INTRINSIC FUNCTIONAL CONNECTIVITY OF THE AMYGDALA IN HEALTHY AND EPILEPTIC BRAINS: A RESTING-STATE FMRI STUDY

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The amygdala is an almond-shaped mass located deep within the medial temporal lobe [1]. It plays a critical role in a variety of cognitive and affective behaviours, including the processing of fear and related emotions, learning, memory, and decision-making. The amygdala is frequently looked at as a single homogeneous unit [2,3]. However, histopathological studies in animal models and human post-mortem data have differentiated the amygdala into laterobasal (LB), centromedial (CM), and superficial (SF) subdivisions, which are known to differ with respect to their connectivity to the rest of the brain and participate in different functional roles [2,4,5].

The structure of the amygdala and its connectivity have been shown to be altered in neurological conditions [3,6,7]. In temporal lobe epilepsy (TLE), the most common drug-resistant epilepsy in adults [8], the amygdala-hippocampal circuitry is considered a core component of the epileptogenic network and is frequently involved in seizure genesis and spread [8]. Histopathological analyses of surgical specimens in TLE patients have consistently reported variable degrees of pathological alterations in the mesiotemporal region [8] - a pathological spectrum collectively referred to as mesiotemporal or hippocampal sclerosis (HS) [9]. Indeed, mesiotemporal pathology was recently classified by the International League Against Epilepsy (ILAE) into several degrees of HS, ranging from marked cell loss and gliosis that is found in the majority of patients (TLE-HS) to only isolated gliosis that may be observed in up to 40% of patients (TLE-G) [10]. Importantly, however, previous studies have generally considered the amygdala as a homogeneous entity, disregarding its individual subdivisions, as well as focusing on its activity as supposed to functional connectivity [11-13]. Moreover, no study has hitherto assessed whether amygdala connectivity is impacted by the overall load of mesiotemporal lobe pathology across patients.

In the present study, we sought to investigate the functional connectivity embedding of amygdalar subdivisions in healthy and epileptic brains by combining resting-state functional magnetic resonance imaging (fMRI) with probabilistic anatomical maps of the amygdala [4]. We hypothesised that our framework would detect significant differences in the functional connectivity of individual amygdala subdivisions with other brain regions in healthy individuals. Furthermore, we expected to observe subdivision-specific disruptions in amygdala connectivity in TLE that may depend on the overall degree of medial temporal pathology**.**

To carry out our investigation, we assessed inter-individual differences in the functional connectivity of these subdivisions in a group of 36 healthy controls using resting-state fMRI, and evaluated the impact of lesions to the medial temporal lobe on connectivity patterns in a group of 34 patients with TLE.

Using systematic quantitative connectivity profiling in our healthy individuals, we observed divergent connectivity profiles among the different amygdalar subdivisions, which were in line with their specific functional roles. We observed that spontaneous activity in LB nuclei positively correlated with activity in frontal and temporal regions, while CM nuclei interacted

mostly with the striatum. Lastly, SF nuclei were shown to be highly coupled with limbic structures. Our connectivity maps generally overlap with previously reported maps in healthy adults [5,14], and show close correspondence with animal amygdala-based circuits [15,16]. By furthermore studying a cohort of TLE patients, in whom degrees of mesiotemporal lobe pathology were verified based on postoperative histopathological analysis, we had the opportunity to assess the impact of pathology on subregional connectivity patterns of the amygdaloid complex. Compared to controls, we observed consistent reductions in connectivity in patients for both SF and CM subdivisions to prefrontal areas, while LB connectivity seemed to be spared at a whole-brain level. However, *post-hoc* analysis that assessed the connectivity between a region formed by intersecting significant regions of frontal disconnectivity from SF and CM and the LB subdivision on the other hand also indicated significant connectivity reductions for the latter ($t>3.9$, $p<0.005$) **(Figure 1)**. Notably, we also observed that the strength of amygdala-prefrontal connectivity disruptions varied as a function of temporal lobe pathology. In fact, connectivity disruptions were significantly more marked in patients with severe mesiotemporal lesions (TLE-HS) compared to those showing rather subtle pathology (TLE-G) for all three subdivisions ($t=1.69$, $p=0.0475$) **(Figure 1)**.

Overall, our findings provide evidence for divergent connectivity profiles among amygdala-subdivisions in healthy controls. Furthermore, in addition to showing a modulation of amygdala network embedding in patients suffering from TLE, they provide first evidence that the severity of amygdala disconnection relates to the degree of temporal lobe pathology.
Additionally, our findings illustrate the importance of not considering the amygdala as a single unit, as most previous neuroimaging studies of the amygdala in healthy individuals and clinical indications have done [17]. Importantly, amygdala-prefrontal connectivity has been implicated in emotion regulation and affective stability [18]. Our findings may be of relevance for the understanding of prevalent affective co-morbidities in epilepsy, with anxiety and depression occurring in up to 60% of patients [19]. Further investigation of the relationship between functional connectivity disturbances in TLE and the expression of co-morbid emotional conditions would therefore be an interesting future area of study.

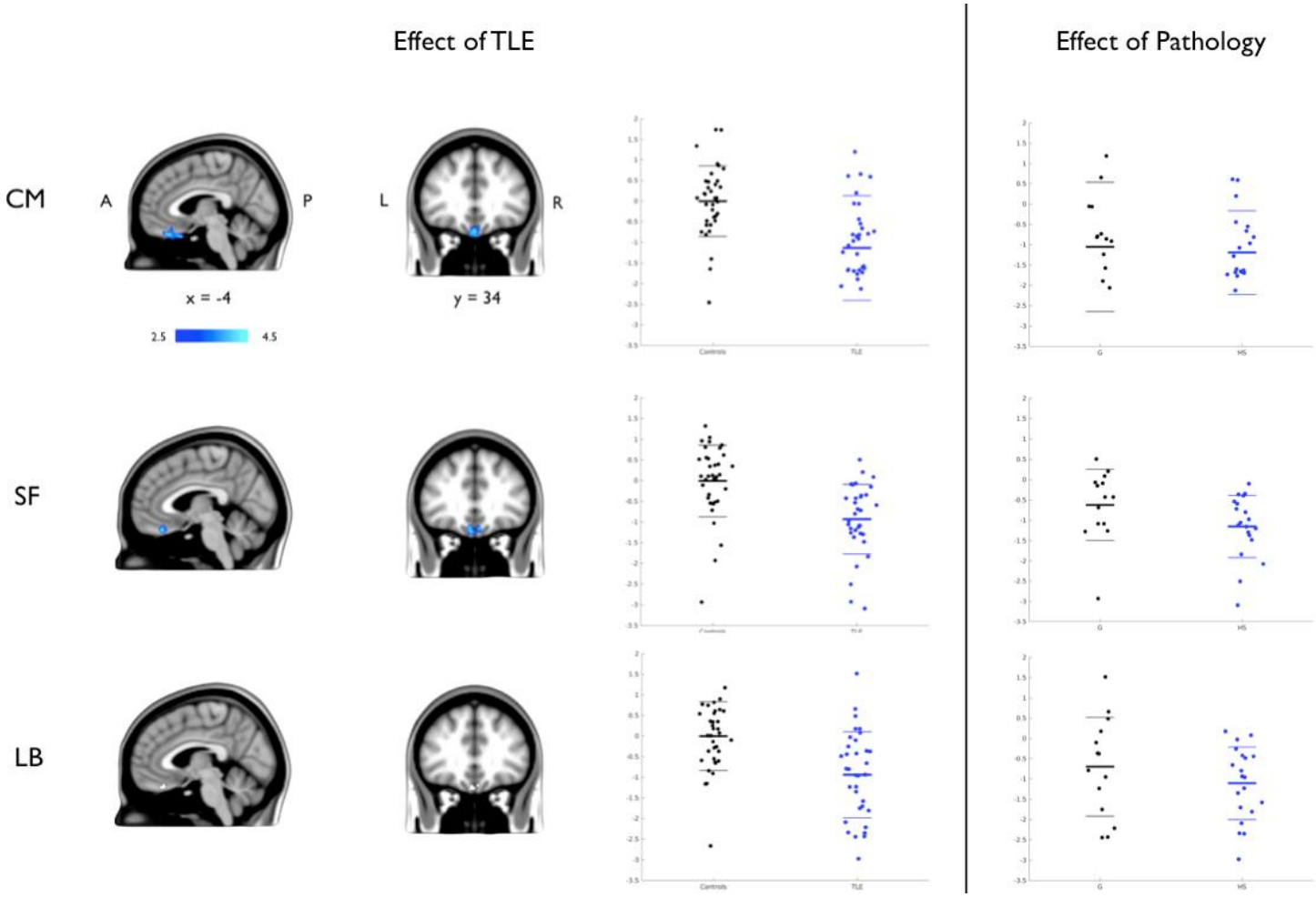

Figure 1. Direct comparison of the functional connectivity of each subdivision in TLE patients with controls, and between patients with varying degrees of hippocampal pathology (G vs. HS). For CM and SF, blue overlay signifies regions of significant under-connectivity in patients compared to controls (*left panel*). For LB, white overlay signifies the area of intersection of these under-connectivity patterns (*left panel*). Sagittal (x = -4) and coronal (y = 34) views are presented. (MNI152 standard space; $p < 0.05$, FWE corrected). Bar graphs illustrate significantly reduced connectivity patterns in TLE patients (blue) between this region of intersection to each subdivision when compared to controls (black) (for LB, t>3.9, p<0.005) (*left panel*). Bar graphs to show a trend between the degree of pathology (G vs. HS; black and blue, respectively) and the level of connectivity disruptions in TLE-patients (*right panel*).

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