for other references). This finding represented the starting hypothesis of the present work in human. We present in this picture preliminary findings (Cerliani, 2009) on a high resolution macaque dataset provided by Alex De Crespigny and Helen D'Arceuil, acquired with the following parameters: 0.43 mm isotropic resolution, TE = 33 ms, 8 shots, TR = 350 ms, 120 isotropically distributed diffusion directions, b(max) = 8000, delta = 6.85 ms, Delta = 10.8 ms, 27 hours total scan time. The brain was fixed with formalin and soaked for 28 days in a solution containing gadolinium (Gd-DTPA) MRI contrast agent to reduce the T1 relaxation time. The insular region of interest was drawn manually on the b=0 image, using the circular sulcus to delimit the boundaries of the ROI. In the rostro-ventral region, the ideal plane connecting the limen insulae with the anterior periinsular sulcus was used as boundary. The methodology followed for the analysis of these data (probabilistic tractography, calculation of the correlation matrix and Laplacian eigenmaps) was identical to that used in human subjects, and detailed in the Materials and Methods part of the main text.

sf2A: In both hemispheres, Laplacian eigenmaps recover a gradual one-dimensional trajectory of variation in the connectivity pattern of insular voxels, spanning from rostro-ventral to dorso-caudal territories. Colors in the graphs of Laplacian eigenmaps encode the distance among voxels on the y (rostro-caudal) direction; colors in the anatomical image represent the distance along the x axis (main trajectory of connectivity variability) from the point with the minimum abscissa. The artwork between the two Laplacian eigenmaps, adapted from (Mesulam and Mufson, 1982), suggests the consistency between our results and the approximate location of the agranular, dysgranular and granular insula.

sf2B: Connectivity maps of the voxels in the 1st (blue), 6th (purple) and 10th (orange) decile of the distance along the main trajectory of connectivity variability. In this maps, the results of distance-corrected probabilistic tractography have been log transformed and thresholded to 5.5, corresponding to ~250 samples for each voxel. The b=0 image was aligned to a recently developed MR macaque atlas (McLaren et al., 2009) and mapped onto the F99 surface available in Caret SumsDB database (`http://sumsdb.wustl.edu/sums/index.jsp`). While samples seeded in the antero-ventral insular cortex reach mostly regions in the paralimbic belt and in the prefrontal cortex, as well as in the inferior temporal lobe, samples from the dorso-caudal insular region mostly reach premotor, primary sensory and posterior parietal regions. Samples from the transitional zone in the insula feature a mixed connectivity pattern between the other two regions.

sf2C: In order to evaluate the magnitude of the rate of change across connectivity patterns in the insula, we also performed probabilistic tractography and build Laplacian eigenmaps of the medial premotor wall, in a region encompassing F1, F3 and F6, above the cingulate cortex, and delimited caudally by the central sulcus, and rostrally by the plane through the genu of the corpus callosum. In this region previous animal studies evidenced the presence of sharp cytoarchitectural, connectional and functional boundaries (See for instance (Picard and Strick, 1996), from which the picture on the right in sf3C was taken). In this case, Laplacian eigenmaps also recovered a one dimensional trajectory of variability, but featuring two interruptions. When replotting the principal component of the Laplacian eigenmaps on the anatomy, the interruptions delimited three main

subregions. Importantly, the separation between F3 and F6 was found just rostral to the plane through the anterior commissure, where it was expected to be located.

sf3: Lateralization analysis: paired t tests were performed to test the hypothesis of an asymmetry in either the amount of samples sent to each target across hemisphere, averaged over all the insular territory, or in the extent of the region of the insula found connected with each target. Asterisks indicate significant difference, corrected for multiple comparison with a false discovery rate of 0.05, corresponding to a corrected alpha threshold of 0.0013.

sf4: Drawing of the insular sulci on each subject's anatomical (T1-weighted) scan at X(MNI) = -38 for (left columns) for the left hemisphere, and X(MNI) = 39 for the right hemisphere. Abbreviations (as in (Ture et al., 1999)): CS = central insular sulcus; SIS = short insular sulcus; preCS = precentral insular sulcus; postCS = postcentral insular sulcus.

sf5: Cytoarchitectonic maps of the insular cortex. The maps of Rose (Rose, 1928), Brodmann (Brodmann, 1909), von Economo and Koskinas (von Economo and Koskinas, 1925), Brockhaus (Brockhaus, 1940), Bonthius (Bonthius et al., 2005) and Kurth (Kurth et al., 2009) were produce as a result of *post mortem* cytoarchitectonic studies. The maps from Flechsig (Flechsig, 1901) shows regions with different temporal progression in the maturation of intracortical myelin. The map of Shaw (Shaw et al., 2008) results from comparing different temporal trajectories in the changes of cortical thickness in a sample of 764 magnetic resonance images obtained from participants in the range 3.5 to 33 years of age.

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