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Network abnormalities in generalized anxiety pervade beyond the amygdala-pre-frontal cortex circuit: Insights from graph theory

Elena Makovac[a,b,c], Matteo Mancini[c,d], Sabrina Fagioli[c,e], David R. Watson[b], Frances Meeten[b,f], Charlotte L. Rae[b,g], Hugo D. Critchley[b,g,h], Cristina Ottaviani[c,i,*]

[a] Centre for Neuroimaging Science, Kings College London, London, UK
[b] Clinical Imaging Sciences Centre, Brighton and Sussex Medical School, University of Sussex, Falmer, UK
[c] Neuroimaging Laboratory, IRCCS Santa Lucia Foundation, Rome, Italy
[d] Centre for Medical Image Computing, University College London, London, UK
[e] Department of Education, University of Roma Tre, Rome, Italy
[f] Department of Psychology, Kings College London, London, UK
[g] Sackler Centre for Consciousness Science, University of Sussex, Falmer, UK
[h] Psychiatry, BSMS Department of Neuroscience, Brighton and Sussex Medical School (BSMS), University of Sussex, Falmer, UK
[i] Department of Psychology, Sapienza University of Rome, Rome, Italy

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ABSTRACT

Generalized anxiety disorder (GAD) has excessive anxiety and uncontrollable worry as core symptoms. Abnormal cerebral functioning underpins the expression and perhaps pathogenesis of GAD:. Studies implicate impaired communication between the amygdala and the pre-frontal cortex (PFC). Our aim was to longitudinally investigate whether such network abnormalities are spatially restricted to this circuit or if the integrity of functional brain networks is globally disrupted in GAD. We acquired resting-state functional magnetic resonance imaging data from 16 GAD patients and 16 matched controls at baseline and after 1 year. Using network modeling and graph-theory, whole-brain connectivity was characterized from local and global perspectives. Overall lower *global efficiency*, indicating sub-optimal brain-wide organization and integration, was present in patients with GAD compared to controls. The amygdala and midline cortices showed higher *betweenness centrality*, reflecting functional dominance of these brain structures. Third, lower *betweenness centrality* and *lower degree* emerged for PFC, suggesting weakened inhibitory control. Overall, network organization showed impairments consistent with neurobiological models of GAD (involving amygdala, PFC, and cingulate cortex) and further pointed to an involvement of temporal regions. Such impairments tended to progress over time and predict anxiety symptoms. A graph-analytic approach represents a powerful approach to deepen our understanding of GAD.

1. Introduction

Generalized anxiety disorder (GAD) is a chronic condition characterized by excessive anxiety, in which uncontrollable anticipation of negative outcomes (i.e. worry) may develop as a response to manage emotional distress. GAD is the most frequent anxiety disorder in primary care, imposing an enormous human and economic burden on society (Hoffman et al., 2008). Abnormal cerebral functioning is evident and implicated in the pathogenesis of anxiety, with a clear role of the amygdala (Mochcovitch et al., 2014). Indeed, functional brain imaging studies show heightened activation of the amygdala across anxiety disorders when compared to healthy controls (HC). Similarly, enhanced amygdala reactivity correlates with trait anxiety in both clinical and healthy populations. Thus, hyper-responsiveness of the amygdala is putatively a trans-diagnostic neural correlate of dispositional anxiety (e.g. Etkin et al., 2009). The role of the amygdala in the pathophysiology of GAD is less clear, with some studies reporting over-reactivity (e.g. greater anticipatory amygdala activity preceding aversive and neutral stimuli; Nitschke et al., 2009), and others diminished activity of the amygdala, for example during the evaluation of angry faces (Blair et al., 2008). Similarly, other studies have failed to report a hyperactivation of the amygdala during the presentation of threatening stimuli in GAD (Monk et al., 2006; Palm et al., 2011). The results appear to be more coherent in pediatric GAD, where hyperactivation of

* Corresponding author at: Department of Psychology, Sapienza University of Rome, Via dei Marsi, 78, 00185 Rome, Italy.
E-mail address: cristina.ottaviani@uniroma1.it (C. Ottaviani).

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the amygdala is evident during the elaboration of emotional stimuli and correlated with the severity of GAD symptoms (Monk et al., 2008; McClure et al., 2007).

On its own, the quantification of amygdala dysfunction yields limited insights to the pathophysiology of anxiety disorders in general and of GAD in particular (Paulus and Stein, 2006). In recent years, understanding of GAD pathophysiology has been enriched by the investigation of abnormal patterns of communication within and between brain networks, capitalizing upon resting state functional connectivity approaches (Sylvester et al., 2012). Moreover, resting-state connectivity tools can be successfully used to demonstrate functional differences and similarities in neural characteristics of distinct anxiety disorders (Peterson et al., 2014). Aberrant communication between amygdala and pre-frontal cortex (PFC) emerges repeatedly as a signature of GAD (Makovac et al., 2016a; Mochcovitch et al., 2014). Crucially, in non-clinical populations, amygdala activity is tonically suppressed by inhibitory inputs from the PFC, enabling the efficient regulation of emotional states (Nomura et al., 2004). Therefore, the emotional dysregulation typical of GAD may plausibly reflect dysfunctional communication between PFC and amygdala, in which the failure of the PFC to down-regulate the amygdala in safe contexts leads to the maintenance of core symptoms of worry and anxiety (Etkin et al., 2009; Makovac et al., 2016a). Such a mechanism illustrates how specific patterns of network dysfunction can contribute to core deficits in cognitive and affective functioning that underlie the expression of clinical symptoms.

Nevertheless, focusing only on the communication between PFC and amygdala (as with focusing on amygdala activation alone) may be too reductive and obscure the recognition of more subtle abnormalities distributed across the brain, of potentially equivalent pathoaetiological significance. Indeed, GAD involves dysfunction of cognitive and emotion regulation processes relying on distributed brain regions spanning multiple lobes (Menon, 2011). For example, other studies have reported a crucial role of the communication between amygdala and temporal pole in GAD (Li et al., 2016). Similarly, recent data have pointed to an involvement of the communication between amygdala and temporal areas in the mediation of the negative affectivity that accompanies worry in GAD (Makovac et al., 2018).

A graph theory analytic approach permits a more global perspective on functional neural connectivity, as only large-scale brain network analytics can provide integrative models of cognitive and affective dysfunction in GAD (Menon, 2011). Within this network-modeling framework, brain regions are represented as *nodes* of a mathematical graph, and the functional couplings between them constitute its *edges* (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). Metrics from graph theory are employed to characterize specific network properties including segregation, i.e. the capability of specialized local processing, and integration, i.e. the capability of distributed global processing. Importantly, a consequence of network organization is that it supports spreading processes between connected regions. It follows that a localized brain dysfunction can cause pathological alterations within regions that are distant, yet functionally linked to the original site of dysfunction (Fornito et al., 2015).

Human 'neural connectomics' has yielded plausible biomarkers for Alzheimer's disease (Bergeron et al., 2016) and psychiatric disorders including schizophrenia (Kambeitz et al., 2016), social anxiety disorder (Yun et al., 2017), post-traumatic stress disorder (Lei et al., 2015), and major depression (Gong and He, 2015). Despite the promise of this approach, and the conceptualization of anxiety disorders as "dysfunction in brain networks" (Sylvester et al., 2012), to date no study has yet applied graph theory to whole brain network connectivity in GAD patients. The present paper addresses this need. We examined whole brain functional connectivity in GAD patients and HC by applying specific quantitative graph measures. We hypothesized that global and local brain network topological properties are disrupted in GAD compared to controls, and that these disruptions extend beyond the PFC-amygdala interactions proposed as a canonical circuit dysfunction. Given the absence of previous studies applying this approach in GAD, we opted for both a data- and theory-driven approach. The latter specifically involved the exploration of brain regions that have emerged as playing a significant role in prior studies on the neurobiology of GAD, i.e., regions within the PFC, and cingulate gyrus (e.g., Makovac et al., 2016a; Via et al., 2018).

The progression of a clinical anxiety disorder is directly coupled to time dependent expression and modification of symptoms (van Beljouw et al., 2010). Correspondingly, we tested for changes in organizational features of whole brain networks at two time points over a 1-year period. Abnormalities in global network organization have the capacity to be clinically important biomarkers for disease progression, for example mapping the transition to psychosis in an at-risk sample (Lord et al., 2012) or mirroring daily affective instability in remitted patients with major depressive disorder (Servaas et al., 2017). In a previous study, we found that longitudinal changes in dorsolateral PFC-amygdala functional connectivity mirrored changes in anxiety symptoms in GAD patients over time (Makovac et al., 2016b). Here, we aimed to extend these findings moving "from connectivity to connectomics".

2. Materials and methods

2.1. Participants

The present study is based on a secondary analysis of data from a larger longitudinal fMRI study (Makovac et al., 2016b). The study was approved by the National Research Ethics Service for the UK National Health Service with university sponsorship granted via the Brighton and Sussex Medical School Research Governance and Ethics Committee. All participants provided written informed consent at both time points. The final sample undergoing both assessments encompassed 16 patients (14 women; mean age = 29.6 ± 7.5 years) who met DSM-IV diagnostic criteria for GAD and 16 HC (13 women; mean age = 28.1 ± 10.1 years). The average illness duration at time 0 was 16.8 ± 8.0 years. Patients and controls were medication free, with the exception of two patients with GAD who used long-term medications (one citalopram, one pregabalin) at both sessions of the study. Wash-out was not applied. At time 0, forty individuals (19 GAD, 21 HC) were recruited by public advertisement; after one-year (time 1), eight participants had dropped-out from the study (3 GAD and 5 HC). All participants were right-handed and native English speakers. Exclusion criteria were: age younger than 18 years, past head injury or neurological disorders, prior history of major medical or psychiatric disorder (other than GAD for the patient group), cognitive impairment, history of substance or alcohol abuse or dependence, diagnosis of heart disease, obesity, pregnancy, claustrophobia or other general magnetic resonance imaging (MRI) exclusions. None of our participants had a formal diagnosis of co-morbid major depressive disorder.

2.2. Procedure

At both time 0 and time 1, all participants underwent the Structured Clinical Interview for DSM-IV to confirm or exclude a current diagnosis of GAD. Then, participants completed a series of online questionnaires and underwent the MRI protocol. Participants completed the same procedure about 1 year later (time 1) (average time between sessions = 10.5 ± 2.2 months). The 1-year time frame was chosen for both practical and theoretical reasons. Given our small sample size and difficulties in recruiting anxious patients for a brain imaging study, we opted for a time frame that allowed us to detect changes in symptoms (DSM-5 criteria require a minimum of 6 months of persistent worry for the diagnosis of GAD), while at the same time minimizing the risk of losing patients at follow-up.