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Brain and behaviour in post-acute stroke: Reduction in seeking and posterior cingulate neuronal variability

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ABSTRACT

Introduction: Stroke is a complex event on both behavioral and neuronal grounds. Recent investigations evidence the central role of subcortical damage on the post-stroke brain and behavior reorganization. We have conducted an exploratory study combining anatomical lesion analysis, functional analysis of resting state fMRI, and behavioral assessment with focus on exploration as represented by SEEKING.

Method: 24 stroke inpatients were studied immediately after their clinical stabilization post-stroke; neuronal variability in fMRI along with behavioral outcomes were assessed. These outcomes were compared with a control group of 22 healthy subjects.

Results: First, we observed predominant subcortical lesions in our sample with all stroke patients showing subcortical lesions and only some exhibiting additional cortical lesions. Second, we observed significantly reduced neuronal variability in the posterior cingulate cortex (PCC) that did not show any structural damage. Third, our stroke subjects showed reduced SEEKING which was related to reduced PCC neuronal variability in an abnormal way (compared to healthy subjects). This last outcome was assessed by considering the subset of 11 stroke subjects for which fMRI and behavioral outcomes were jointly measured.

Conclusions: Taken together, our findings suggest that damage in subcortical regions may play a central role in abnormalities in both cortical activity (PCC) and associated behavior of post-stroke reorganization. Accounting for these aspects may have significant implications to optimize multidisciplinary rehabilitation processes, particularly during the early steps of recovery, reducing the impact of stroke on the patient and caregiver quality of life.

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Post-acute stroke; SEEKING; PCC; neuronal Variability; resting State functional neuroimaging

1. Introduction

1.1. Subcortical regions in stroke

Cerebral stroke is one of the major causes of long-term disability in the world (e.g., Johnson et al., 2016). Stroke survivors present with and sustain many changes and deficits from the acute phase and post-acute phase forward, which affect daily life and sense of self. Changes to outward behavior with subsequent reduction in social functioning has a negative impact on quality of life and wellbeing for both patients and caregivers (Dabrowska-Bender et al., 2017).

Early comprehensive rehabilitation based on a proper multidisciplinary approach is necessary to improve recovery and reduce the impact of stroke (Duncan et al., 2005). It should be based on a proper assessment that connects post-stroke brain functioning to behavior. To this purpose, Corbetta et al. (2015) suggest going beyond the nearly exclusive focus on single cortical brain lesions causing specific deficits and behavioral disorders. In fact, clinical practice and recent neuroimaging show that post-stroke outcomes and conditions are only partly dependent on specific focal brain lesions (Ramsey et al., 2017). Concerning neuropsychiatric and affective-behavioral outcomes, Hackett et al. (2014) demonstrated that considerable overlap occurred among numerous symptoms and syndromes in stroke patients. The lack of direct causal relation between symptoms and brain lesion raises the question of shared underlying mechanisms. Corbetta et al. (2015) suggested that arousal regulation deficits may be associated with a more basic brain lesion location (i.e., subcortical regions). Subcortical lesions may not be associated with specific symptoms, but rather provide a basis or neural predisposition (Northoff & Heiss, 2015)

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for the various symptoms observed in stroke. Therefore, Corbetta et al. (2015) conceptualizes stroke as a subcortical, rather than a cortical, disease and stipulates a novel model of subcortical and white matter damage associated with clusters of behavioral alteration. This novel model is helpful for understanding the consequences of stroke as a complex disease.

1.2. Subcortical regions and behavior

Subcortical diseases may also impact motivation, which plays a major role in the rehabilitation phase. In fact, subcortical networks have been associated with "basic emotions" (Panksepp, 1998; Panksepp & Biven, 2012) that provide individuals with primary motivational resources for surviving, adapting to the environment, and recovering after traumatic events as well as illness stroke damages. Four basic emotional systems (SEEKING, ANGER, FEAR, and LUST) that have evolutionally deep reptilian roots and three basic emotional systems (CARING, SADNESS, and PLAY) that reflect uniquely mammalian adaptations have been identified. The basic emotional systems cited above not only generate instinctual behavioral responses but are also closely linked to subjectively experienced primal affective consciousness that accompanies these types of emotional arousal (Panksepp, 2011a, 2011b). In contrast, learning and higher brain functions are critically dependent on higher, more recently evolved brain functions that are conceptualized as secondary and tertiary processes (Northoff et al., 2010; Panksepp, 2011a).

Among basic emotions, SEEKING is crucial during early phases of post-stroke plastic reorganization because it provides instinctual motivation for recovery and rehabilitation. Neuroscientific researchers (Alcaro & Panksepp, 2011; Wright & Panksepp, 2012) suggest that "SEEKING" is rooted in psycho-behavioral and neurobiological processes that drive organisms to spontaneously explore and interact with a variety of different and specific environmental objects. This intrinsic motivational system in mammals depends on dopamine transmission and release (as an essential modulator) and is active before the organism has formed perceptual and cognitive representations of those objects. After it has been molded by learning, more activation is registered in anticipation of rewards, rather than during the pleasure accompanying consumption of rewards. Considering its innate positive affective valence, the activation of SEEKING is experienced and pursued by organisms as a positive affectively desirable state, "rewarding" in itself.

Further neuroscientific studies indicate that SEEKING behaviors are associated with intrinsic activity of subcortical and medial regions of the brain (e.g., Northoff & Panksepp, 2008; Panksepp & Northoff, 2009). Rehabilitation processes aim to not only recover functional abilities and independence in adaptation to the environment, but also to promote the patient's ability to positively engage with the world again while maintaining a sense of wellbeing. In this regard, clinical practice often shows that persons in post-stroke condition lack energy and basic motivation from early through late phases of the disorder. Proper treatments and supportive interventions are required to address these emotional components, potentially taking into account the role of SEEKING system activity in the interactive relational context.

In one of our previous studies, we investigated the impact of subcortical and cortical lesions with a focus on SEEKING which was consistently reduced in stroke patients, thus evidencing the patients' often-elevated degrees of depression (Farinelli et al., 2013). In another study (Farinelli et al., 2015), we showed that brain damages in the same regions were associated with emotion regulation. These studies suggested the neuronal correlates of SEEKING, which are the focus of the present study.

1.3. Aim and hypotheses

The general aim of our study was to investigate the neuro-functional and behavioral correlates of stroke by examining resting state fMRI and SEEKING. To this purpose, resting state fMRI was conducted by focusing on a novel measure (i.e., neuronal variability; Zang et al., 2007). In our recent studies on brain lesion patients suffering from loss of consciousness (i.e., unresponsive wakefulness) we observed activity changes in the posterior cingulate cortex (PCC), though the PCC was not affected by the lesion (Huang et al., 2014, 2016; Wu et al., 2015; Zhang et al., 2017). The PCC is an important node within the default mode network with a rich structural connectivity to many other cortical regions of the brain, suggesting its role as the cortical hub (Leech et al., 2012; Leech & Sharp, 2014). As a trans-modal region, the PCC receives and integrates information from multiple functional networks (Braga & Leech, 2015; Leech et al., 2012; Leech & Sharp, 2014). The PCC is posited to be involved in complex emotional cognitive integration through connections to heteromodal associations and paralimbic areas. Moreover, PCC activity is considered central for arousal, awareness, and for regulating the balance between internal and external attention, with more activation in internally-directed thought. Thus, level of PCC activity can be related to exploratory and outward (i.e., externally-directed) behavior as measured by SEEKING.

Therefore, we hypothesized that stroke patients with lesions predominantly located in subcortical regions would show decreased neuronal variability in the PCCstroke though cortical regions, including the PCC, would be unaffected. Hence, even if not directly damaged, cortical regions like the PCC may nevertheless be affected by the lesion indirectly, indicating functional reorganization as it is typically observed in stroke patients (Drevets et al., 2008; Lassalle-Lagadec et al., 2012; Sharp et al., 2014). Our resting state fMRI was complemented by investigating the behavioral correlates of stroke focusing on basic emotions like SEEKING. Based on our previous studies (Farinelli et al., 2015, 2013), we hypothesized reduced SEEKING would be associated with reduced neuronal variability in unaffected cortical regions, specifically the PCC (Leech & Sharp, 2014), in subcortical stroke patients.

2. Method

2.1. Study group (stroke patients)

After obtaining approval by the local ethical committee (Ethical Committee of City of Bologna, Italy, Local Health Trust file Protocol N. 141/CE), 24 stroke inpatients (see Table 1) in the post-acute phase were recruited. Patients had all been discharged from the stroke unit following stabilization and were admitted and recruited stroke at the rehabilitation hospital stroke (15 days to 1 month after the acute event). Focusing on both the affective/behavioral assessment and functional neuroimaging in the post-stroke early phases (soon after general medical stabilization) can help track neuroplastic post-stroke reorganization, including the affective behavioral aspects. This information is important in the planning of multidisciplinary rehabilitation, which is integral from the early post-stroke phases onward (Duncan et al., 2005). In particular, Coleman et al. (2017) suggested the presence of a window of pronounced neuroplasticity soon after stroke, "during which the brain's dynamic response to injury is heightened and rehabilitation might be particularly effective". Communication of the diagnosis to the patient was managed by the stroke-unit MDs before their entry into the rehabilitation hospital.

Exclusion criteria included the presence of receptive aphasia indicated by scores less than 26 on the Token Test (De Renzi & Faglioni, 1978; Spinnler & Tognoni, 1987; Zaidel, 1977). Patients with Mini Mental State Examination (MMSE) scores (Folstein et al., 1975) less than 21, corresponding to moderate/high cognitive impairments, were excluded. Patients with previous stroke events or with concomitant neurologic disease (i.e., chronic, acute or degenerative) were also excluded. Administration of all tests and questionnaires was performed within the first week of admission by trained clinical psychologists working in the rehabilitation hospital in collaboration with the neuropsychology service. Patient ability to read and understand the meaning of items were checked at the beginning of administration and supported during the compilation process; testing breaks were allowed to prevent patient burden and its potential consequences on the validity of the results.

2.2. The control group

In addition to the stroke patients, a control group was also recruited. Thus, twenty-two healthy subjects (see Table 1) without history of neurological injuries and psychiatric disorders were asked to take part in this research. They provided written informed consent and completed the battery of self-report questionnaires.

2.3. Psychometric evaluations Two self-report questionnaires were utilized

(a) AFFECTIVE NEUROSCIENCE PERSONALITY SCALE (ANPS) by Davis et al. (2003). In the Italian version of the ANPS by Pascazio et al. (2015), there is a self-report questionnaire of 110 items developed and based on evidence for brain affective systems. It measures the three basic positive emotions (ANPS-SEEKING, ANPS-PLAY, and ANPS-CARE) and the three basic negative emotions (ANPS-FEAR, ANPS-ANGER, and ANPS-SADNESS) that play a role in the development of personality. In particular:

Table 1. General statistics relative to stroke patients (stroke) and control groups. M = male; F = female. A t-test has been used to evaluate differences in age and gender. A chi-square test has been used to test differences in the other features. Significance *p* of the corresponding statistics is reported for each feature.

	Stroke (N = 24)	Control (N = 22)	t; χ^2	p
Age (years, average/SD)	60.29/10.03	45.55/12.25	1.823	0.07
Gender (M/F)	18/6	11/11	3.079	0.08
Education (primary/secondary/high/degree/unknown)	5/7/9/3/0	0/2/9/8/3	6.904	0.08
Marital status (married/divorced/separated/widowed/unmarried/unknown)	14/2/2/1/5/0	7/2/0/0/10/3	5.155	0.16

- SEEKING was defined as feeling curious, liking to explore, striving for solutions to problems and puzzles, positively anticipating new experiences, and a sense of being able to accomplish almost anything.
- PLAY was defined as having fun versus being serious, playing social games with physical contact, humor, laughter, and being generally happy and joyful.
- CARE was defined as nurturing, feeling softhearted toward animals and people in need, being drawn to young children and pets, feeling empathy, feeling affection and liking to care for others, as well as liking to be needed by others.
- FEAR was defined as experiencing anxiety, worrying, feeling tense, struggling with decisions, ruminating about past decisions and statements, losing sleep, and not typically being courageous.
- ANGER was defined as feeling hot-headed, being easily irritated and frustrated, experiencing frustration leading to anger, expressing anger verbally or physically, and remaining angry for long periods.
- SADNESS/SEPARATION DISTRESS was defined as feeling lonely and feeling distress when not with loved ones, crying frequently, thinking about loved ones and past relationships.

(a) BECK DEPRESSION INVENTORY-II (BDI-II; Beck et al., 1996; Sica & Ghisi, 2007) is a self-report questionnaire which measures the severity of depressive symptomatology. Each of the 21 items is rated on a 4-point scale ranging from 0 to 3. The total score is calculated by adding the scores of each item; the total ranges from 0 to 63. A score of 14 or above indicates the presence of depressive symptoms, which are categorized as: minimal/moderate (range 14–19), moderate-severe (range 20–29), and severe (30–63).

2.4. MRI data acquisition

A General Electric (Milwaukee, WI, USA) 3 Tesla Signa HDxt system and an eight-channel phased array coil were used to acquire fMRI and morphological sequences with the following parameters in all stroke patients and control healthy subjects. Stroke patients and control healthy subjects were tested in the same scanner.

2.4.1. Morphological MRI

(1) Whole brain high definition volumetric sagittal Spoiled GRadient Recall – Inversion Recovery (3D SPGR-IR) T1-weighted (T1-w) sequence, with Repetition Time 6916 ms, Echo Time 3104 ms, Inversion Time 750 ms, Field of View 290 mm, Matrix 512 × 512 (5.664 x 5.664 mm^2 in-plane resolution), Thickness 1.0 mm, Gap 0.0 mm, Number of EXcitations 1.0, Flip Angle 10°, Acquisition Time 6 min 35 s.

(2) Whole brain high definition volumetric sagittal CUBE T2-weighted (T2-w) sequence, with Repetition Time 3000 ms, Echo Time 66.5 ms, Inversion Time 0 ms, Field of View 256 mm, Matrix 512 × 512 (5.000 × 5.000 mm^2 in-plane resolution), Thickness 1.0 mm, Gap 0 mm, Number of Excitations 1.0, Flip Angle 90, Acquisition Time 5 min 10 s.

(3) Whole brain (36 slices) axial FLAIR (Fluid-Attenuated Inversion Recovery) T2-weighted, with Repetition Time 8002 ms, Echo Time 101.304 ms, Inversion Time 2100 ms, Field of View 240 mm, Matrix 512 × 512 (4.688 ×' 4.688 mm^2 in-plane resolution), Thickness 4.0 mm, Gap 0.0 mm, Number of Excitations 1.0, Flip Angle 90°, Acquisition Time 4 min 31 s.

(4) Whole brain (36 slices) coronal GRE (Gradient-Echo) T2-star weighted, with Repetition Time 800 ms, Echo Time 15 ms, Inversion Time 0 ms, Field of View 240 mm, Matrix 512 × 512 (4.688 x 4.688 mm^2 in-plane resolution), Thickness 4.0 mm, Gap 0 mm, Number of EXcitations 1.0, Flip Angle 17°, Acquisition Time 3 min 16 s.

Patient number 19 (Pt 19) had a claustrophobic attack shortly after starting the exam, so only the T1-w volumetric sequence was acquired for this participant. Therefore, although Pt 19 is considered in Figure 2(b), Supplementary Figure 1, and Tables 1 and 2, she was not included in other analyses or figures.

To evaluate the presence of possible midline shifts in the PCC area, we used the following procedure: for each patient, before the Talairach normalization, we traced a red cross, having the vertical line passing through the midline at the cingulate gyrus level (see Supplementary Figure 1). After a careful inspection, we noted that no stroke patients had a midline shift at the PCC level including Pt 19 who had the biggest lesion and was removed from the patient population due to a claustrophobic attack.

2.4.2. Functional MRI (fMRI)

Whole brain, 36 interleaved slices, axial (bicommissural AC-PC plane), single-shot, GE-EPI (Gradient-Echo – Echo-Planar Image) T2-star weighted, sensitive to Blood Oxygenation Level Dependent (BOLD) contrast, with Thickness 4.0 mm, Gap 0.0 mm, Field of View 240 mm,

Table 2. Lesion locations and respective classification for each patient (Pt) based on the corresponding lesion site: cortical, subcortical (SubCort), anterior (Ant), posterior (Post), lateral (Lat), medial (Med), and side (Right and Left). Cortical locations are written in red, subcortical locations are written in blue.

Pt	Lesion Locations	Cortical	SubCort	Ant	Post	Lat	Med	Right	Left
Pt01	Right occipital (+ right temporo- mesial)	x	x		x	x	x	x	
Pt02	Right fronto-parieto-insular medially up to right lateral ventricle and basal ganglia, upward to corona radiata	x	x	x	x	x	x	x	
Pt03	Right radiate crown (+ posterior part of lenticular nucleus and posterior limb of the internal capsula)		x				x	x	
Pt04	Left lenticular nucleus (+ head of caudate nucleus, external capsule, radiate crown, inferior temporal lobe)	x	x		x	x	x		x
Pt05	Left radiate crown + posterior lenticular nucleus (small lesion)		x				x		x
Pt06	Left pontine		x				x		x
Pt07	Right temporal (with minimal parietal involvement) and right capsulo-lenticular (extending to the head of caudate nucleus anteriorly and to the radiate crown upwards)	x	x		x	x	x	x	
Pt08	Left lenticulo-capsular, extending upwards to radiate crown		x				x		x
Pt09	Right parietal (postcentral gyrus) extending downwards to radiate crown	x	x		x	x	x	x	
Pt10	Right putamen, extending upward to radiate crown		x				x	x	
Pt11	Left thalamic, extending upward to radiate crown		x				x		x
Pt12	Left occipital (cortico-subcortical)	x	x		x	x	x		x
Pt13	Left lenticular nucleus and adjacent radiate crown		x				x		x
Pt14	Left semioval center, parasagittal, extending from frontal to parietal lobe		x	x	x		x		x
Pt15	Bilateral (Median/paramedian) pontine small lesions, prevailing on the right side		x				x	(x)	(x)
Pt16	Bilateral capsulo-lenticular ischemic microlacunae		x				x	(x)	(x)
Pt17	Right capsulo-lenticular, extending upward to radiate crown		x				x	x	
Pt18	Left lenticular nucleus, left radiate crown		x				x		x
Pt19	Right cortico-subcortical fronto-insular (claustrophobic Pt)	x	x	x		x	x	x	
Pt20	Left cerebellum		x				x		x
Pt21	Left thalamo-capsular		x				x		x
Pt22	Left post-central gyrus extending to pre-central gyri (at vertex only)	x		x	x	x			x
Pt23	Right fronto-temporo-parietal, extended to basal nuclei	x	x	x	x	x	x	x	
Pt24	Right lenticular nucleus, caudate nucleus, internal capsule (anterior limb), radiate crown		x				x	x	

Matrix 64 × 64 (3.75 × 3.75 mm2 in-plane resolution), Number of EXcitations 1.0, Flip Angle 90°, Repetition Time 2000 ms, Interslice Time 55 ms, Echo Time 30 ms, Acquisition Time of 5 min 10 s (150 volumes) were acquired. Two kinds of acquisitions were applied: all participants were instructed to relax, to move as little as possible, and to keep their eyes: 1) closed for 5 min (Resting State with Eyes Closed condition, RS-EC) and 2) open for 5 min (Resting State with Eyes Open condition, RS-EO).

2.5. fMRI data analysis

Pre-processing steps were implemented using the software Analysis of Functional Images, AFNI (Cox, 1996, http://afni.nimh.nih.gov/afni). The GE 3 T machine automatically deletes the first chosen number of saturated volumes (5 volumes in this case, 10 s of dummy scans) and automatically corrects interleaved acquisitions. The resulting functional images were then aligned (see Figure 1 and Supplementary Figure 1(a-c), temporally, spatially smoothed (8-mm Full Width at Half Maximum Gaussian blur), and spatially transformed into Talairach space (Talairach & Tournoux, 1988).

After the head motion correction procedure, the magnitude of head motion at each time point for 6 parameters (3 for shift and 3 for rotation) was obtained for each subject. The averaged head motion parameter for shift and rotation was calculated as the mean of the absolute frame-wise displacement across the entire scan (see Zang et al., 2007). The standard deviation (SD) of shift and rotation across all subjects was then calculated. Subjects with head motion (shift or rotation) exceeding 4 times of the Standard Deviation across all subjects were excluded from further analysis. The averaged head motion parameter for shift and rotation per subject can be translated as the average FD or degrees of rotation across the entire scan.

The estimated six parameters of head motion and mean time series from the white matter (WM) and cerebrospinal fluid (CSF) were regressed out. To minimize partial voluming with gray matter, the WM and CSF masks were eroded by one voxel (Chai et al., 2012).

Motion artifacts were addressed rigorously, as minor group differences in motion have been shown to artefactually create between-group differences (Power et al., 2012; Van Dijk et al., 2012). For this reason, the magnitude of head motion at each time point for six parameters (three for shift and three for rotation) was obtained for each fMRI run and each subject. The averaged head motion parameters and standard deviation (SD) for shift and rotation were then calculated (Zang et al., 2007). Runs with head motion (shift or rotation) exceeding 4 SDs were excluded from further analysis. Using this criterion, the following data were excluded: Stroke patients 9 and 23 for RS-EC condition (20 stroke

Figure 1. Co-registrations between morphological and functional series in the five most damaged stroke patients (Pt). In Pt 2, 7, 9 and 14, brain damages are so severe enough to interrupt the functional epi-slice, whereas in Pt 23, the damaged area is one of the widest. For each stroke patients' number, ten axial slices are shown in the regions where lesions are more evident. Inter-slice distance is 4 mm for Pt 2, 7, 9 and 14, 6 mm for Pt 23.

patients remaining); stroke patients 1, 2, 9, 15, 16 and 18 for RS-EO condition (12 stroke patients remaining). This relatively small number of stroke patients left, in RS-EO condition, certainly represents a limiting factor, but, of course, it is better to remove more stroke patients and have a smaller, cleaner sample, rather than retain more stroke patients and obtain less reliable results. In our experience, RS-EO condition is often characterized by a higher number of head movements than RS-EC condition because, when a patient keeps his eyes open, it is instinctive to look around and unintentionally moves one's head).

Data were then filtered with a band-pass filter preserving signals between 0.01 and 0.1 Hz (standard frequencies) which is thought to reflect fluctuations of spontaneous brain activity (Biswal et al., 1995; Fox &

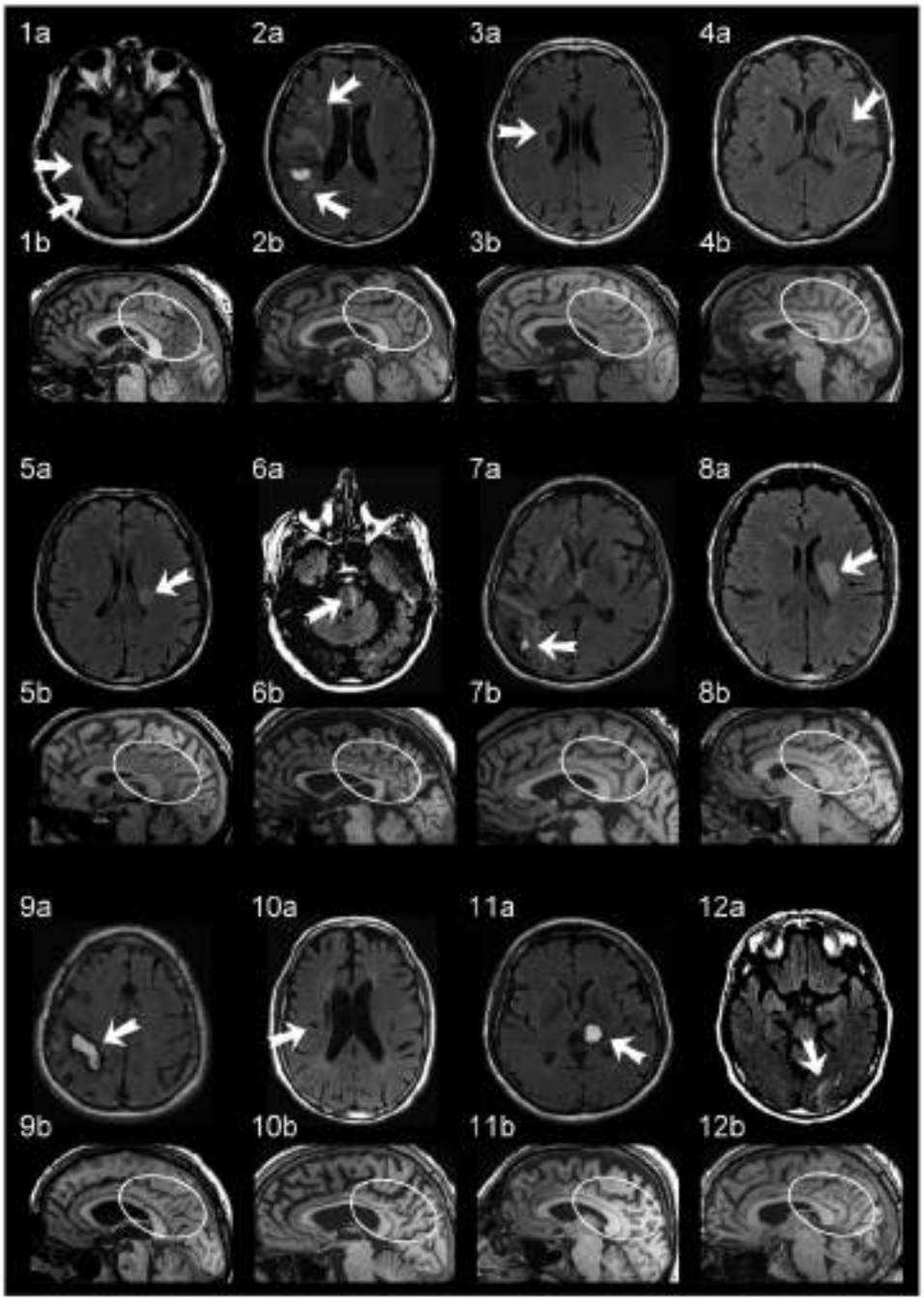

Figure 2. (a, b). Main lesion locations in the 24 stroke patients who underwent fMRI. For each patient number (1–24), the letter "a" (e.g., "1a", "2a", etc.) shows the lesion site (white arrows help identify lesion localization) in axial FLAIR sequence and the letter "b" (e.g., "1b", "2b", etc.) shows a near-midline slice in sagittal SPGR-IR T1-w sequence, where a white oval line includes the PCC region. In the claustrophobic patient (n. 19), the only SPGR-IR T1-w acquired sequence is shown. Note that, wherever the lesion is, PCC is not damaged.

Raichle, 2007; Fox et al., 2005; Zhang & Raichle, 2010). As our main hypothesis was focused on the PCC (see above), we pursued a strict region-of-interest approach. For that purpose, we drew spherical ROIs with a radius of 6 mm and placed them in the Talairach coordinates of the PCC (x = 4, y = 49, z = 25) and the Perigenual Anterior Cingulate Cortex (PACC, x = 0, y = −45, z = 0), serving as specificity control. For a given voxel, we calculated the averaged square root of the power spectrum to yield a measure of the Amplitude of Low-Frequency Fluctuations (ALFF) for the frequency range considered (0.01–0.1 Hz) (Zang et al., 2007). The ALFF of each voxel was further divided by the global mean value to reduce the potential global effects of variability across participants (Han et al., 2011; Zang et al., 2007). The resulting ALFF maps for each subject were tested in two-sample t-tests

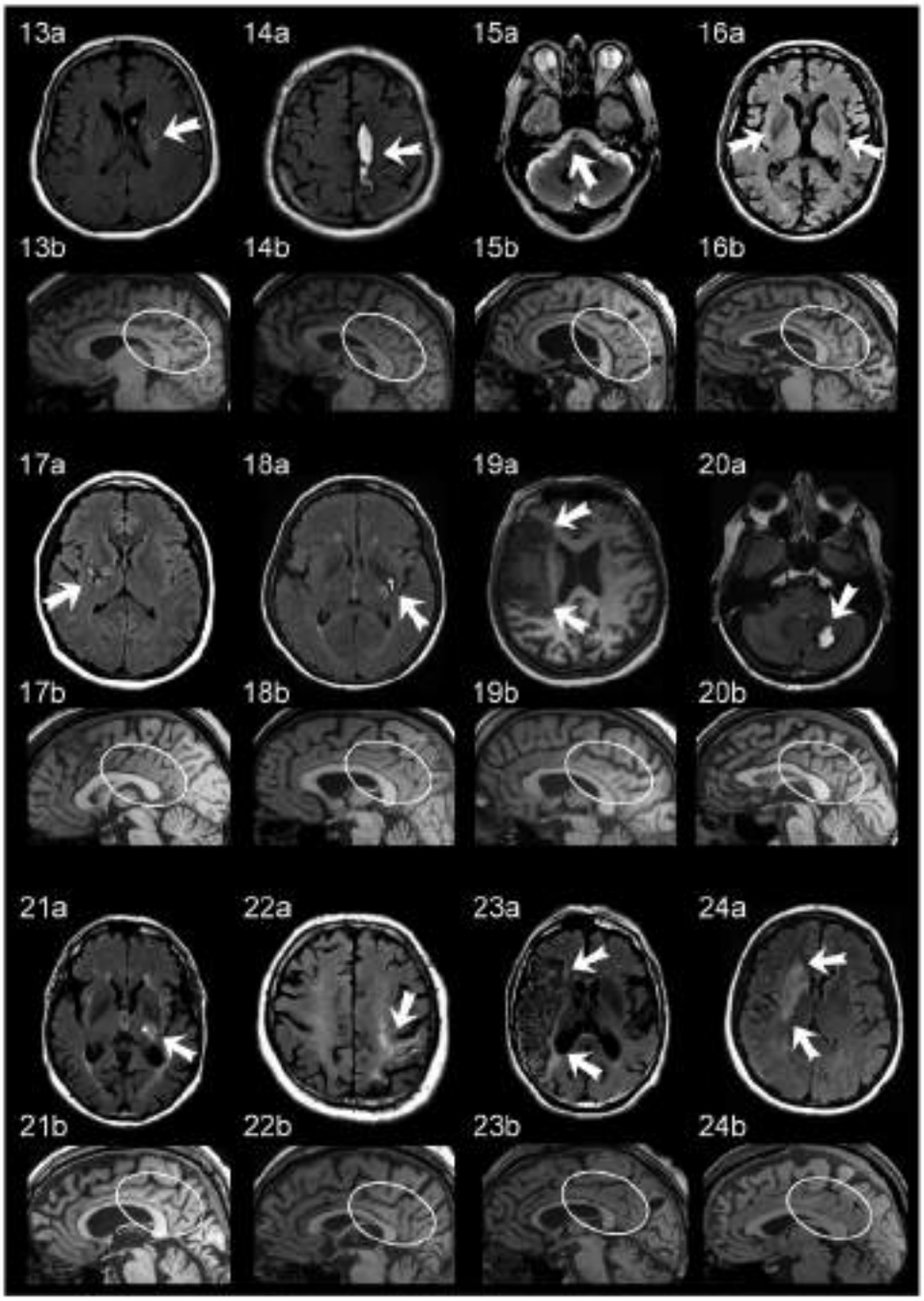

Figure 2. (continued.)

(with age and motion as irrelevant factors) to examine the group differences between control healthy subjects and stroke patients for the frequency band considered (0.01–0.1 Hz).

2.6. Statistical analysis

Frequencies, means, and percentages were used to describe socio-demographic characteristics of the stroke patients and the control group (Table 1). Statistically significant differences between control and patient groups for possible covariates (i.e., age, gender, education, marital status, and occupation) were evaluated with t-tests and chi-square comparisons, with a significance threshold of $p < .05$. No statistically significant ($p < .05$) differences were revealed between the two samples for these variables. Though there were no significant gender differences in our sample (which may be related, in part, to the small size of the sample), previous studies have reported gender differences in SEEKING in healthy subjects (Davis et al., 2003). To this

end, we conducted a multivariate analysis of covariance (MANCOVA) considering gender as a covariate to examine possible differences in the average scores of stroke and control groups. T-tests and MANOVA analysis were also applied to fMRI outcomes to investigate differences between stroke patients and control subjects. Finally, for psychometric and neuroimaging indices found to significantly differ between stroke and control groups, Pearson and Spearman correlation tests (two-tailed) were conducted to identify potential associations between the indices.

3. Results

3.1. Lesion location – Subcortical vs cortical

The main lesion locations in the 24 stroke patients who underwent MRI are described in the left part of Table 2 (second column, lesion locations) and are better illustrated in Figure 2(a,b), where the main lesions are shown, patient by patient, in an axial FLAIR sequence ("a"); the respective PCC area is also shown, included in a white oval line, patient by patient, in a sagittal paramedian SPGR-IR T1-w sequence ("b"). Notably, although stroke patients had lesions in various locations (mostly subcortical), no stroke patients showed structural damage in the PCC area, even when cortical lesions were present.

Lesion classification is better specified in the right part of Table 2, where location (cortical, subcortical, lateral, medial, anterior, posterior) and side (right and left) are considered. Classification criteria of the lesions followed those described in our previous papers (Farinelli et al., 2015, 2013). Briefly, "cortical lesions" were defined as damages restricted to cerebral gyri and sulci, while "subcortical lesions" were restricted to damages below cerebral cortex; "anterior and posterior lesions" were named with respect to the sensorimotor cortex; "medial lesions" referred to damage around midline structures (i.e., typically involving basal ganglia in subcortical regions and cingulate gyrus at cortical levels), whereas "lateral lesions" referred to brain damage starting as far as at least 30 mm from the midline, defined by drawing a line from septum pellucidum, crossing the third ventricle up to pineal gland (Liao et al., 2018). On this basis, the number of lesions found were the following (including lesions involving more than one location): cortical/subcortical 9/23; anterior/posterior: 5/9; lateral/medial: 9/23; right/left: 10/12 (excluding bilateral lesions). As to cortical/subcortical subdivision, all stroke patients but one (Pt 22) showed subcortical lesions whereas only 8 stroke patients showed lesions involving both cortical and subcortical areas (see also Table 2). They are shown in Figure 3(a,b), three axial slices for each patient.

3.2. Psychometric tests

To identify significant differences in scores obtained by stroke and control groups, a MANCOVA test was performed on psychometric data using gender as a co-variate. This analysis revealed a statistically significant difference in psychometric outcomes in the two groups ($F = 2.938$, $p = 0.011$; Wilk's $\Lambda = 0.57$, partial $\eta^2 = .43$), as well as an effect of gender, though this was not significant ($F = 1.854$, $p = .098$; Wilk's $\Lambda = 0.68$, partial $\eta^2 = 0.32$). The Tests of Between-Subjects Effects (ANOVA) are reported in Table 3. The η^2 parameter indicates that the dimension showing the greatest differences between stroke and control is the ANPS-SEEKING while the other scales (of ANPS and other scales) showed minor differences only. In particular, scores obtained by stroke patients were significantly lower than those of the control group.

3.3. fMRI

Differences between stroke and control groups in the two functional conditions (i.e., RS-EC and RS-EO) in the frequency interval studied (0.01–0.1 Hz) are shown in Figure 4. More specifically, Figure 4(a) shows color maps resulting from whole-brain voxel-wise 3dttest analysis at $p < .01$, FDR-corrected. Note that the significant difference in the whole-brain voxel-wise comparison between groups occurred mainly in a location corresponding to PCC. In contrast, no other cortical regions including the PACC showed any statistically significant differences in SD between the two groups. Due to the direction of the comparison (control healthy subjects vs stroke patients), yellow-red regions indicate supra-threshold areas, where activity of control healthy subjects is higher than activity of stroke patients: the yellower the color, the greater the difference. Figure 4(b) shows the results of PCC ROIs statistical analysis independent of the whole-brain voxel-wise analysis. A statistical two sample t-test comparing the spatial means of temporal variabilities (SD) of control healthy subjects vs stroke patients was performed. Note that both tests are statistically significant (at $p < .02$). The same statistical analysis was performed on PACC ROIs, but yielded no statistically significant results.

3.4. Correlation between PCC activity and ANPS-SEEKING scores

To evaluate the possible relationship between ANPS-SEEKING scores and SD in PCC in Stroke and Control groups, we conducted Pearson and Spearman correlation analyses. A post-hoc test for evaluating significance of observed differences between the correlations in the two groups was also performed by following Lenhard and

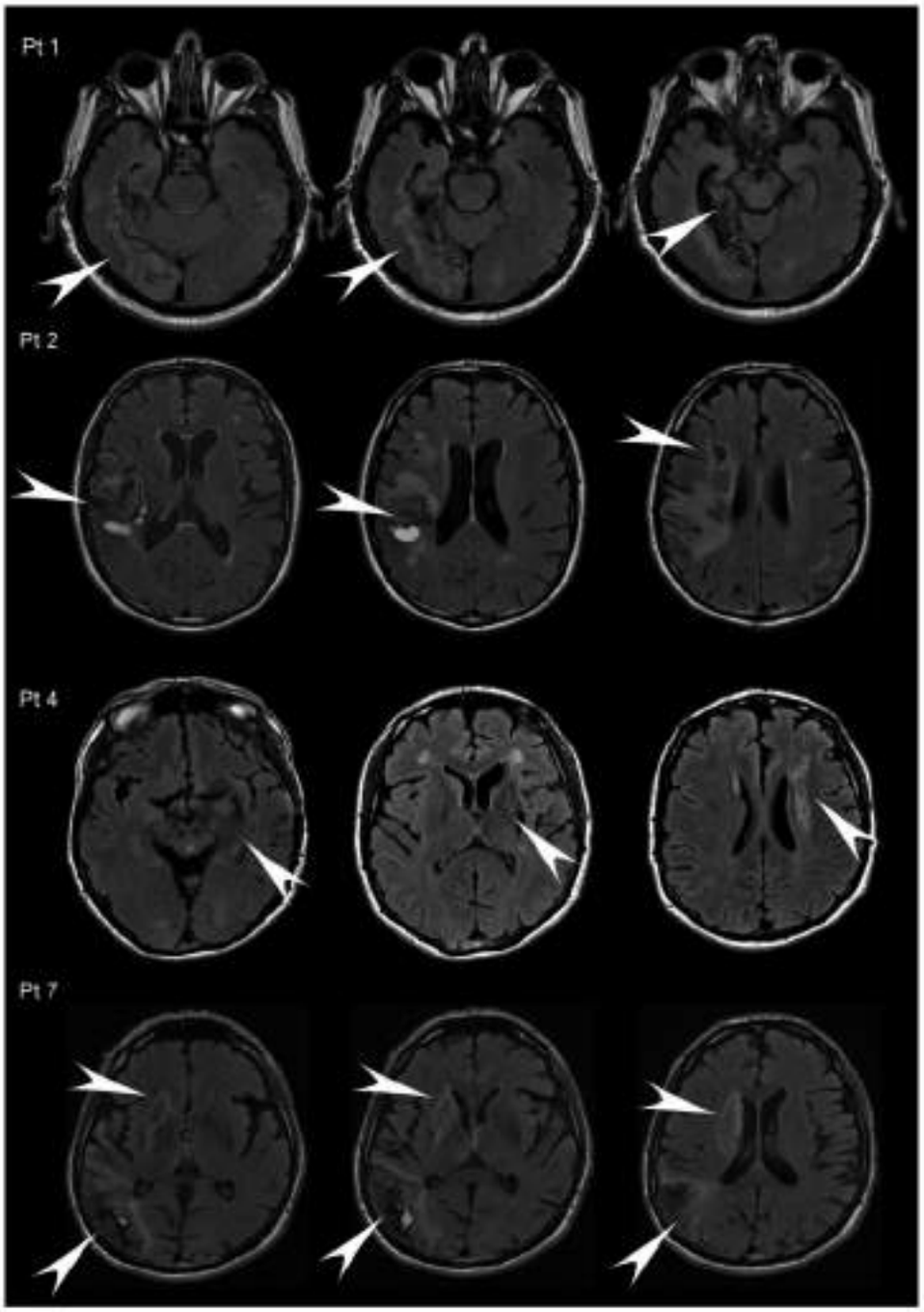

Figure 3. (a, b). The eight Stroke patients having both cortical and subcortical lesions (cp. Table 2). For each stroke patients' number, three axial slices are shown in the areas where lesions are more evident. White arrowheads help to localize lesions.

Lenhard (2014) and Zar (1999) for Pearson and Spearman coefficients respectively. Results are reported in Table 4; they show a significantly negative correlation between ANPS-SEEKING scores and PCC SD (in eyes open condition) in the Control group only: the higher neuronal variability in PCC, the lower the degree of SEEKING in the Control subjects (Figure 5). The correlation between ANPS-SEEKING and PCC in the control group was also more evident when using Spearman correlation coefficient (Table 4). In both cases, the difference between the correlation observed in the two groups was statistically significant. This finding suggests that a non-linear monotonic relationship exists between the two parameters. Most interestingly, we observed a positive correlation (relatively high but not statistically significant) between SEEKING and PCC SD in the stroke group: the higher the neuronal SD variability in PCC, the higher the degree of SEEKING in the stroke group (Figure 5). While these results were

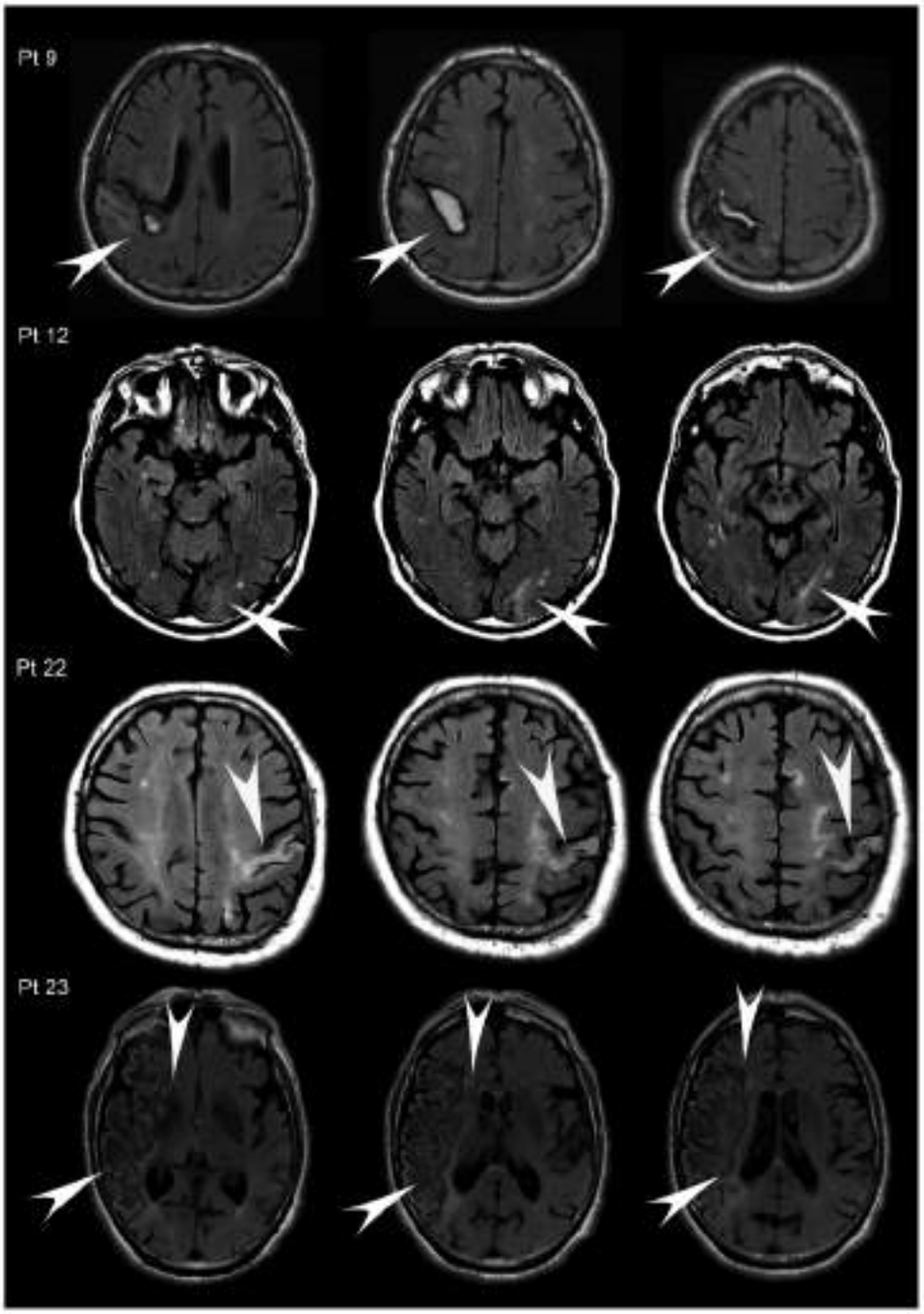

Figure 3. (continued.)

obtained for the eyes open condition, a similar pattern, albeit non-significant, was also observed in eyes closed condition.

4. Discussion

4.1. Main findings

We investigated a sample group of 24 stroke patients in the post-acute neuro-rehabilitative phase by examining both fMRI and behavior. The aim was to report how possible SEEKING system alterations relate to neural resting state dysfunction and reorganization after stroke. Both stroke patients and healthy control subjects were evaluated through self-report questionnaires and structural and functional neuroimaging. Lesions were located mainly subcortically and medially. Main outcomes of the statistical analysis show decreased ANPS-SEEKING in the stroke group compared to the control group, reduced resting state neuronal variability in lesion-unaffected

Table 3. MANOVA tests of between-subjects (stroke patients vs. control healthy subjects) Effects relative to psychometric measures. Statistically significant outcomes ($p < .05$) are reported in bold. p is the significance level associated to the F value.

	stroke (N = 24)	control (N = 22)			
	Average (SD)	*Average(SD)*	*F*	*p*	*Partial* η^2
ANPS-SEEK	35.63 (4.41)	39.41 (3.59)	**5.233**	**.009**	**.196**
ANPS-FEAR	35.13 (4.95)	36.50 (5.33)	.409	.667	.019
ANPS-CARE	42.08 (5.08)	40.95 (3.90)	1.148	.327	.051
ANPS-ANGER	32.63 (4.56)	35.05 (6.36)	1.253	.296	.055
ANPS-PLAY	36.71 (4.90)	37.73 (4.78)	.306	.738	.014
ANPS-SEP.D	34.29 (4.13)	33.00 (3.45)	1.994	.148	.085
BDI-II	8.46 (5.43)	8.50 (5.09)	1.934	.157	.083

PCC in the stroke group, and a significant inverse (i.e., negative) correlation between ANPS-SEEKNG and PCC activity in the healthy control group that was reversed (i.e., positive) in stroke patients.

4.2. Subcortical lesions – Lesion location

Some of the "classical" lesion subdivisions (anterior/posterior, right/left) appear somewhat forced and misleading. For instance, the five right anterior lesions were not all "pure" as they also extended posteriorly and/or medially. Note, on the contrary, that all stroke patients but one (Pt 22) showed subcortical medial lesions, which were associated with lateral cortical lesions in some stroke patients (9/23).

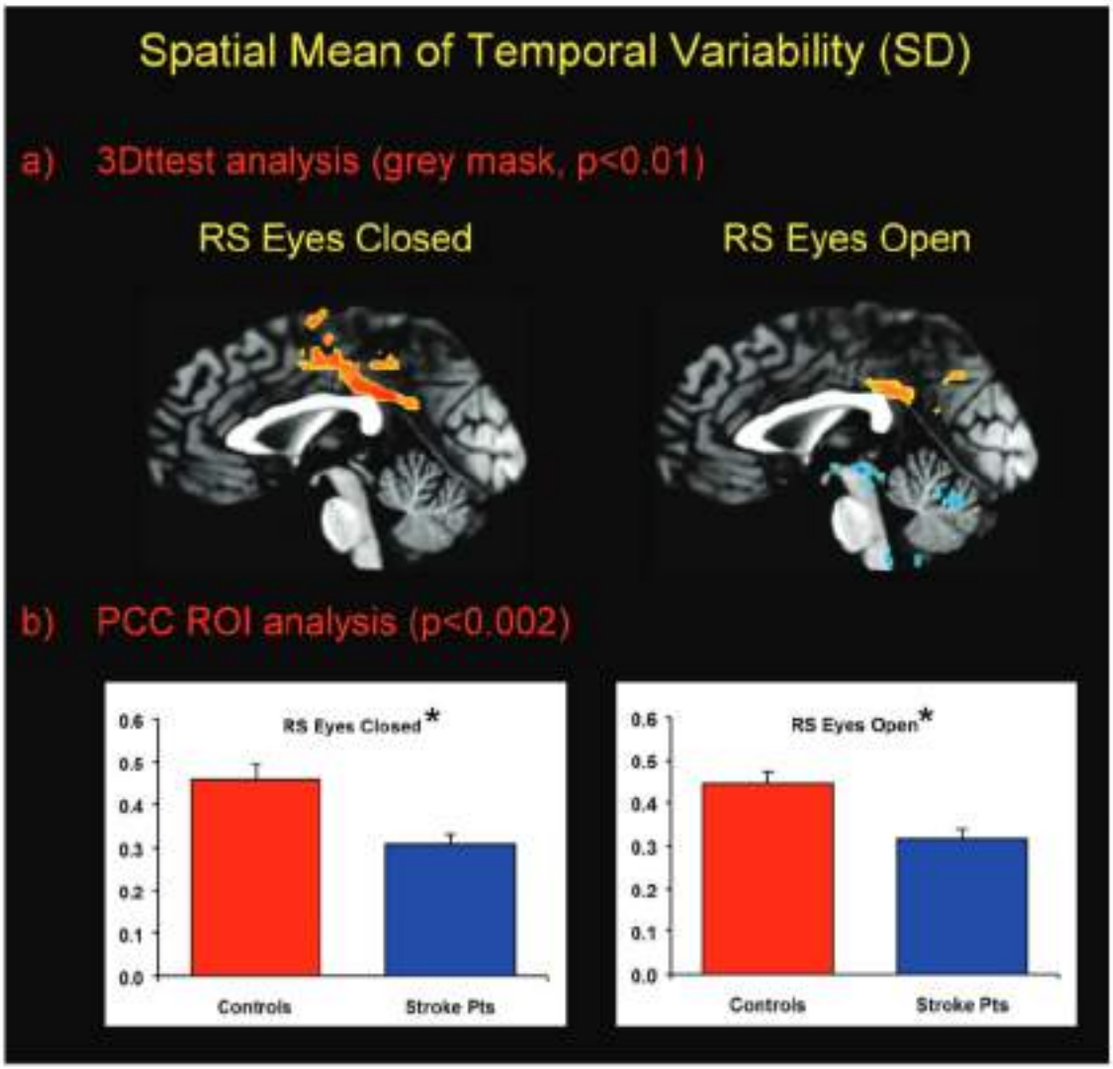

Figure 4. Results of the comparison between Control group vs Stroke group. 4a) Maps of Group differences (3dtt++ test), at $p < .01$, between Control subjects and Stroke patients, in the two functional conditions considered, RS-EC (on the left) and RS-EO (on the right), and in the frequency interval studied (0.01–0.1 Hz, standard frequency). Maps indicate the spatial mean of temporal variability (SD) of the gray masks considered. 4b) Histograms showing the results of PCC ROIs statistical two samples t-test, between the spatial means of temporal variabilities (SD) of Control subjects vs Stroke patients' ones, in the two functional conditions considered, RS-EC (on the left) and RS-EO (on the right), at $p < .002$. Note that both comparisons are statistically significant (asterisk *).

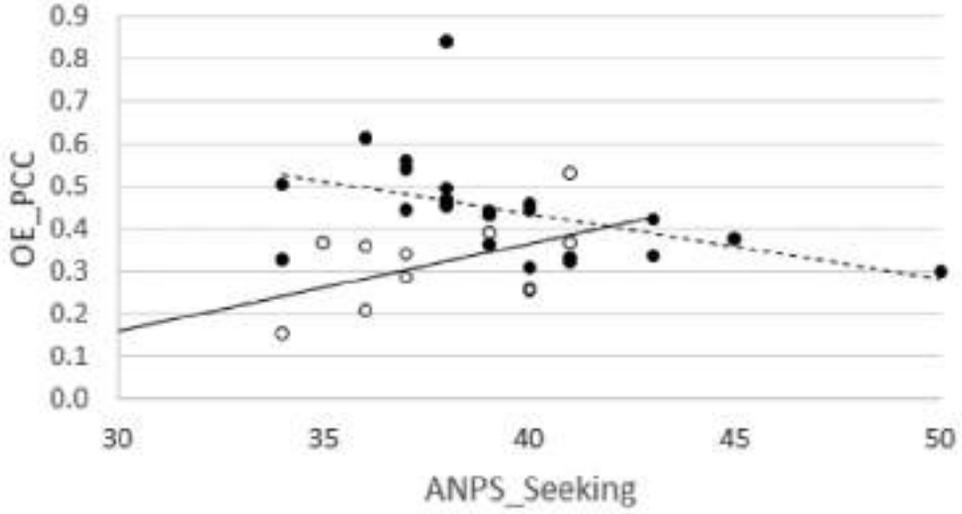

Figure 5. Correlation of ANPS-SEEKING scores and PCC activity (in the open-eyes condition) in the healthy control healthy subjects (black dots) and Stroke (empty circles). The linear regression model for Control (dashed line) and Stroke (continuous line) groups are also reported in the figure.

Table 4. Pearson correlation analysis considering ANPS-SEEKING scores and PCC activity (OE: open-eyes condition; CE: closed eyes condition) in the stroke and control groups. r, and ρ respectively represent the Pearson and Spearman correlation coefficients. N is the number of included subjects. In the last column, outcomes of a post-hoc Fisher's test for differences between the correlation values in the two groups are reported. In the case of Spearman coefficient, the correction proposed by Zar (2005) has been applied to compute Z. *p* is the relevant significance level (two-tailed). Statistically significant outcomes are reported in boldface.

	stroke	control	Post hoc test
	r (*p*),*N*	*r* (*p*), *N*	*Z* (*p*)
PCC-OE vs. ANPS-SEEKING	0.496 (0.121), 11	**−0.452 (0.034), 22**	**2.45 (0.01)**
PCC-CE vs. ANPS-SEEKING	0.013 (0.964), 16	−0.196 (0.382),22	0.50 (0.615)
	ρ (*p*),*N*	ρ (*p*), *N*	(*p*)
PCC-OE vs. ANPS-SEEKING	0.404 (0.218), 11	**−0.641 (0.001), 22**	**2.74 (0.006)**
PCC-CE vs. ANPS-SEEKING	0.07 (0.798), 16	−0.217 (0.331),22	0.67 (0.503)

Finally, lesions on the right side appeared very wide in extension and overlapped at many points. On the left side, conversely, lesions were smaller and less overlapped. Note also the complete absence of left frontal cortical and left temporal lesions in the superior and middle temporal gyri. This is due to including only verbally capable stroke patients (i.e., the absence of significant verbal comprehension/production deficits), a necessary ability to complete the neuropsychological tests administered (see material and methods). This could be considered a bias in stroke patients selection as these stroke patients with left frontal cortical or superior-middle temporal lesions were automatically excluded.

4.3. Subcortical lesions – Behavioral correlates

In the current patient group, post-stroke lesions were in prevalence wide and involving subcortical medial regions of the brain (Table 2). This aligns with Corbetta et al.'s (2015) study which indicates that stroke should be better conceptualized as a subcortical than cortical disease. In their paper, Corbetta et al. (2015) suggest that scientific literature often highlights those neurological deficits and behavioral syndromes that are related to focal cortical disruption of specific highly modular functional systems. Thus, Corbetta et al. (2015) assumes that current research biases us toward neglecting the central relevance of subcortical lesions in stroke; this is further supported by the fact that only 15% of strokes are purely cortical (see Bogousslavsky et al., 1988; Kang et al., 2003; Wessels et al., 2006). Our data are well in line with the central role of subcortical and, specifically, medial subcortical lesions in stroke. Together with the cited literature, these findings raise the question about the behavioral and functional correlates of these subcortical lesions in stroke.

Subcortical medial regions of the brain are associated with basic emotions (Panksepp, 1998; Panksepp & Biven, 2012) and core self (e.g., Northoff & Panksepp, 2008; Panksepp & Northoff, 2009), in particular the SEEKING disposition, promoted by dopamine transmission within intermediary "associative" subcortical neural areas connecting sensory and

motor processing. Most interestingly, we observed significantly reduced SEEKING in stroke patients, which means that they show less exploratory-driven behavior. Most importantly, this was not related to depression and thus represents a deficit in SEEKING itself (rather than a symptom or consequence of depression). In fact, no significant difference between stroke and control groups were revealed by the MANOVA analysis when considering BDI-II scores. This implies that, at least in the current sample, reduced SEEKING scores in stroke patients are not related to depression but, possibly, to post-stroke brain damage as related to specific subcortical regions. A proper assessment of SEEKING in post-stroke is important due to its potential role in brain/behavior neuroplastic reorganization. Findings obtained by ANPS need to be contextualized in a complex frame. As evidenced in our previous studies (Farinelli et al., 2015, 2013), the questionnaire scales assess higher levels of mental functioning, reflecting the primary ones and representing an integration of lower and higher brain functions. Furthermore, neurorehabilitation practice and research highlight that the post-stroke reorganization starts very early, involving multiple levels, and depends on several interacting neuropsychosocial factors in continuity or discontinuity with previous states (Chang et al., 2013). Especially in the acute and the post-acute phases, the personal identity, subjectively perceived and experienced, can be impacted by the interaction of previous traits and psychosocial factors with brain damage consequences, traumatic event impact, and caregiver mirroring of the personal and relational experience of the change. ANPS should be supported by more direct measures, systematically detecting affective behaviors and primary traits of personality and processes, including the interaction with caregivers.

4.4. Cortical reorganization – Posterior cingulate cortex (PCC)

Previous studies on stroke and traumatic brain injury (TBI) have investigated brain functional alterations in such stroke patients in relation to affective- behavioral dysregulation (Drevets et al., 2008; Lassalle-Lagadec et al., 2012; Sharp et al., 2014). Our study is the first to investigate neuronal variability in stroke. As noted in our results, stroke patients showed significantly lower neuronal variability in the PCC specifically, which was further confirmed by the independent region of interest analysis. The central role of PCC in stroke is well in line with a recent study by Matsuoka et al. (2015) that discovered a relationship between PCC volume and stroke duration. Most importantly, our results suggest that reduced neuronal variability must be purely functional, as our subjects did not show any structural changes in PCC. This raises the question of the functional role of PCC in stroke and its behavioral influence.

The PCC has the highest cerebral blood flow in the brain (Pfefferbaum et al., 2010) and is among the most metabolically active regions (Raichle et al., 2001). Even if the PCC shows higher vulnerability in cases of diffuse brain ischemia (DeVolder et al., 1990), acute focal ischemic lesions of this area are very rarely reported in the literature (e.g., Addis et al., 2007; Katayama et al., 1999; Leech & Sharp, 2014; Takahashi et al., 1997), which may be due to the particular vascular configuration of this region. That being the case, the complete absence of lesions in PCC in any of our stroke patients is not surprising, but rather well in line with research on both vascularity and physiology of blood flow, as well as the lesion and stroke literature.

As stated above, PCC is a central node within the default mode network (DMN) that is highly connected to many other cortical regions and integrates information from multiple functional networks of the brain. PCC activity is thought to be crucial for emotional-cognitive integration and focus of attention in the internal/external balance. Zhang et al. (2017) showed that PCC activity is correlated with the level of consciousness in brain lesioned patients, whereas, in the present study, the PCC remained structurally unaffected. The level of arousal and awareness as well as the internal/external balance (i.e., directedness of behavior toward the external world,) are closely related to exploratory and outward (i.e., externally-directed) behavior as measured by SEEKING.

Our data showing reduced SEEKING suggest that the very same behavior is reduced in stroke patients; they show a much lower level of arousal/awareness of the external world and reduced exploratory behavior directed toward the external world.

Importantly, we observed a significant negative correlation between SEEKING and PCC neuronal variability in healthy subjects, with less variability in PCC leading to increased SEEKING. Supposing that SEEKING is mainly driven by subcortical activity (Panksepp, 1998; Panksepp & Biven, 2012), this suggests that cortical variability may need to be suppressed during subcortical activity related to SEEKING. One may thus want to assume inverse or reciprocal relationships between subcortical and cortical/PCC activity during SEEKING in the healthy brain.

That same reciprocal or inverse relationship between subcortical and cortical activity does not appear in the case of stroke. Instead of a negative (i.e., inverse PCC-SEEKING) relationship, we observed a positive correlation between neuronal variability in PCC and SEEKING: the higher the PCC neuronal variability, the higher the degree

of SEEKING. This may be interpreted as compensation for the reduced PCC neuronal variability we observed in PCC. Alternatively, it may be related to abnormal subcortical-PCC relation. The strong presence of subcortical lesions may make a reverse or inverse relationship between subcortical regions and PCC impossible. Due to the subcortical lesion, the cortical regions (i.e., the PCC) may have to drive SEEKING in stroke patients. More generally, subcortical lesions seem to lead to functional reorganization of the cortical levels including the PCC activity, measured in terms of its neuronal variability at least in the acute and sub-acute adjustments. Future follow-up studies may explore long-term reorganization of the brain and affective behavioral function.

4.5. Limitations

The heterogeneity of lesions makes resting state investigation of stroke patients difficult. That same heterogeneity is also apparent in our study sample. However, confirming a recent study by Corbetta et al. (2015), our stroke patients showed predominant lesion in subcortical regions, while fewer lesions were observed in cortical regions. The heterogeneity of lesions in our sample reflects a clinical reality while, at the same time, makes detailed and systematic scientific investigation difficult, if not impossible. For that reason, we refrained from investigating functional connectivity between subcortical lesion and cortical regions like the PCC. Analysis of subcortical-cortical/PCC functional connectivity would have been desirable to support our assumption of cortical-behavioral functional reorganization (as evidenced by our abnormal PCC-SEEKING correlation) on subcortical grounds. However, the heterogeneity of subcortical lesions made such analyses impossible.

Moreover, the presence of structural lesions makes it difficult to investigate and interpret functional parameters in the damaged regions. Our results revealed reduction in neuronal variability in one region, the PCC, that did not show structural damage in any of the included stroke patients. Therefore, our findings of reduced neuronal variability can be interpreted as primarily functional, rather than being a secondary deficit due to primary structural lesion. However, more detailed structural investigation, including DTI, would be necessary to rule out structural lesion appropriately.

Another limitation is that our sample was somewhat biased as we required stroke patients to be able to understand and follow verbal instructions. This excluded stroke patients with lesions in Wernicke's and/or Broca' regions (i.e., with severe verbal language disorders). Therefore, future studies should include stroke patients with lesions in language-related regions as well to investigate whether they show a similar pattern of neuronal and behavioral results.

Finally, one must be aware that, despite the fact that control and stroke groups did not show statistically significant differences in terms of age and education, some differences trending toward significance were noted. Possibly, the lack of significance may be due to an underpowered test more than true lack of differences. Future studies using larger groups might clarify the role of these potential baseline differences when assessing similar outcomes.

5. Conclusion

We investigated behavior and resting state activity in a post-acute sample of stroke patients. Our sample showed predominantly subcortical (rather than cortical) lesions as well as reductions in both SEEKING and PCC neuronal variability with the latter two also correlating in an abnormal way (i.e., positive, rather than negative). Our findings add to the current literature regarding both subcortical regions and PCC involvement in stroke and more generally, suggest cortical-behavioral reorganization of resting state activity and associated behavior (e.g., SEEKING) associated with subcortical lesions in stroke.

Highlights

- Predominant subcortical lesions in the stroke sample
- Reduced SEEKING in stroke patients with respect to healthy control subjects
- Reduced neuronal variability in the posterior cingulate cortex (PCC) in stroke
- SEEKING is abnormally correlated with PCC neuronal variability in stroke
- Subcortical alterations may play a central role in post-stroke reorganization

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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