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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/94232> since 2016-01-11T13:09:08Z

Published version:

DOI:10.1007/s00429-012-0382-9

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UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

Brain Structure and Function (2013) 218:1–20

DOI 10.1007/s00429-012-0382-9

The definitive version is available at:

<http://www.springer.com/biomed/neuroscience/journal/429>

Functional anatomy of cortical areas characterized by Von Economo neurons

Franco Cauda · Diana M. E. Torta · Katuscia Sacco · Federico D'Agata · Elisabetta Geda · Sergio Duca · Giuliano Geminiani · Alessandro Vercelli

Abstract Von Economo's neurons (VENs) are large, bipolar or corkscrew-shaped neurons located in layers III and V of the frontoinsular and the anterior cingulate cortices. VENs are reported to be altered in pathologies such as frontotemporal dementia and autism, in which the individual's self control is seriously compromised. To investigate the role of VENs in the active human brain, we have explored the functional connectivity of brain areas containing VENs by analyzing resting state functional connectivity (rsFC) in 20 healthy volunteers. Our results show that cortical areas containing VENs form a network of frontoparietal functional connectivity. With the use of fuzzy clustering techniques, we find that this network comprises four sub-networks: the first network cluster resembles a "saliency detection" attentional network, which includes superior frontal cortex (Brodmann's Area, BA 10), inferior parietal lobe, anterior insula, and dorsal anterior cingulate cortex; the second cluster, part of a "sensory-motor network", comprises

the superior temporal, precentral and postcentral areas; the third cluster consists of frontal ventromedial and ventrodorsal areas constituted by parts of the "anterior default mode network"; and the fourth cluster encompasses dorsal anterior cingulate cortex, dorsomedial prefrontal, and superior frontal (BA 10) areas, resembling the anterior part of the "dorsal attentional network". Thus, the network that emerges from analyzing functional connectivity among areas that are known to contain VENs is primarily involved in functions of saliency detection and self-regulation. In addition, parts of this network constitute sub-networks that partially overlap with the default mode, the sensory-motor and the dorsal attentional networks.

Keywords Von Economo neurons · Resting state · Functional connectivity · Clustering · FMRI

Abbreviations

VENs	Von Economo's neurons
FI	Frontoinsular cortex
ACC	Anterior cingulate cortex
ATF3	Activating-transcription factor 3
IL4Ra	Interleukin 4 receptor
NMB	Neuromedin B
DTI	Diffusion tensor imaging
rsFC	Resting state functional connectivity
BOLD	Blood oxygen level-dependent
EPI	Echoplanar sequences
TR	Repetition time
FoV	Field of view
AC-PC	Anterior-posterior commissure
FFE	Fast field echo sequence
GLM	General linear model
FCM	Fuzzy-mean clustering technique

Electronic supplementary material The online version of this article (doi:[10.1007/s00429-012-0382-9](https://doi.org/10.1007/s00429-012-0382-9)) contains supplementary material, which is available to authorized users.

F. Cauda · K. Sacco · F. D'Agata · E. Geda · S. Duca · G. Geminiani
CCS fMRI, Koelliker Hospital, Turin, Italy

F. Cauda (✉) · D. M. E. Torta · K. Sacco · F. D'Agata · E. Geda · S. Duca · G. Geminiani
Department of Psychology, University of Turin, Turin, Italy
e-mail: franco.cauda@unito.it

A. Vercelli
Department of Anatomy, Pharmacology and Forensic Medicine,
Neuroscience Institute of the Cavalieri Ottolenghi,
University of Turin, Orbassano, Italy

Introduction

Von Economo's neurons (VENs), occasionally observed by Betz (1874, 1881) and Ramon y Cajal (1900, 1904), were first described in detail by Von Economo and Koskinas [Von Economo and Koskinas 1925] as large, bipolar/corkscrew-shaped neurons located in layers III and V of the fronto-insular (FI) and anterior cingulate cortices (ACC; Brodmann area BA 24, see also (Butti et al. 2011; Seeley et al. 2011; Nimchinsky et al. 1995)]. These neurons have been described also in other areas, such as the dorsolateral prefrontal cortex (Brodmann's area 9) (Fajardo et al. 2008), and the subicular and entorhinal cortices (Butti and Hof 2010; Butti et al. 2009; Ngowyang 1932). The vertical orientation of VENs and the narrow lateral extent of their dendritic arbors suggest that they may relay the output of cortical columns (Watson et al. 2006). VENs express the peptides neuromedin B and gastrin releasing peptide, both of which are involved in the regulation of appetite. Based on these findings, some authors (Allman et al. 2010) have supposed that VENs originate from a phylogenetically ancient population of neurons in the insular cortex that are involved in the control of appetite. VENs also exhibit the highest immunoreactivity for activating-transcription factor 3 (ATF3), interleukin 4 receptor (IL4Ra), and NMB within the cortex, in a higher proportion compared to the brains of other apes (Stimpson et al. 2011). These peptides are related to pain, immune modulation and digestion, and their expression suggests an involvement of VENs in the interoception of one's own homeostatic condition (Stimpson et al. 2011). In the developing human, the VENs can first be identified at late stages of gestation (36 weeks post-conception), and their number increases in the first eight postnatal months (Allman et al. 2011) reaching the adult number at 4 years of age (Allman et al. 2010).

VENs in the anterior cingulate cortex (BA 24) can be retrogradely labeled in vitro (in fixed brains) from DiI crystals placed in the cingulum bundle, indicating that they are projection neurons similar to pyramids (Nimchinsky et al. 1995), or even a special type of pyramidal neurons—this is also supported by biochemical data (Butti et al. 2009). Nevertheless, DiI cannot trace long-range connections in fixed tissue, and it is not possible to use neuronal tracers in vivo in the human brain (Vercelli et al. 2000). Instead, diffusion tensor imaging (DTI) has been used to study some types of long-range connectivity: DTI reveals that VEN-containing regions of the cortex connect with the frontal pole, as well as with other parts of frontal and insular cortices, the septum, and the amygdale (Allman et al. 2011). However, we cannot directly infer from these data the pattern of projection of VENs, either in terms of corticocortical connectivity or in terms of subcortical projection as their relation to the regulation of visceral functions would suggest.

VENs have been identified in humans, chimpanzee, bonobos, gorillas, and orangutan, i.e., in the great apes (Nimchinsky et al. 1999). VENs are also found in whales (Hof and Van der Gucht 2007), and in a number of different cetaceans (with different brain sizes) including the bottlenose dolphin (*Tursiops truncatus*), Risso's dolphin (*Grampus griseus*), the beluga whale (*Delphinapterus leucas*), and humpback whale (*Megaptera novaeangliae*) (Butti et al. 2009). And, finally, they have been observed in the brain of the elephant (Hakeem et al. 2009). This phylogenetic distribution has led to the hypothesis that the presence of VENs is correlated to the brain size. However, other authors have argued that the presence of VENs is related to special functions such as mirror self recognition. On the other hand, VENs are the 5.6% of layer V pyramidal neurons in humans and decrease to 0.6% in the orangutan (Nimchinsky et al. 1999). Their soma size is strongly correlated to the encephalization quotient (a measure of observed brain size relative to expected brain size derived from a regression of brain weight on body weight) thus leading some authors to the hypothesis that VENs are linked to cognitive functions supported by the prefrontal cortex (Nimchinsky et al. 1999).

Emerging data have involved VENs in the pathogenesis of specific neurological and psychiatric diseases. VENs are selectively affected in a behavioral variant of frontotemporal dementia in which empathy, social awareness, and self control are seriously compromised (Kim et al. 2011; Seeley 2008; Seeley et al. 2006, 2007a). A reduction in the number of VENs is associated with agenesis of the corpus callosum (Kaufman et al. 2008), whereas ischemic lesions of the corpus callosum do not affect the number of VENs (Allman et al. 2010). Contrasting findings have been reported about the number of VENs in autism (Simms et al. 2009): compared to controls, in three out of nine autistic cases VENs were increased whereas in the remaining six they were decreased. In contrast, Santos and colleagues found an increase in the number of VENs (Santos et al. 2011) and Kennedy et al. (2007) reported no changes in fronto-insular VENs in autistic brains.

The protein DISC1, which is putatively involved in the pathogenesis of schizophrenia, has been found to be preferentially expressed in the VENs of psychologically healthy individuals compared to its expression in other pyramidal cells (Allman et al. 2010). Consistent with a possible role of VEN in the pathophysiology of schizophrenia, they have been found in reduced density in the ACC of patients with an early onset of the disorder (Brüne et al. 2011).

According to several authors, VENs and their related circuitry in the insular cortex may be involved in monitoring changes in the physiological network that regulates

an individual's own body and also an individual's social behavior. In both cases, VENs may be involved in initiating homeostatic corrections to re-set network states (Allman et al. 2005).

In a previous study (Cauda et al. 2011), we have used resting-state functional connectivity (rsFC) to analyze the connectivity of the insula in terms of cortical areas and lateralization, documenting two major complementary networks involving the ventral-anterior and the dorsal-posterior insula. One network links the anterior insula to the middle and inferior temporal cortex and anterior cingulate cortex, and is primarily related to limbic regions which play a role in emotional aspects. The second network links the middle-posterior insula to premotor, sensorimotor, supplementary motor, and middle-posterior cingulate cortices, indicating a role for the insula in sensorimotor integration. These partitions and patterns of connectivity were recently confirmed with use of diffusion tensor imaging (Cloutman et al. 2011; Jakab et al. 2011). In addition, correlation maps were found to be clearly lateralized with the salience network, related to the ventral anterior insula, displaying stronger connections with the anterior cingulate cortex on the right side, and with the frontal cortex on the left side; the posterior network having stronger connections with the superior temporal cortex and the occipital cortex on the right side (Cauda et al. 2011).

The goal of this study was to explore the functional role of VENs and the functional connectivity of the brain areas containing VEN neurons. Notably, such an approach did not allow us to provide a direct evidence that VENs are anatomically involved in the connectivity of such regions.

To calculate the functional connectivity of VENs-containing areas, we first investigated whether in the current scientific literature the term "Von Economo Neurons" is found in association with specific brain areas and other terms that are frequently cited together. This was done through two different ontological tools, PubBrain and PubAtlas (Bilder et al. 2009), with the creation of phenotype maps. Then we used rsFC (Beckmann et al. 2005; Damoiseaux et al. 2006; Raichle and Snyder 2007) to investigate the functional relationships among cortical areas containing VENs. Functional connectivity defines "the temporal correlations across cortical regions", and represents an index of brain function (Friston et al. 1993; Horwitz 2003). Spontaneous resting state fluctuations of the blood oxygen level-dependent (BOLD) fMRI signals yield patterns of spatiotemporal synchrony that are temporally coherent within anatomically and functionally related areas of the brain (Damoiseaux et al. 2006; Fox et al. 2005a; Greicius et al. 2003; Hampson et al. 2002; Vincent et al. 2007). Since evidence exists for a hierarchical functional modularity of resting state networks

(Ferrarini et al. 2009; Meunier et al. 2009; Bassett et al. 2008; He et al. 2009), we also investigated whether the network characterized by the distribution of VENs is comprised of sub-networks: this was done with use of the fuzzy clustering technique (Smolders et al. 2007; Cauda et al. 2010a) for analysis of the timecourses of the voxels in the network. Finally, using the BrainMap database (Fox et al. 2005b; Laird et al. 2005), we analyzed the engagement of VEN-rich areas in specific behavioral domain profiles.

Materials and methods

Phenotype maps

To inspect the areas of the brain that are more frequently associated with VENs in the scientific literature (as indexed in Pubmed) we performed a PubBrain (Bilder et al. 2009) query. PubBrain is a search aid and meta-analysis tool developed using the data provided in the PubMed database hosted by the National Library of Medicine. To examine as to which terms the VENs are more frequently associated in the scientific literature (as indexed in Pubmed) we created a series of phenotype maps using PubAtlas. PubAtlas is a web-based application with which one can examine and visualize cognitive concepts in the scientific literature (as represented in PubMed). PubAtlas attempts to provide "phenotype maps", using a grid to map associations of large sets of terms. We employed the following lexica: "Von Economo neurons", "gut", "pain", "attention", "emotions", "salience", "social brain" and "theory of mind". PubAtlas allows the user to specify general phenotypes (i.e., terms or lexica) and maps these terms or lexica, into heatmaps or topologies (graphs) that reflect strengths of association. Each graph is thresholded by association strength, according to "velocity plots" that show the history of co-occurrences, by year. The association strength we employed for this study is the natural log of the Jaccard similarity index (Jaccard 1901): it measures similarity between sample sets, and is defined as the size of the intersection divided by the size of the union of the sample sets:

$$J(A, B) = \frac{A \cap B}{A \cup B}$$

PubAtlas permits users to either select from a number of pre-established lexica, or to input their own lexica, as the queries to PubMed. Nodes in these graphs reflect results of individual query expressions, while edges represent measures of association between the nodes (e.g., the Jaccard coefficient).

Subjects

Twenty-four healthy, right-handed volunteers (12 males; mean = 26 years old; SD = 7.2 years), free of neurological or psychiatric disorders, not taking medications known to alter brain activity, and with no history of drug or alcohol abuse, participated in the study. Written informed consent was obtained from each subject, in accordance with the Declaration of Helsinki. The study protocol was approved by the Institutional Committee of the University of Torino on ethical use of human subjects.

Task and image acquisition

Subjects were instructed to keep their eyes closed, think of nothing in particular, and not to fall asleep. After the scanning session, participants were asked if they had fallen asleep during the scan; no subjects with positive or doubtful answers were excluded from the study.

Images were gathered on a 1.5 Tesla INTERA™ scanner (Philips Medical Systems) with a SENSE high-field, high-resolution (MRIDC) head coil optimized for functional imaging. Resting state functional T2* weighted images were acquired using echoplanar (EPI) sequences, with a repetition time (TR) of 2,000 ms, an echo time (TE) of 50 ms, and a 90° flip angle. The acquisition matrix was 64 × 64, with a 200 mm field of view (FoV). A total of 200 volumes were acquired, with each volume consisting of 19 axial slices, parallel to the anterior–posterior (AC–PC) commissure; slice thickness was 4.5 mm with a 0.5 mm gap. To reach a steady-state magnetization before acquiring the experimental data, two scans were added at the beginning of functional scanning: data from these scans were discarded. Within a single session for each participant, a set of three-dimensional high-resolution T₁-weighted structural images was acquired, using a fast field echo (FFE) sequence, with a 25 ms TR, an ultra-short TE, and a 30° flip angle. The acquisition matrix was 256 × 256, and the FoV was 256 mm. The set consisted of 160 contiguous sagittal images covering the whole brain. In-plane resolution was 1 × 1 mm and slice thickness 1 mm (1 × 1 × 1 mm voxels).

Data analysis

BOLD imaging data were analyzed using the Brain Voyager QX software (BrainInnovation, Maastricht, The Netherlands). To reduce artifacts and improve statistical analysis we processed functional images as follows: First, we performed a slice-scan time correction using a sinc interpolation algorithm. Successively, we corrected 3D motion. To this aim, we spatially aligned (Miezin et al. 2000) all volumes to the first volume by rigid body

transformations, using a trilinear interpolation algorithm; the roto-translation information was saved for subsequent elaborations. We then performed a spatial smoothing using a Gaussian kernel of 8 mm FWHM. Lastly, we applied temporal filters: linear trend removal and band pass filter of 0.01–0.1 Hz was used as several previous studies (Biswal et al. 1995; Greicius et al. 2003) have found the range of frequency (0.1–0.01 Hz) to have the greatest power in revealing the underlying connectivity (Achard et al. 2006; Biswal et al. 1995; Fransson 2006; Greicius 2008; Haggmann et al. 2008).

After pre-processing, the following series of steps was used for precise anatomical location of brain activity, and to facilitate inter-subject averaging: First, each subject's slice-based functional scan was co-registered to the 3D high-resolution structural scan. Second, the 3D structural dataset for each subject was transformed into Talairach space. Third, the volume time course for each subject was created in the subject-specific anatomic space.

Selection of ROIs

Regions of interest (ROIs) were placed in the areas containing a high density of the Von Economo Neurons according to the results of Allman et al. (2010) on the distribution of VENs in the human (Fig. 1). In Allman et al. (2010) Von Economo Neurons were identified on Nissl stained and immunoreacted sections, and reconstructions of the brain obtained from serial sections, thus defining fronto-insular (in the anterior inferior insula) and limbic anterior cingulate cortex as the VEN-containing areas. The ROI were manually drawn on the basis of the data provided by the Allman paper. Then in each subject, the selected voxels were restricted to the individual's gray matter surface, using the results of the BrainVoyager brain segmentation tool (See Fig. 2).

Functional connectivity analysis

Functional connectivity maps of the VEN-containing areas were computed according to Margulies et al. (2007). BOLD time courses were extracted from the ROIs by averaging over voxels within the three regions of interest (one for each insula and one for the cingulate cortex). Several nuisance covariates were included in the analyses, to reduce effects of physiological processes such as fluctuations related to cardiac and respiratory cycles (Bandettini and Bullmore 2008; Napadow et al. 2008) or to motion. We included nine additional covariates that modeled nuisance signals from white matter, cerebrospinal fluid, global signal (Fox et al. 2009; Weissenbacher et al. 2009), as well as from six motion parameters (three rotations and three translations). All seed-based predictors

Fig. 1 Spatial distribution of the ROIs used as seed regions for rsFC analyses (only left hemisphere is represented)



were z -normalized. Temporal autocorrelation correction (pre-whitening) (Woolrich et al. 2001) was used. Seed ROI-driven functional connectivity maps were computed on a voxel-wise basis for each previously selected region. The individual participant multiple regression analysis was carried out using the general linear model (GLM) (Penny et al. 2006) and resulted in a t -based map (SPM- t) corrected for multiple comparisons at the cluster level using a Monte Carlo simulation (Forman et al. 1995; Goebel et al. 2006), see supporting online materials) ($p < 0.05$), leading to a cluster threshold $K > 22$ voxels in the native resolution).

Group statistical map

Random effect group-level analyses, controlling for age and gender effects, were carried out with use of the ANCOVA analysis tool, implemented in BrainVoyager QX. Corrections for multiple comparisons were performed at the cluster level using a Monte Carlo simulation (Forman et al. 1995; Goebel et al. 2006), see supporting online materials) ($p < 0.05$), leading to a cluster threshold $K > 22$ voxels in the native resolution); the resulting maps were then projected on a 3D representation of a template, created with use of the BrainVoyager QX cortical tool.

To evaluate the spatial consistency of functional connectivity patterns across subjects, we computed spatial probabilistic maps, calculated separately for each ROI-generated network. These maps describe the relative frequency (expressed in percentage) with which the same network is represented over different brain areas.

Voxelwise parcellation

Since in the same cortical area other neuron types are intermixed with VENs and participate in several networks, we performed a fuzzy clustering to achieve a voxel-wise segregation of the main VEN network. Voxels belonging to the VEN network were submitted to a voxel-wise

unsupervised fuzzy clustering technique, as implemented in the BrainVoyager QX Fuzzy clustering plug-in (Smolders et al. 2007). Clustering techniques allow grouping together elements that share similar characteristics. In our case, we used an unsupervised clustering, meaning that we did not choose a priori criteria to assign elements to clusters. Voxels were grouped into clusters sharing a similar temporal profile, which suggests a functional similarity. Fuzzy clustering partitions a subset of n voxels into c “clusters” of activation (Smolders et al. 2007; Zadeh 1976). The z -standardized signal time courses of all voxels are considered simultaneously, compared, and assigned to a representative cluster time courses (cluster centroids). The extent to which a voxel belongs to a cluster is defined by the similarity (measured by correlation) of its time course to the cluster centroid. At the end of the clustering procedure we obtained c clusters each containing a certain number of voxels sharing a similar temporal profile (standardized timecourse).

In this method, “fuzziness” relates to the fact that a voxel is generally not uniquely assigned to one cluster, but, instead, some voxels can be classified in more than one cluster; this percentage is controlled by the parameter “ m ”. We ensured an optimal implementation of the fuzzy clustering by performing an unsupervised search for the optimal number of clusters (see Supplementary Method section) leading to a number of four clusters. As suggested in the literature (Fadili et al. 2000, 2001; Golay et al. 1998; Moller et al. 2002), we set the parameter “ m ”, which controls the degree of fuzziness, to a value within the range of values commonly used in Fuzzy C-Means algorithms using fMRI datasets (0.4), thus allowing some voxels to be classified in more than one cluster. Principal component analysis was applied to the datasets to reduce dimensionality, while capturing at least 90% of the total variance/covariance. Single subject maps were grouped using the SogIca method (see supplementary material (Cauda et al. 2010b), and group-level results were visualized using probabilistic maps. The resulting probabilistic maps were reported in the interval [10–100%] and superimposed on the inflated representation of a template brain (average brain).

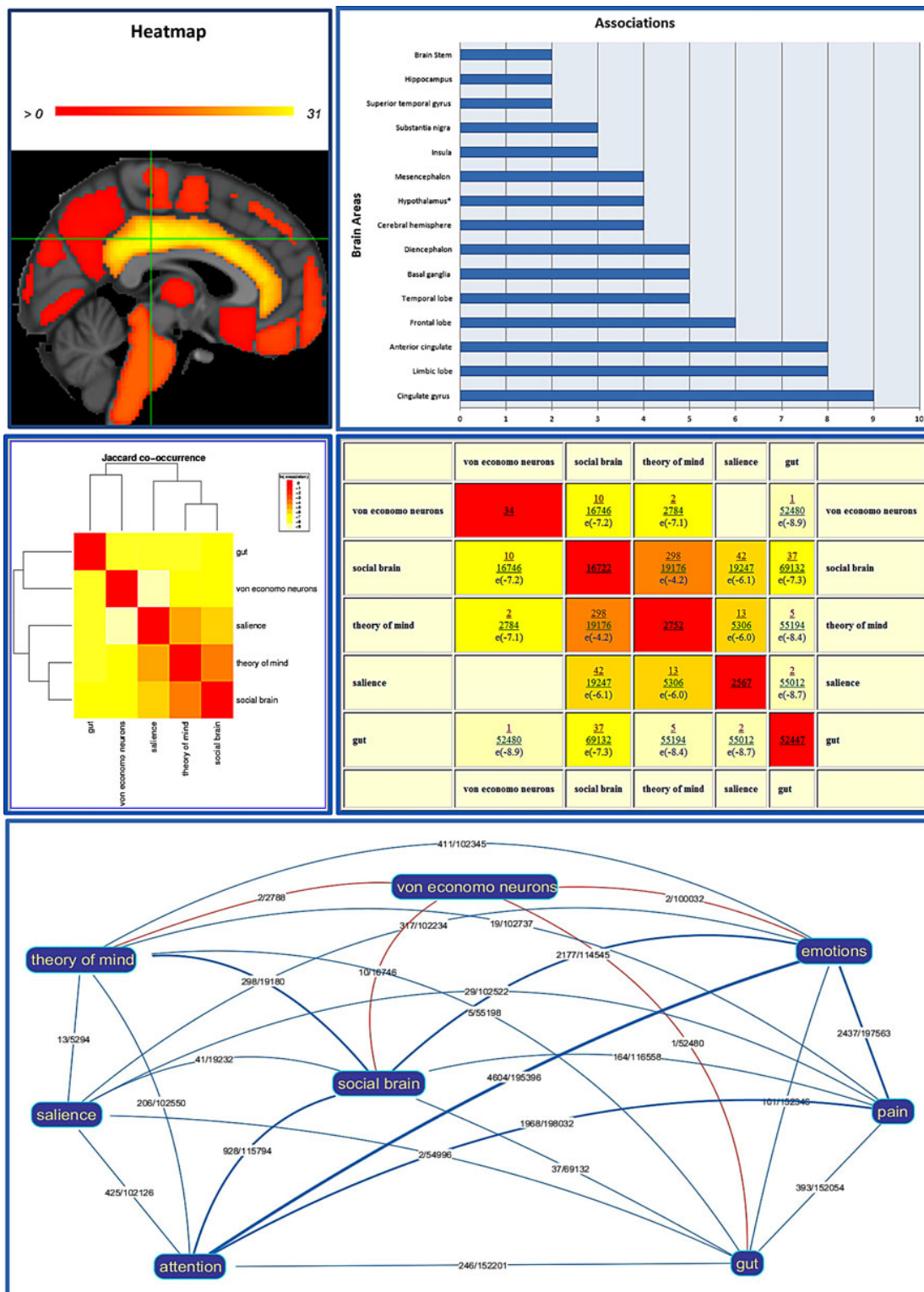


Fig. 2 Cognitive Ontologies PubBrain output for the query “Von Economo neurons”, illustrating the retrieval results for an arbitrary location (crosshairs positioned in the cingulate gyrus). *Upper left panel* shows heat maps reporting the co-occurrence of the search term and brain anatomical locations; *upper right panel* shows a *bar-graph* depicting the co-occurrence of the search term and brain anatomical

locations; *middle panels* show graphs that report the co-occurrence and hierarchical clustering of the search terms “Von Economo neurons”, “gut”, “pain”, “attention”, “emotions”, “salience”, “social brain” and “theory of mind”, thresholded to reveal only the strongest associations (natural log of Jaccard coefficient > -10); *lower panel* shows a graph of the network of associations among terms

Behavioral domain profiles

Since we were interested in examining what brain functions are associated with the activation of our ROIs we employed the BrainMap Database (Fox et al. 2005b; Laird et al. 2005), which is publicly accessible, and which archives peak coordinates from published neuroimaging studies along with the corresponding metadata that summarize the experimental design. BrainMap currently contains 1,950 papers generated by 9,166 experiments that express 81 paradigm classes; these papers indicate 73,133 local maxima of activation (locations).

We queried BrainMap for papers involving normals that present a co-activation of all the three chosen ROIs. The query was: (Diagnosis = Normals) AND [ROIs = “right-insula”, Black, (25, -3, -14) to (45, 17, 6) AND “left-insula”, Black, (-45, -3, -14) to (-25, 17, 6) AND “acc”, (-10, 21, 12) to (10, 41, 32)].

In BrainMap, metadata are organized under three experiment-level fields: context, paradigm class, and behavioral domain. “Context” represents the purpose for which an experiment was designed; possible contexts include: normal mapping, age effects, disease effects, etc. “Paradigm class” is the experimental task isolated by the contrast; for a given experiment, multiple paradigm classes may apply. Paradigm classes include, among others, action observation, episodic recall, task switching, etc. “Behavioral domain” describes the categories and subcategories of mental operations likely to be isolated by the experimental contrast; these domains are classified in six main categories: cognition, action, perception, emotion, interoception or pharmacology, but multiple behavioral domains may apply for a given experiment: e.g., cognition-memory, cognition-attention, etc. A complete list of BrainMap’s behavioral domains can be accessed at <http://brainmap.org/subscribe/>.

Results

Phenotype maps

In Fig. 1 we inspected the areas of the brain that are more frequently associated with VENs in the scientific literature (as indexed in Pubmed). The PubBrain output for the query “Von Economo neurons”, indicated that the cingulate gyrus, the limbic lobe and the frontal lobe are the areas that are most often found associated with VENs.

The “phenotype map”, obtained by studying the association between the terms “Von Economo neurons”, “gut”, “pain”, “attention”, “emotions”, “salience”, “social brain” and “theory of mind”, and thus corresponding to individual literature queries of heatmaps or

topologies (graphs) that reflect strength of association, shows that the terms referring to salience, theory of mind and social brain are often found in association, but are less frequently linked with VENs. The term that is most often used in association with VENs is “social brain” followed by “theory of mind” and “gut”.

Functional connectivity

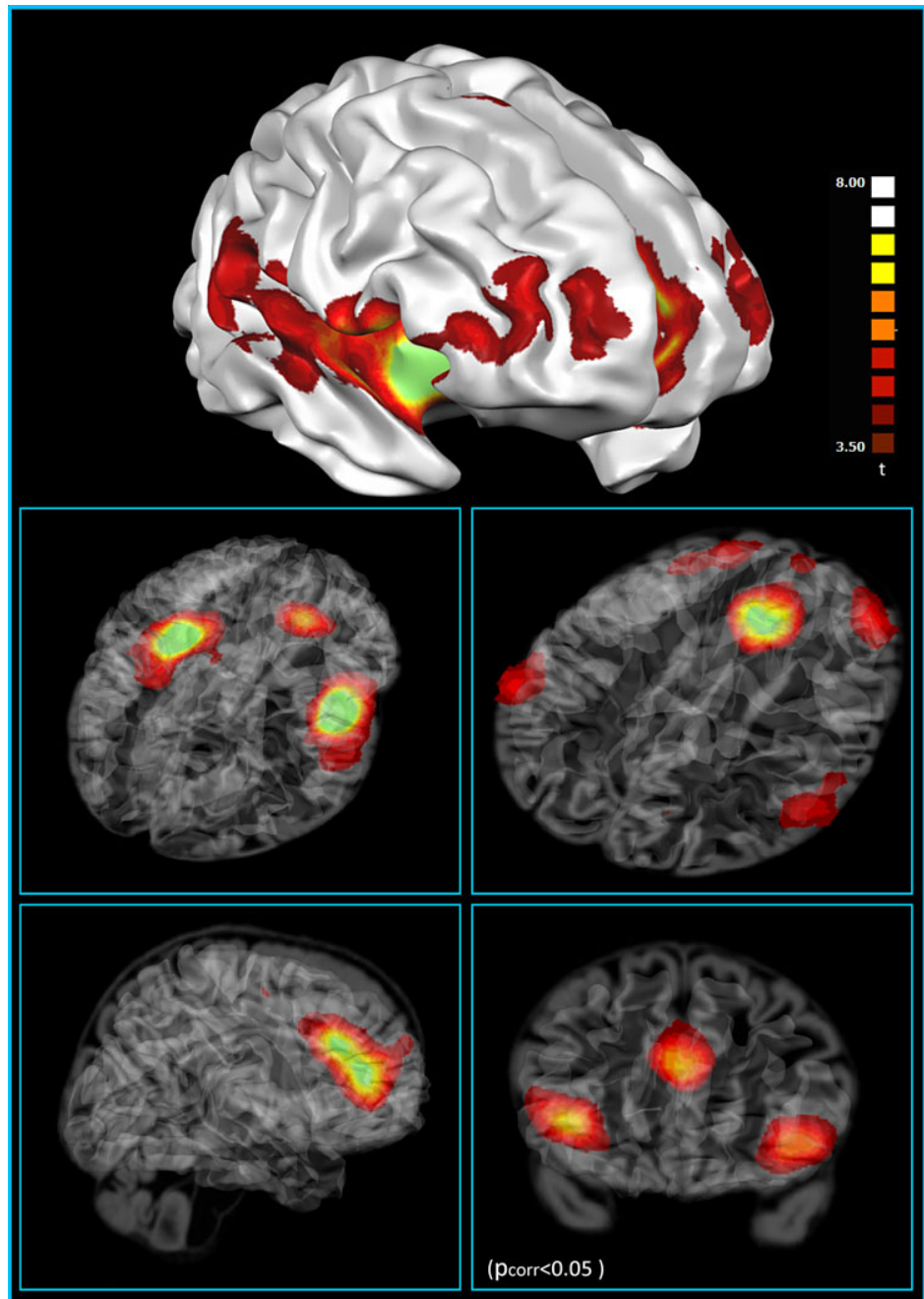
First, we inspected the functional connectivity of the three ROIs placed in the two areas that reportedly have a dense population of VENs: the ventral anterior insula and the ACC. The network comprising these ROIs showed a frontotemporal network (Fig. 3; Table 1), involving the anterior insula, the dorsal ACC, the superior, middle and inferior frontal gyrus and the inferior parietal lobule, along with some temporal thalamic and cerebellar locations.

This network was found to be prevalently bilateral, with strong right-sided lateralization in only a small portion of the middle temporal gyrus. To discover further network properties, we asked whether this functional network could be parcellated into two or more sub-clusters. To this aim, we submitted the results of the rsFC to an unsupervised fuzzy *c*-mean clustering technique (FCM) with the goal of parcellating groups of voxels that share a common time course. FCM parcellated the network into four sub-networks (Figs. 4; 5). The first cluster included superior frontal cortex (BA 10), inferior parietal lobe, anterior insula, and dorsal ACC; the second cluster comprised superior temporal, precentral and postcentral areas; the third cluster included frontal ventromedial and ventrodorsal areas; the fourth cluster encompassed dorsal ACC, dorsomedial prefrontal and superior frontal (BA 10) areas.

To characterize these four sub-networks, we visualized their full brain connectivity, removing the spatial constraints that were imposed on the previous clustering (in which only the areas that showed rsFC within the VEN-rich areas were visualized). To this aim for each subject we extracted the mean time course of each cluster, and performed a whole brain regression with this predictor (Fig. 6).

The first cluster showed a frontotemporal connectivity, and was moderately right lateralized, particularly in the insula, medial and superior frontal gyri (Fig. 7). This sub-network was found to have the highest overlap with VEN-containing areas. The second cluster showed a temporal and sensorimotor connectivity pattern, with little lateralization (Fig. 8). The third cluster included frontopolar cortices, ACC, and ventrolateral frontal cortices. As for cluster 2, the third cluster was scarcely lateralized (Fig. 9). The fourth cluster resembled the anterior part of the dorsal attentional network (Corbetta and Shulman 2002), and was poorly lateralized (Fig. 10).

Fig. 3 rsFC of the VEN-containing areas. rsFC of areas containing VENs (one sample t -test, corrections for multiple comparisons performed at the cluster level using a Monte Carlo simulation, see supporting online materials) ($p < 0.05$), leading to a cluster threshold $K > 22$ voxels in the native resolution; maps are projected on a 3D average brain with use of the Brainvoyager QX surface tool



Behavioral domain profiles

The query to the database returned 33 papers (complete list in supplementary Table 1) in which 35 experiments were performed on 584 subjects, yielding to 636 locations. We used the profiles generated by the automatic database query to characterize behavioral domains that are more often associated with the network under investigation. Seven

classes of behavioral domains were found to be related more often to the activity of this network: memory, emotions, attention, interoception, pain, action execution, and other, see Fig. 11. The predominant classes were emotions and memory, each of which expresses 21% of the activations, followed by attention (15%), interoception (12%), pain (9%) and action execution (9%); the other/variously classified studies express about 12% of the activations.

Table 1 rsFC of the VEN-containing areas

Area	Voxels	L/R%	Left BA	Right BA
Bilat out of gyrus	16,292	51/49	BA 13 BA 10 BA 47 BA 32	BA 13 BA 10 BA 47 BA 32
Bilat anteriorcingulate	7,414	48/52	BA 32 BA 24 BA 33	BA 32 BA 24 BA 33 BA 10
Bilat transverse temporal gyrus	1,574	45/55	BA 41 BA 42	BA 41 BA 42 BA 40
Bilat insula	18,524	44/56	BA 13 BA 22 BA 47 BA 44	BA 13 BA 22 BA 47 BA 44
Right middle temporal gyrus	427	0/100	–	BA 21 BA 22 BA 39
Bilat superior temporal gyrus	18,118	42/58	BA 22 BA 42 BA 41 BA 13	BA 22 BA 42 BA 41 BA 13
Bilat inferior frontal gyrus	14,375	53/47	BA 47 BA 45 BA 44 BA 46	BA 47 BA 45 BA 44 BA 46
Bilat supramarginal gyrus	2,526	24/76	BA 40	BA 40
Bilat cingulate gyrus	4,838	50/50	BA 32 BA 24 BA 9	BA 32 BA 24 BA 9
Bilat inferior parietal lobule	6,001	47/53	BA 40 BA 13 BA 22	BA 40 BA 13 BA 22
Bilat middle frontal gyrus	14,569	48/52	BA 10 BA 46 BA 9	BA 10 BA 46 BA 9
Bilat post central gyrus	2,952	43/57	BA 40 BA 43 BA 42 BA 41	BA 40 BA 43 BA 42 BA 41
Bilat pre-central gyrus	6,426	50/50	BA 44 BA 6 BA 43 BA 22	BA 44 BA 6 BA 43 BA 22
Bilat superior frontal gyrus	9,844	49/51	BA 10 BA 9	BA 10 BA 9
Bilat medial frontal gyrus	7,129	49/51	BA 9 BA 10 BA 6 BA 32	BA 9 BA 10 BA 6 BA 32
Bilat putamen	5,831	48/52	–	–

rsFC of the areas containing VENs (one sample test, corrections for multiple comparisons performed at the cluster level using a Monte Carlo simulation; see supporting online materials) ($p < 0.05$), leading to a cluster threshold $K > 22$ voxels in the native resolution

BA Brodman’s area, *Bilat* bilateral, *L/R%* percentage of voxels in the left or right hemisphere, *Out of gyrus* blobs that have a local maxima that falls out of the gray matter mask

Discussion

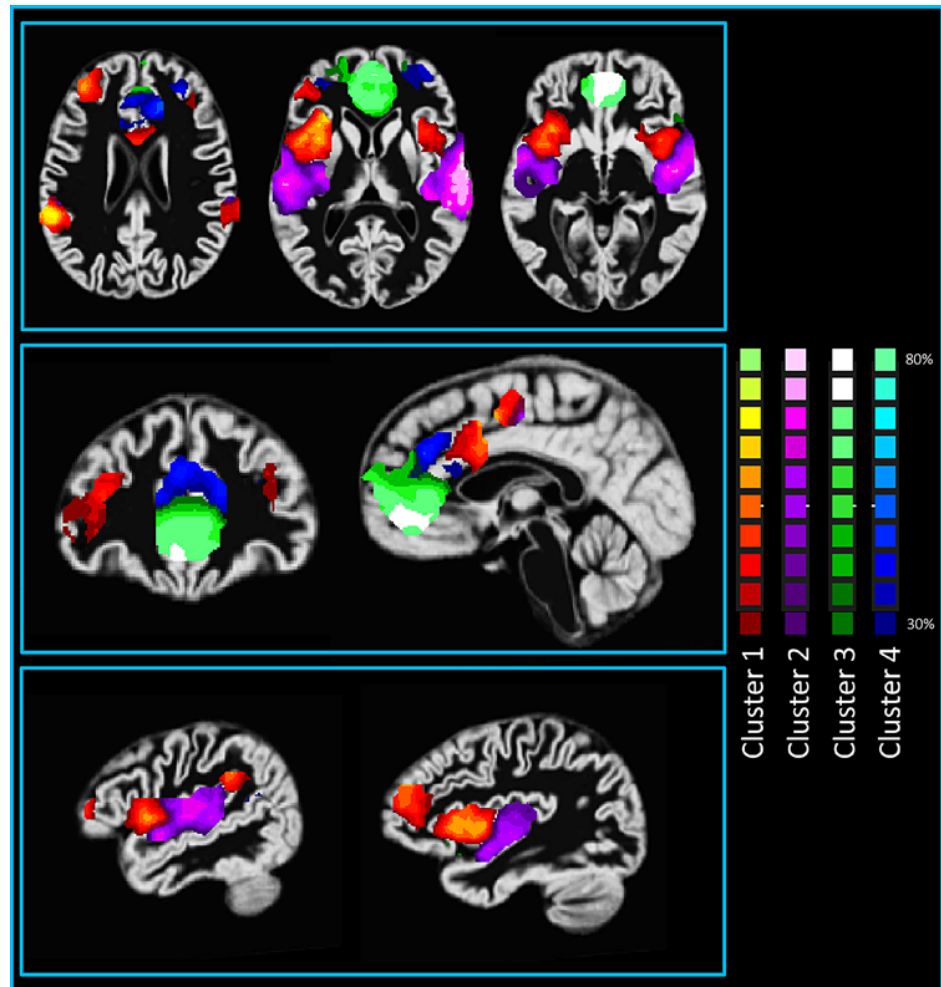
The goal of our study was to perform a literature mapping (Parker et al. 2009) using association-based techniques and to characterize the functional connectivity of human brain areas that contain VENs. Such areas have been identified by Allman et al. (2010), who showed that some areas of the human cortex contain a high density of VENs. Indeed, since tract-tracing techniques cannot be used on humans, functional connectivity of the areas containing VENs is the only approach for investigating the possible function of VENs. We first inspected functional connectivity of two regions that have the densest populations of VENs: the rostroventral insula and the ACC. Our analyses show that both areas are part of a frontoparietal network that involves the anterior insula, the dorsal ACC, the superior, middle, and inferior frontal gyri, the inferior parietal lobule and locations in the temporal cortex, thalamus, and cerebellum (Fig. 3). By parcellating groups of voxels that share a common time course, such a network can be divided in four sub-networks, with distinct connectivity profiles (Figs. 4, 5): the first includes the superior frontal lobe (BA 10), the inferior parietal lobe, the anterior insula and the dorsal ACC; the second comprises superior temporal, precentral and postcentral areas; the third includes frontal ventromedial and ventrodorsal areas; the fourth encompasses dorsal ACC, dorsomedial prefrontal and superior

frontal (BA 10) areas. Lastly, an analysis of full brain connectivity (see “Materials and methods”) showed that the first cluster is moderately lateralized on the right, particularly in relation to the insula and the medial and superior frontal gyri (Fig. 7); this sub-network also has the highest overlap with VEN-containing cortical areas, as mapped in Allman et al. (2010). The second cluster exhibits a temporal and sensory–motor connectivity pattern, with little lateralization (see Fig. 8). The third resembles the anterior part of the default mode network, including frontopolar cortices, ACC, and ventrolateral frontal cortices, and as for cluster 2, it is scarcely lateralized (Fig. 9). The fourth resembles the anterior part of the dorsal attentional network, and is poorly lateralized (see Fig. 10). The brain areas and the networks that emerge from this study only partially overlap the areas and lexica that are commonly related to VENs in literature. Indeed, as evidenced by cognitive ontologies (Bilder et al. 2009), the VENs are often cited as probable part of the social brain or related to theory of mind, but are scarcely linked to salience or to a frontoparietal control network.

Functional connectivity of the areas containing high densities of VENs

The first analysis on the functional connectivity of the three ROIs with a high density of VENs shows that they are part

Fig. 4 Voxelwise parcellation of the VEN network (2D representation). Connectivity-based parcellation of the VEN network. Probabilistic maps for functional connectivity-defined clusters. The *color scheme* represents the probability (30–80%) of overlapping brains in each voxel across the 22 subjects

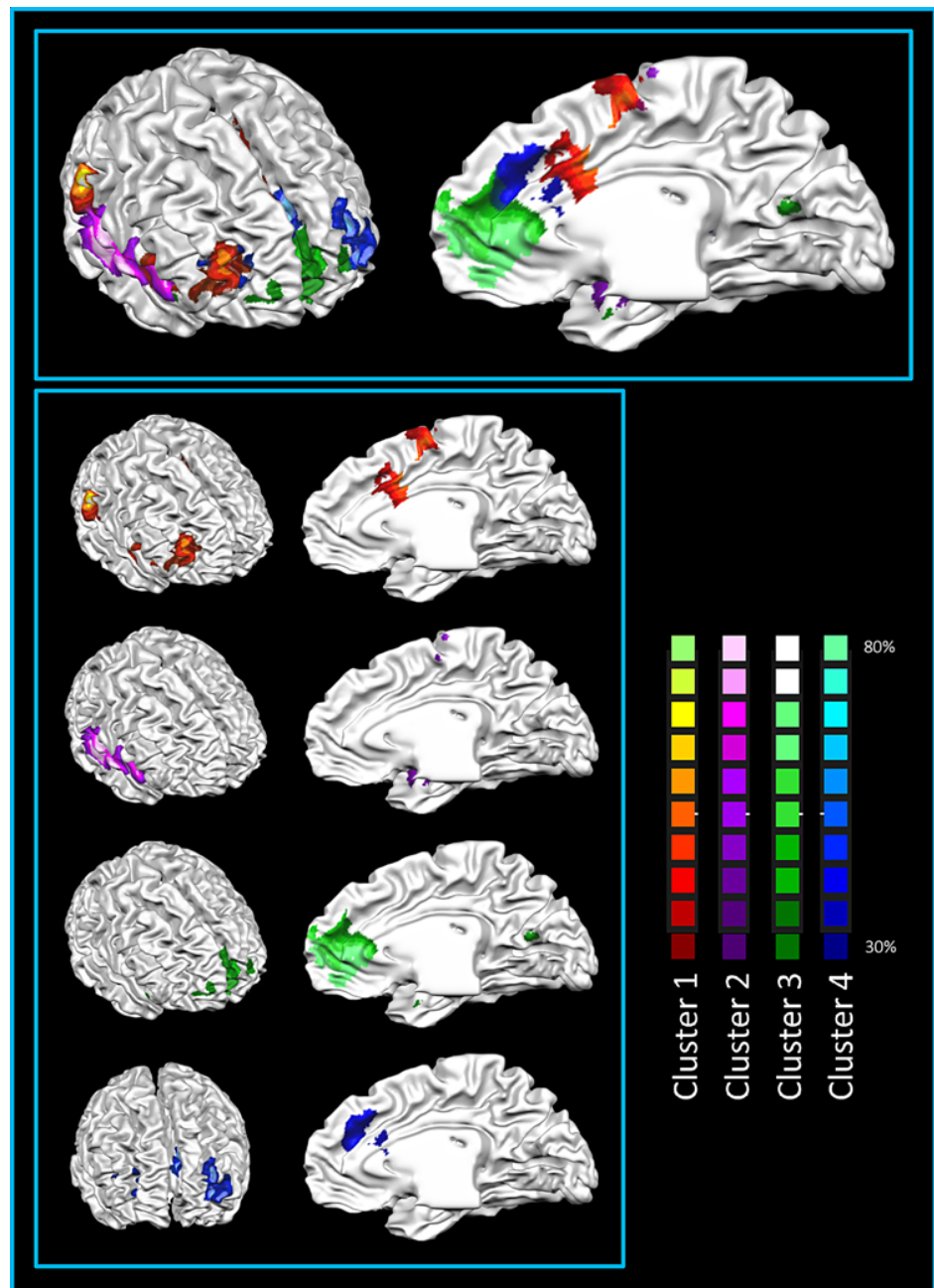


of a frontoparietal control network (Spreng et al. 2010; Vincent et al. 2008), which comprises areas of the “saliency detection network” (Seeley et al. 2007b), the “control network” (Fox et al. 2006; Seeley et al. 2007a) and the network encompassing the posterior insula (Cauda et al. 2011). This finding is in line with previous studies that relate the activity of VENs to error monitoring (Dehaene and Cohen 1994; Seeley et al. 2007b), evaluation of unexpected stimuli, and homeostatic functions (Allman et al. 2010; Greicius et al. 2003) for a review). Indeed, the anterior insula, one of the areas with a high density of VENs, has been repeatedly found to be active in highly uncertain situations (Seeley et al. 2007b). VENs are also associated with basic functions of homeostatic regulation (e.g., in the regulation of hunger), and to the homeostasis of social interpersonal relationships. Related to this, it has been noted that VENs are found in species with a highly developed social life (Allman et al. 2010). Our findings on functional connectivity push further the results of the phenotype maps that show that the group of terms that involve salience, theory of mind and social brain are often

found together, even though less frequently linked with VENs. The term that is most often used in association with VENs was “social brain” followed by “theory of mind” and “gut”. Moreover, it is interesting, from an evolutionary point of view, to note that the circuits involving areas in which VENs are located comprise ventralmost areas in the frontal and parietal lobes and the insula, which are particularly developed in humans, even compared to great apes (Preuss 2011).

According to Craig’s theory (Craig 2009, 2010), the involvement of the anterior insular cortex in a plurality of activities such as the evaluation of the emotional aspects of pain, empathy for pain, metabolic stress, hunger, pleasant touch, viewing faces of loved ones or allies, and social rejection (Seeley et al. 2007b) can be explained if we consider the anterior insula to be a site of convergence for the proprioceptive, interoceptive, emotional, cognitive, homeostatic, and environmental information originating in the posterior insula. The anterior insula would therefore build a coherent representation of the self in space and time, and the circuit that encompasses the anterior insula

Fig. 5 Voxelwise clustering of the VEN network (3D representation). Connectivity-based parcellation of VEN network. Probabilistic maps for functional connectivity-defined clusters. The *color scheme* represents the probability (30–80%) of overlapping brains in each voxel across the 24 subjects. 3D maps are projected on an inflated 3D brain surface with use of the BrainVoyager QX surface tool

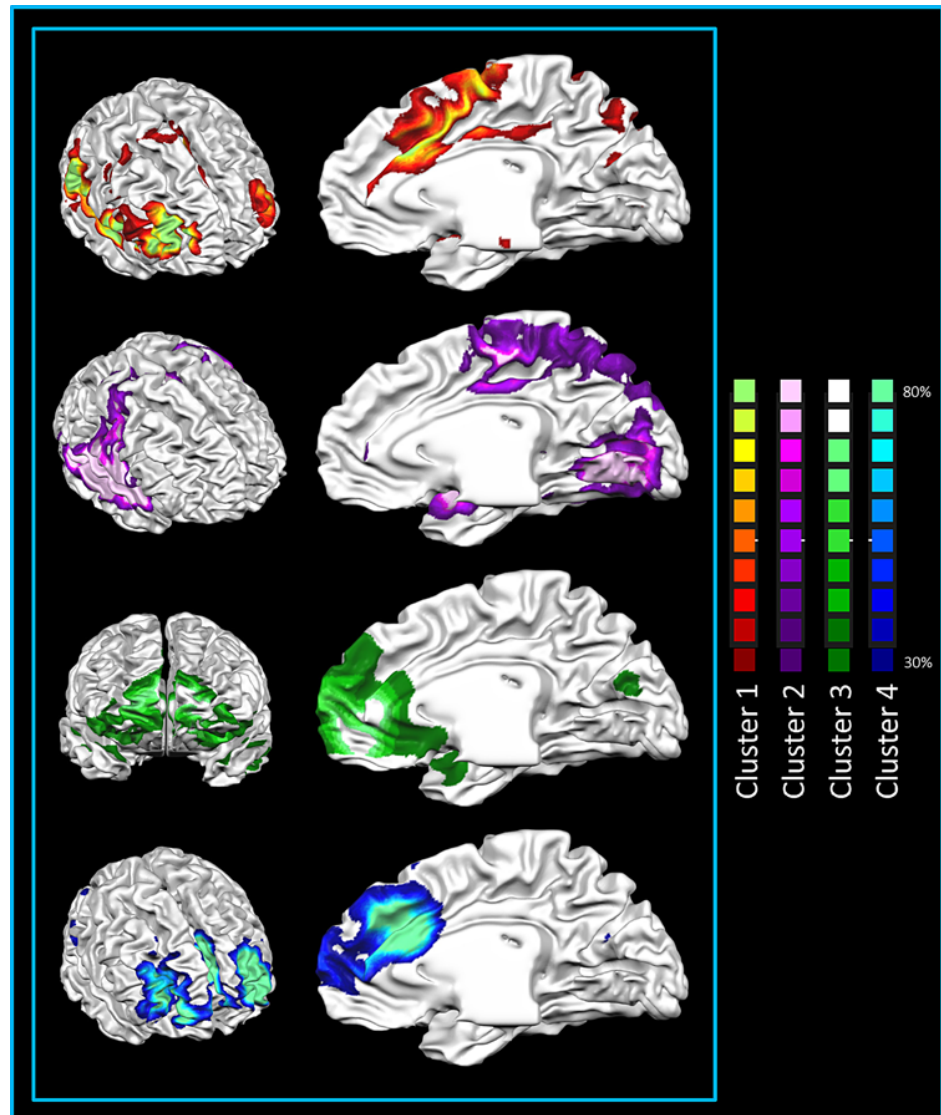


would greatly contribute to the awareness of homeostatic changes, either stimulus-driven or stimulus-independent. This and other recent theories relate the activity of the insula to different kinds of awareness (Corbetta and Shulman 2002; Craig 2010; Menon and Uddin 2010), such as motor awareness or sense-of-knowing. Recently Allman and Nelson (Allman et al. 2005; Nelson et al. 2010) implicated VENs in the rapid intuition that relies on an immediate awareness, without the engagement of deliberative processes. These authors therefore specifically relate the VENs, not just to the areas wherein they are frequently

observed, but to awareness. Such ability for “insight” is greatly reduced in patients affected by autism (Ben Shalom et al. 2006) and frontotemporal dementia. Importantly, in the brains of individuals with these disorders, a pathological reduction of VENs has been proposed (Santos et al. 2011; Seeley et al. 2006), perhaps explaining their impaired discrimination between self and other, and between self and the external environment.

Our results also show that functional connectivity between areas with a high density of VENs is not limited to the “saliency detection” system, but involves other areas of

Fig. 6 Whole brain rsFC of each cluster. Whole brain rsFC of the four clusters (one sample t -test, corrections for multiple comparisons performed at the cluster level using a Monte Carlo simulation; see supporting online materials) ($p < 0.05$), leading to a cluster threshold $K > 22$ voxels in the native resolution; maps projected on a 3D average brain with use of the Brainvoyager QX surface tool

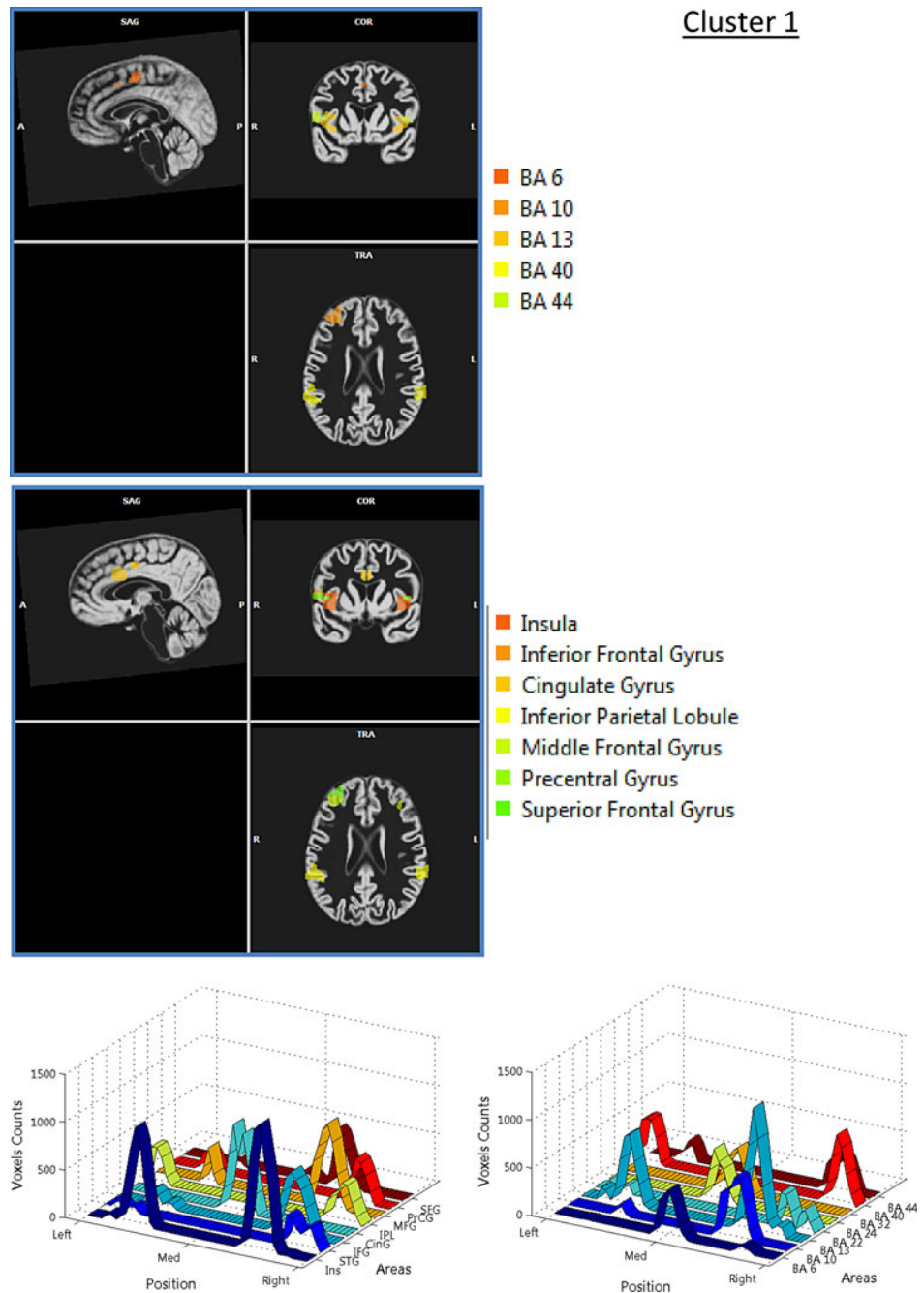


the frontoparietal control network. Recently, Sridharan et al. (2008) demonstrated that the activity of the right anterior insula precedes and causally influences the activity of other areas that belong to saliency and control networks, determining the subsequent state of these two anti-correlated systems. Additional support for the involvement of VENs in control networks comes from the report that these cells strongly express a protein encoded by the DISC1 gene, variations in which are associated with changes in cognitive functions, particularly in relation to tasks that involve maintaining and shifting attention (see Chubb et al. (2008) for a review).

An evolutionary perspective on the involvement of VENs neurons in saliency detection tasks is supported by the finding that the cells are found mostly in animals with a large brain, but their density is not correlated with relative

brain size and encephalization (Allman et al. 2010). In fact, the increase in brain size could cause a conduction delay i.e., longer time required for the transmission of information, due to the increased distance between connected cell groups. Large VENs, with large diameter axons and high conduction speed, could allow rapid information flow, and would represent an adaptive response to the brain enlargement. Therefore, VENs could provide long-range axons for conveying information as part of a saliency network that may have emerged as a consequence of a larger brain size (Allman et al. 2010). Thus, the VENs in FI cortex might serve to rapidly relay information to other parts of the brain (Allman et al. 2011). Indeed VENs seem to be especially tailored to convey such information within restricted cortical domains (Buxhoeveden and Casanova 2002).

Fig. 7 Voxelwise clustering (Cluster 1). *Upper panel* shows the subdivision of the cluster 1 in Brodmann's areas; *middle panel* shows the subdivision of the cluster 1 in gyri; lower panels show the lateralization of the BA and gyri forming the cluster 1. See supporting online materials for more detailed methods

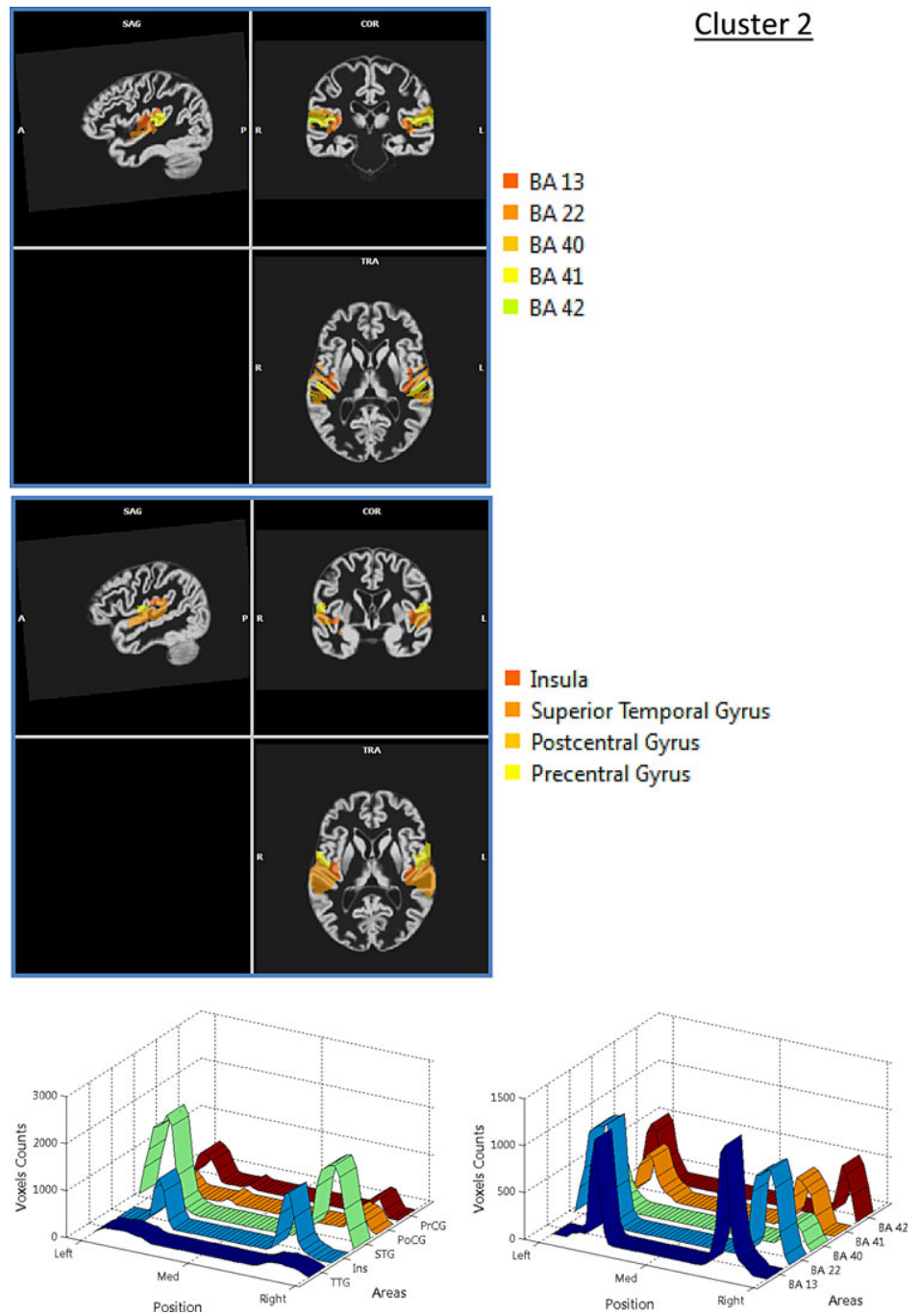


Sub-networks in the VENs' functional connectivity and lateralization

Recent findings underscore the hierarchical structural organization of cerebral networks. Indeed, the majority of cerebral networks may be further divided into sub-clusters (Bassett et al. 2008; Ferrarini et al. 2009; He et al. 2009; Meunier et al. 2009). This sub-division would allow a more effective functioning of the networks. We therefore

ran the additional analysis on the network of frontoparietal VENs' to investigate if it could be further parcellated: by applying fuzzy clustering (Cauda et al. 2010a) techniques we further divided the network encompassing the anterior insula and the ACC into four sub-networks, which were largely superimposable to those previously found studying functional connectivity. The main sub-network was composed of areas of the saliency system, excepted for posterior cingulate and sensorimotor areas—not strictly

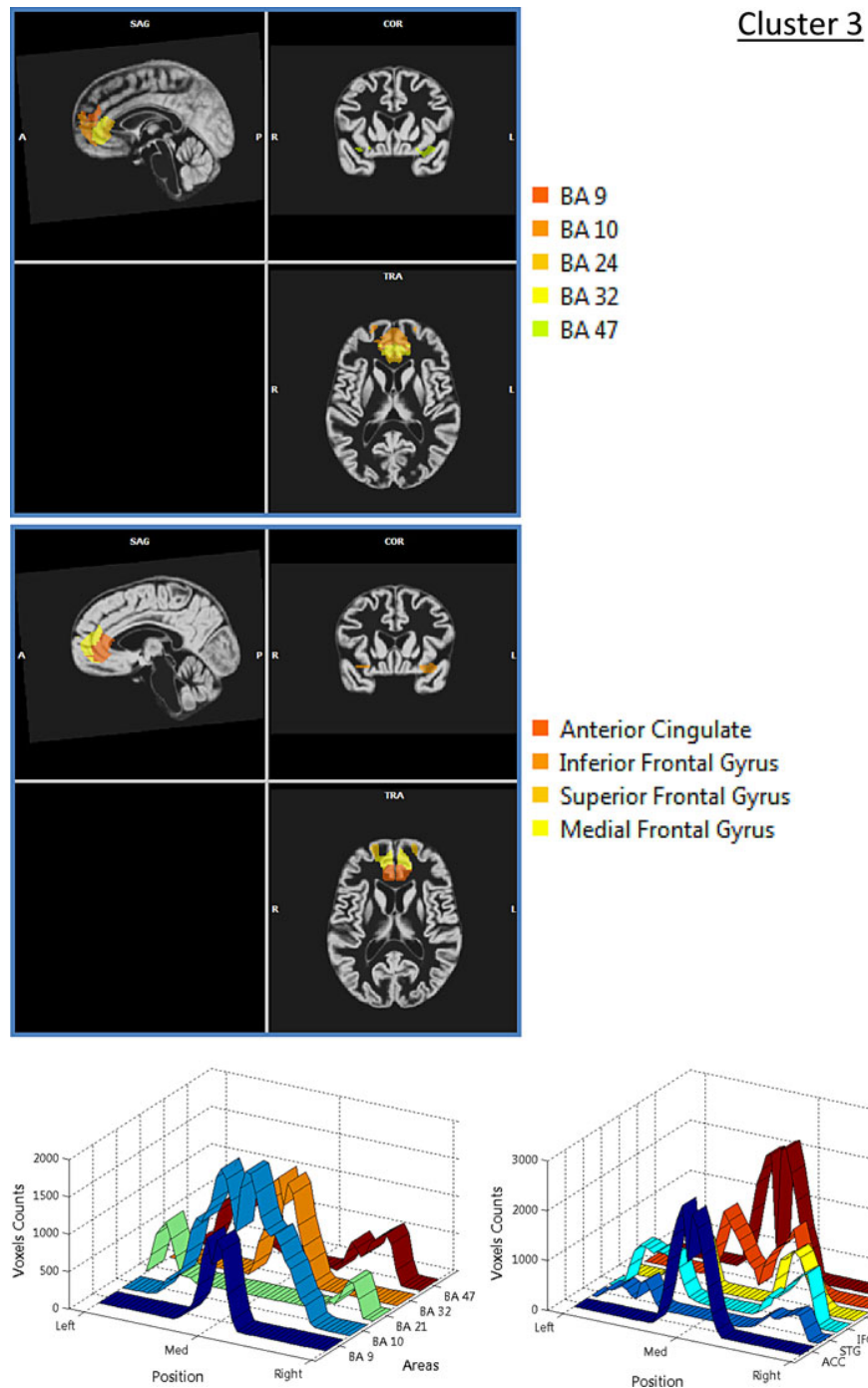
Fig. 8 Voxelwise clustering (Cluster 2). *Upper panel* shows the subdivision of the cluster 2 in Brodmann's areas; *middle panel* shows the subdivision of the cluster 2 in gyri; *lower panels* show the lateralization of the BA and gyri forming the cluster 2. See supporting online materials for more detailed methods



pertaining to the saliency system that we identified with the previous classification. This network showed a right lateralization, consistent with the finding of a higher density of VENs in the right-insula and cingulate cortex (Allman et al. 2011), and with the report that these cortical areas are thicker in the right hemisphere of normal subjects. Such asymmetry may be related to the asymmetry of the autonomic nervous system. The right hemisphere is more related to sympathetic activation, whereas the left

hemisphere is more related to parasympathetic activation; such asymmetry is also consistent with the right lateralization of the saliency detection function that evaluates the potential dangerousness of a stimulus for the survival of the organism. The other three clusters, while pertaining to the frontoparietal control network, may also be considered as parts of other important cerebral networks such as the default mode network (Raichle and Snyder 2007), control network (Menon and Uddin 2010) and posterior insular

Fig. 9 Voxelwise clustering (Cluster 3). *Upper panel* shows the subdivision of the cluster 3 in Brodmann's areas; *middle panel* shows the subdivision of the cluster 3 in gyri; *lower panel* shows the lateralization of the BA and gyri forming the cluster 3. See supporting online materials for more detailed methods

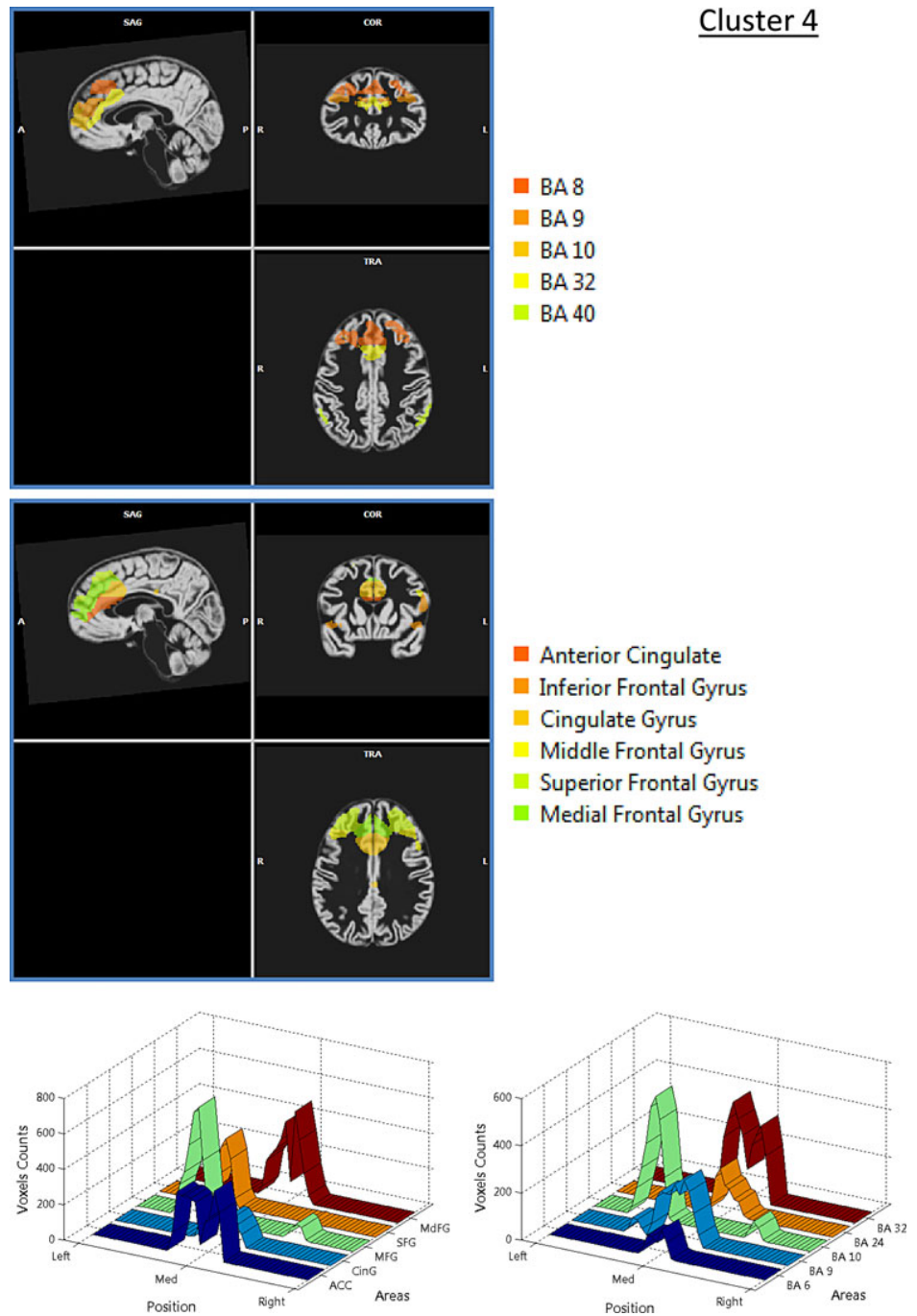


network (Cauda et al. 2011). In fact, the brain networks constantly communicate with each other and have correlated activities (Deshpande et al. 2011; Jafri et al. 2008). Some areas exert a causal influence on the communication between networks, as does anterior insula on the control and default mode networks (Sridharan et al. 2008). It has been suggested that the communication between brain networks may happen through “hubs” (Buckner et al.

2009; Sporns et al. 2007; Zamora-Lopez et al. 2010) that are common to all networks that permit the transport of information.

Behavioral networks that more frequently activate cortical areas that have VENs are those associated with memory, emotions, attention, interoception, pain, and action execution. All of these domains are coherent with the salience processing function and with subsequent

Fig. 10 Voxelwise clustering (Cluster 4). *Upper panel* shows the subdivision of the cluster 3 in Brodmann's areas; *middle panel* shows the subdivision of the cluster 3 in gyri; lower panels show the lateralization of the BA and gyri forming the cluster 3. See supporting online materials for more detailed methods



activation of effector circuits related to the insula and dorsal cingulate cortex. Here we found also an activation of the anterior insula and of the ACC (see also (Torta and Cauda 2011)). In fact, all studies with fMRI paradigm require high attention from the subject. Indeed, anterior insula and ACC are major components of the system for the flexible control of goal-directed behavior, as recently suggested by Dosenbach et al. (2006). This network for

goal-directed behavior is therefore necessarily activated during an activity such as fMRI task that requires sustained attention. It should be mentioned that, although our results cannot be considered specific for VENs neurons, as different types of neuron are found in the areas under exam, however, in this study we demonstrate that the areas characterized by VENs have a specific connectivity and a evident hierarchical sub-network structure.

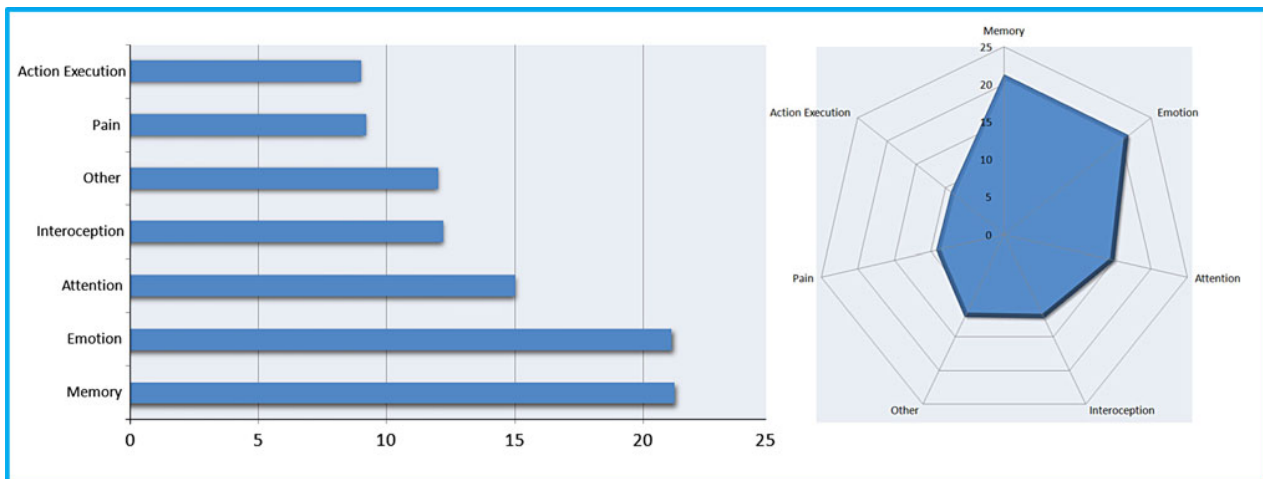


Fig. 11 Behavioral statistics. Behavioral domains that lead to activations in the VEN areas. The graphs demonstrate the number of experiments (in percent) coded in each behavioral domain, within the behavioral domain breakdown of the papers included in this meta-analysis

Conclusion

This study was designed to perform a literature mapping using association-based techniques and to characterize the functional connectivity of human brain areas that contain VENs, and thus to investigate the functional role of VEN-rich areas in humans. Our results show that areas containing VENs have a frontoparietal connectivity profile, and can be sub-divided in networks that overlap with the previously identified “saliency network”, “control network” and part of the “default mode network”. Together with the analysis of behavioral domains, and the other studies on the expression of the protein encoded by the gene *DISC1*, these results indicate that VENs are probably located in networks of saliency detection and self-regulation, confirming the possible role of these neurons in homeostatic functions. Caution should however be applied to the interpretation of the results: by describing the functional connectivity of the regions containing VENs, we suggest a possible role for such neurons, but we cannot directly infer that the functional connectivity of the VEN-containing areas reflects the activity of the VENs. Although it is plausible that at least a part of the activity in those regions is driven by the activity of the VENs, direct evidence on this is lacking.

Acknowledgments This research was supported by the Regione-Piemonte, bandoScienzeUmane e Sociali 2008, L.R. n. 4/2006.

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