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GABA content within medial prefrontal cortex predicts the variability of fronto-limbic effective connectivity

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Abstract

The amygdala-medial prefrontal cortex (mPFC) circuit plays a key role in social behavior. The amygdala and mPFC are bidirectionally connected, functionally and anatomically, via the uncinate fasciculus. Recent evidence suggests that GABA-ergic neurotransmission within the mPFC could be central to the regulation of amygdala activity related to emotions and anxiety processing. However, the functional and neurochemical interactions within amygdala-mPFC circuits are unclear. In the current study, multimodal magnetic resonance imaging techniques were combined to investigate effective connectivity within the amygdala-mPFC network and its relationship with mPFC neurotransmission in 22 healthy subjects aged between 41 and 88 years. Effective connectivity in the amygdala-mPFC circuit was assessed on resting-state functional magnetic resonance imaging data using spectral dynamic causal modelling. State and trait anxiety were also assessed. The mPFC was shown to be the target of incoming outputs from the amygdalae and the source of exciting inputs to the limbic system. The amygdalae were reciprocally connected by excitatory projections. About half of the variance relating to the strength of top-down endogenous connection between right amygdala and mPFC was explained by mPFC GABA levels. State anxiety was correlated with the strength of the endogenous connections between right amygdala and mPFC. We suggest that mPFC GABA content predicts variability in the effective connectivity within the mPFC-amygdala circuit, providing new insights on emotional physiology and the underlying functional and neurochemical interactions.

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Keywords

Amygdala; Anxiety; Emotions; γ -Aminobutyric acid (GABA); Medial prefrontal cortex (mPFC)

Introduction

A key unresolved question in social and affective neuroscience is how emotions are processed in the brain (Kragel and LaBar 2016). It is well known that the amygdala is an integrative hub for emotions, receiving inputs on potentially threatening stimuli from the cortico-thalamic pathway and sending this information to sub-cortical regions and brainstem to drive the autonomic and sensorimotor response (Calhoun and Tye 2015). It is also widely reported that the interaction of the amygdala with the medial prefrontal cortex (mPFC), including the anterior cingulate cortex (ACC), is central for shaping limbic activity and emotional regulation (Blair et al. 2008; Taylor and Whalen 2015). Ex-vivo animal studies showed bidirectional connections between the amygdala and mPFC (Ghashghaei and Barbas 2002; Ghashghaei et al. 2007). In humans, diffusion tensor imaging studies in-vivo demonstrated that the ventral portion of the uncinate fasciculus connects the amygdala with the ventral regions of mPFC and ACC (Bracht et al. 2009) and that this branch is involved in emotional regulation and anxiety disorders (Kim et al. 2011; Phillips et al. 2008). Functionally, mPFC receives incoming information from the amygdala and it exerts top-down control on the basolateral nuclei activity by stimulating the glutamatergic pyramidal neurons (Rule et al. 2002; Nuss 2015). In this context, it has been observed in animal models that GABA-ergic interneurons within the mPFC exert a powerful inhibitory control over the glutamatergic pyramidal neurons, influencing mPFC activity and autonomic response to threatening stimuli (Constantinidis et al. 2002; Akirav and Maroun 2007; Courtin et al. 2014; Chefer et al. 2011; Moscarello and LeDoux 2013; Nuss 2015). However, because of the invasive methods that are used to assess brain connections and neurochemistry (lesion and tracing studies), less is known on humans. Recently, we reported that mPFC GABA-ergic balance is related to trait anxiety and functional coupling between the amygdala and mPFC (DelliPizzi et al. 2016, 2017). Interestingly, imaging studies suggested that the GABA-ergic neurotransmission could be central in synchronising brain circuit activity, both locally and potentially at long-range network levels (Duncan et al. 2014). Particularly, it has been shown that the γ -aminobutyric acid (GABA) concentrations within a key node may predict the variability of the functional connectivity across brain networks (Donahue et al. 2010; Duncan et al. 2011; Falkenberg et al. 2012; Horn et al. 2010; Muthukumaraswamy et al. 2012; Kapogiannis et al. 2013; Northoff et al. 2007). Hence, in the current study, we hypothesized that mPFC GABA content could predict the variability of functional interactions within the amygdala-mPFC network. Moreover, considering that this network acts as a feedback control on limbic activity, high GABA content within the mPFC could be associated with weaker functional connections within the amygdala-mPFC network and lower modulation of the limbic activity, leading to greater anxiety. Thus, we combined proton Magnetic Resonance Spectroscopy (^1H -MRS) and resting-state functional MR imaging (rs-fMRI) to assess effective connectivity within the amygdala-mPFC loop, in-vivo and non-invasively, and relationships with neurotransmission within the mPFC. Spectral Dynamic Causal Model (sDCM) (Bastos-Leite et al. 2015; Friston et al. 2014; Razi et al.

2015) was applied to rs-fMRI data to investigate the directionality and causal relationships of the interactions within the amygdala-mPFC circuit. sDCM is derived by a deterministic model that generates predicted crossed spectra which allow effective connectivity to be assessed (Friston et al. 2014). The advantage of this approach lies in its computational efficiency; it also provides the opportunity to study effective connectivity and the characteristics of the neuronal fluctuations.

Materials and methods

Study sample

This study was approved by the Local Institutional Ethics Committee and was performed according to the Declaration of Helsinki (1997) and subsequent revisions. All experiment participants gave written informed consent.

Twenty-two healthy subjects aged between 41 and 88 years (11 males and 11 females) underwent an MRI session and a neuropsychological evaluation. Exclusion criteria were: prior history of major medical or psychiatric disorders; head injury or neurological problems; any contraindication to MRI scanning, including metal implants and claustrophobia; history of substance abuse; any pharmacological treatment; and tobacco addiction. Alcohol and caffeine consumption were prohibited for 12 h prior to the MR acquisition (Gao et al. 2013). Furthermore, all participants were screened for substance use disorders via a non-structural clinical interview. Subjects with any drug or alcohol abuse were excluded. Pre-menopausal female subjects were recruited in the follicular or luteal phase to take into account the possible effect of the menstrual cycle phase on GABA (De Bondt et al. 2015).

Neuropsychiatric and neuropsychological evaluation

Current or past psychiatric diagnosis and family history of psychiatric disorders were assessed with a clinical evaluation carried out by medical specialists using a non-structured clinical interview according to 5th edition of the Diagnostic and Statistical Manual of Mental Disorders diagnostic criteria (American Psychiatric Association guidelines 2013). Before MR scanning, the state and trait anxiety were respectively assessed using the subscale Y1 and Y2 of the State-Trait Anxiety Inventory (STAI) (Spielberger 1983). All subjects were assessed to establish normal brain functioning. Global cognitive assessment was performed using the Mini-Mental State Examination (MMSE) (Magni et al. 1996). The presence of frontal dysfunction was studied using the Frontal Assessment Battery (FAB) (Appollonio et al. 2005). A verbal fluency test (FAS) was used to investigate lexical production and phonemic verbal fluency as well as attention (Oppenheimer 2008). Trail Making Test (TMT)-A and -B were performed to assess attention skills, sustained attention, divided attention, task coordination, and set-shifting (Giovagnoli et al. 1996). Attentional matrices were used to assess speed and attention (Abbate et al. 2007). Short-term and long-term verbal memories (BSRT) (Babcock Story Recall Test; Horner et al. 2002) were investigated as well as auditory working memory (Baddeley and Wilson 2002). Visuospatial memory and ability were also evaluated (Shin et al. 2006). Finally, auditory working memory was assessed using the forward and backward Digit Span test (Wechsler 1939).

MR protocol

MR data were acquired by means of a Philips Achieva 3T scanner (Philips Medical Systems, Best, The Netherlands) using a whole-body radio-frequency coil for signal excitation and an 8-channel phased-array head coil for signal reception. T₁-weighted images were acquired using a 3D Turbo Field-Echo sequence (TFE, TR/TE = 11/5 ms, slice thickness of 0.8 mm). T₂-weighted fluid attenuation inversion recovery (FLAIR, TR/TE = 12,000/120 ms, slice thickness of 4 mm, FOV = 230 mm × 140 mm × 190 mm) images were also acquired to exclude subjects with concomitant vascular pathology or with white matter (WM) abnormalities. ¹H-MRS spectrum was acquired from a voxel of 2.0 (anterior-posterior) × 3.0 (left-right) × 3.0 (cranio-caudal) cm³ centred on the mPFC (Delli Pizzi et al. 2016, 2017). A MESHCHER-GARWOOD POINT RESOLVED SPECTROSCOPY (MEGA-PRESS) sequence (TR/TE = 2000/68 ms, 320 averages) was used to acquire 1024 points with a spectral width of 2000 Hz. MEGA-PRESS generates two sub-spectra, with the editing pulse ON in one and OFF in the other. Specifically, an editing pulse is applied to GABA spins at 1.9 ppm to selectively refocus the evolution of J-coupling to the GABA spins at 3.02 ppm (ON spectra). In the other, the inversion pulse is applied elsewhere, so that the J-coupling evolves freely throughout the TE (OFF spectra). Subtracting OFF spectra from ON spectra, overlying total creatine (tCr) signal was removed from the edited spectrum, revealing the GABA signal in the difference spectrum (Mullins et al. 2014). Resting-state Blood Oxygen Level Dependent (BOLD) fMRI data were acquired using gradient-echo T₂^{*}-weighted echo-planar (EPI) sequences with the following parameters: FOV 230 mm, matrix size 64 × 64, in-plane voxel size 3.6 mm × 3.6 mm, Sensitivity Encoding (SENSE) factor 1.8 anterior-posterior, slice thickness 5 mm, and TE = 30 ms. For each subject, two runs of 300 functional volumes consisting of 21 trans-axial slices were acquired with a TR of 1100 ms. Subjects were instructed to lie still and keep their eyes closed during acquisition.

Data analysis

¹H-MRS—Based on its stable levels reported in normal conditions (Bogner et al. 2010) and its independence from trait anxiety (Delli Pizzi et al. 2016), tCr was used as an internal standard reference. Of note, this method has been shown to have performance equal to, or better than, water referencing (Bogner et al. 2010). Because the GABA signal detected at 3.02 ppm is also expected to include contributions from both macromolecules and homocarnosine, in the rest of the manuscript, this signal is labelled as GABA + to highlight the potential presence of these other compounds (Gao et al. 2013; Rothman et al. 1997). GANNET, a MATLAB-based tool (Edden et al. 2014), was used to quantify GABA+/tCr in each spectrum using default parameters, including frequency and phase correction of time-resolved data using spectral registration (Near et al. 2015). GANNET-estimated signal for GABA is shown in suppl. Fig. 1. For each subject, a mask of ¹H-MRS voxel was obtained using a GANNET extension (Harris et al. 2015). Structural T₁-image was segmented using the “recon-all -all” command line integrated in FreeSurfer (Dale et al. 1999). FLIRT tool (FMRIB Software Library, FSL; Smith et al. 2004) was used to co-register FreeSurfer’s output and ¹H-MRS voxel mask in a common (native) space. FSL command lines (fslmaths and fslstats) were used to define the GM within the ¹H-MRS voxel and to calculate the

tissue volumes. All generated images were viewed in FSL View (Jenkinson et al. 2012) to validate the location of the MRS voxel and the confidence of the tissue segmentation.

fMRI—Resting-state fMRI data were analysed using AFNI (<http://afni.nimh.nih.gov/afni>). The first 4 volumes of each functional run were discarded to allow T_1 equilibration of the MR signal. The initial pre-processing included slice scan time correction and motion correction. Motion correction was performed using a rigid body registration of EPI images to a reference image represented by the fifth volume of the first run. The root mean square (RMS) of the six realignment parameters (three translations and three rotations) was used as a parameter to inspect for runs affected by excessive motion. All runs showed a RMS value well below 1.5 mm. In addition, for each subject, the mean frame-wise displacement (FD) was calculated from derivatives of the realignment parameters, as a summary statistic of motion during the fMRI run to inspect potential correlations between motion and connectivity values (Power et al. 2012). The pre-processed EPI time series were coregistered with the corresponding structural data set using an affine transformation and resampled to a $3 \text{ mm} \times 3 \text{ mm} \times 3 \text{ mm}$ grid. Motion correction and co-registration transformations were applied together to the EPI images to prevent the blurring effect of multiple resampling steps. Then, additional pre-processing was performed removing the six motion regressors and two nuisance regressors (obtained by extracting the EPI average time course within the cerebrospinal fluid (CSF) and white matter (WM) masks) from the EPI time series. CSF and WM masks were obtained from the FreeSurfer-segmented structural scans (<http://surfer.nmr.mgh.harvard.edu>). Further pre-processing steps included spatial smoothing with an isotropic Gaussian kernel (full width at half maximum = 6 mm) and temporal band-pass filtering in the frequency band considered of interest for resting-state fMRI ($0.01 < \text{Hz} < 0.1$). Finally, the two runs were concatenated resulting in voxel time courses with 592 time points. Masks from three regions of interest (ROIs) including the GM within the MRS voxel, right, and left amygdalae were defined (Fig. 1a). Specifically, the right and left amygdalae were defined using FMRIB's Integrated Registration and Segmentation Tool (FIRST), whereas the GM within the MRS was obtained according with the method described above in the ^1H -MRS section. All these masks were resampled in the functional space using the 3dAllineate tool. For each subject, spectral DCM was performed according with methods described by Razi et al. (2015), following these steps: (1) extraction of BOLD fMRI time series from the three ROIs (mPFC, right amygdala, and left amygdala); (2) specification of the model space based on a fully and reciprocally connected model (Fig. 1b); (3) estimation of the specified model; and (4) implementation of a post-hoc model selection routine.

Statistical analysis

The demographic, psychometric, and imaging outcomes are shown as mean $\pm$ standard deviation.

One-sample t test was used to define the significant connections within and between nodes. Gender differences were assessed using non-parametric Mann-Whitney–Wilcoxon test. Pearson's correlation was used to investigate the relationship between the demographic features and the sDCM outcomes and among sDCM outcomes. General regression model was used to verify whether the GABA+/tCr content within the mPFC predicts the effective

connectivity variability within the amygdala-mPFC circuits. The sDCM outcomes were included as dependent variables whereas the MRS measures, age, and GM volume within the MRS voxel were included as independent variables. Gender was added as categorical predictor. The statistical results were adjusted for multiple comparisons using False Discovery Rate (FDR). Linear regression (block-wise) was used to assess the relationship between STAI scores (dependent variables) and sDCM outcomes (independent variables).

Results

Table 1 summarizes the participant demographic details and outcomes from structural MRI and ¹H-MRS data. Table 2 summarizes the psychometric scores. When comparing for gender, no differences were found in demographic, psychometric, or imaging outcomes. The STAI-Y1 and STAI-Y2 were significantly correlated ($r = 0.58$; $p = 0.005$).

Spectral dynamic causal modelling

Figure 1b and Table 3 show the effective connectivity within the mPFC-amygdala network. A significant self-inhibition was found within the mPFC and amygdalae (right and left) nodes. Furthermore, extrinsic connections (bottom-up and top-down) were found between the amygdalae and the mPFC and between the right and left amygdalae. The connectivity strengths of bottom-up connections, and between right and left amygdalae, were positive, whereas the connectivity strength of top-down connections was negative. Comparing for gender, no differences were found on sDCM outcomes (suppl. table 1). No differences for STAI and imaging outcomes were found between subjects under 65 years and subjects over 65 years of age (suppl. table 2).

Correlation analysis

Figure 1c shows the relationships between the sDCM outcomes.

Table 4 and suppl. Fig. 2 show the relationships between the sDCM outcomes and mPFC GABA+/tCr measures. The mPFC GABA+/tCr levels explained about half of the variance in the strengths of top-down connection. Specifically, the strength of the negative extrinsic connection from the mPFC to the right amygdala was negatively correlated with the mPFC GABA+/tCr content. Table 5 and suppl. Fig. 3 show the relationships between imaging outcomes and STAI-Y1 scores. The STAI-Y1 was negatively related to the strength of the negative extrinsic connections projecting from the mPFC to the right amygdala. No significant correlation was found between the sDCM outcomes and the STAI-Y2. The GABA+/tCr content was significantly related with the STAI-Y1 ($r = 0.43$; $p = 0.04$) and the STAI-Y2 ($r = 0.64$, $p = 0.001$).

No significant correlation was found between the sDCM outcomes and the demographic features including age, educational level, and between the sDCM outcomes and the GM within the ¹H-MRS voxel (suppl. table 3).

Finally, no significant relationship was found between sDCM outcomes and mean FD (suppl. table 4).

Discussion

In the present study, we found a close relationship between the effective connectivity at rest within the amygdala-mPFC circuit, the mPFC GABA amount, and state anxiety scores. Our findings were not influenced by demographic features and structural changes.

Several studies described functional interactions between amygdalae and mPFC (Somerville et al. 2013; Kim et al. 2003; Urry et al. 2006; Johnstone et al. 2007). Recently, we observed that amygdala-mPFC functional coupling varied according to mPFC GABA content and trait anxiety (Delli Pizzi et al. 2017). Nevertheless, functional connectivity is unable to provide information regarding causality, or direction of connection. Indeed, it only gives information on “the statistical association or dependency among two or more anatomically distinct time series” (Friston et al. 1996; Friston 2011). In contrast, DCM applied to resting-state data is a useful method to study the effective connectivity at rest, providing a powerful tool to “summarize the causal influence one neural system exerts over another using a model of neural interactions that best explains the observed signals or their functional connectivity” (Razi et al. 2015). Hence, by combining sDCM and MRS measures, we were able to segregate the functional contributes of bottom-up and top-down pathways, investigating the relationship between metabolite levels and effective connectivity of the amygdala-mPFC network.

The architecture of our amygdala-mPFC circuit was remarkably consistent with the recently proposed models (Calhoun and Tye 2015; Nuss 2015). Specifically, we observed that the mPFC is the target of incoming positive outputs from the amygdalae, and the source of exiting negative inputs to the amygdalae. In sDCM, positive values of extrinsic connectivity strength are interpreted as excitatory, whereas negative values are interpreted as inhibitory (Crone et al. 2015; Razi et al. 2015). Notably, this interpretation is not referring to cellular level signalling, which cannot be assessed using fMRI. Rather, we refer to the interactions between neuronal populations within amygdalae and mPFC as modelled by sDCM. Indeed, sDCM models these influences as either inhibitory or excitatory (Razi et al. 2015; Crone et al. 2017). Thus, the observation of positive exogenous connections from the amygdalae to the mPFC was consistent with the excitatory role of the bottom-up pathway, which is triggered by external stimuli and cortico-thalamic input (Calhoun and Tye 2015; Nuss 2015). Conversely, the observation of negative exogenous connections from the mPFC to the amygdalae was consistent with the inhibitory role of the top-down pathway, which controls and regulates amygdala activity (Bishop 2007; Calhoun and Tye 2015; Likhtik et al. 2005; Krettek and Price 1978; McDonald 1998; Quirk and Gehlert 2003; Russchen 1982; Sesack et al. 1989). The presence of bidirectional exogenous connections between amygdalae is consistent with their functional coupling at rest (Zald et al. 1998; Roy et al. 2009). Interestingly, we observed significant self-intrinsic (endogenous) connections within the mPFC. In sDCM studies, the effective connectivity “within node” is considered always inhibitory on biological constraints (Friston et al. 2014; Li et al. 2011; Razi et al. 2015). Specifically, positive values of intrinsic connectivity mean that the self-connections are highly inhibitory, whereas negative values mean that the self-connections are weakly inhibitory (Crone et al. 2015). Combined fMRI and ¹H-MRS studies have recently demonstrated a key role of GABA in modulating intrinsic neuronal activity on humans

(Duncan et al. 2014; Kapogiannis et al. 2013; Staggs et al. 2014). GABA-ergic interneurons orchestrate synchrony between microcircuits (Bonifazi et al. 2009) and higher GABA concentration in the human anterior cingulate cortex predicts negative BOLD responses in the same region upon emotional stimulus presentation (Northoff et al. 2007). Thus, we speculate that mPFC GABA may be central in synchronising neuronal activity in that region and, therefore, greater inhibitory activity within the mPFC could be related to higher GABA concentration within it. Importantly, we found that GABA content within the mPFC predicts the variability of exogenous connection between the amygdalae and the mPFC. Specifically, we observed that high mPFC GABA level is associated with low inhibitory strength of the top-down connections. Thus, considering that the top-down projection from mPFC to amygdala acts as “a feedback system”, which shapes the amygdala outputs to brainstem and other relevant regions deputed to the autonomic response, we posit that high intrinsic connectivity within top-down connection could result in strong amygdala modulation (Fig. 2a), whereas weak intrinsic connectivity could lead to low amygdala modulation (Fig. 2b). In agreement with this hypothesis, we showed a negative relationship between the state anxiety and the strength of the top-down endogenous connection between the mPFC and the right amygdala.

Ex-vivo animal studies suggested that aberrant top-down modulation of amygdalae can lead to amygdalae hyper-responsiveness, down-regulation of fear extinction, and anxiety (Adhikari et al. 2015). Furthermore, chronic stress may lead to an increase of synaptic inhibition onto prefrontal glutamatergic outputs neurons, limiting the influence of PFC in the regulation of top-down control and offering a potential mechanisms for increased anxiety-related behaviors (McKlveen et al. 2016). Human fMRI studies on emotional processing confirmed the close link among PFC recruitment, amygdala activity, and top-down processing (Ochsner et al. 2004). Particularly, it was reported that the degree of recruitment of the ACC/mPFC predicts the degree of exaggeration of amygdala response (Somerville et al. 2013; Kim et al. 2003; Urry et al. 2006; Johnstone et al. 2007). Nevertheless, recent models on emotional dysregulation have hypothesized that anxiety disorders are related to hypofunctionality of the mPFC/ACC, which, in turn, triggers deficient top-down control system during the emotional regulation (Mochcovitch et al. 2014). Indeed, patients with generalized anxiety disorder fail to activate ACC during the regulation of emotional conflict (Etkin et al. 2009) and the inhibition of fear (Greenberg et al. 2013). In the same way, the forward connections from the mPFC to the amygdala shifted from inhibitory in healthy controls to excitatory in patients with social anxiety disorder during an emotional discrimination task (Sladky et al. 2015). Therefore, we hypothesized that GABA-induced imbalance of the intrinsic connectivity within the amygdala-mPFC network could cause a failure of amygdala activity regulation, leading to an excessive autonomic response and greater anxiety (Fig. 2b).

In the current study, we observed a lateralization of our findings to the right amygdala. Hemispheric lateralization of emotional processes is a matter of debate. Although amygdalae are bilaterally involved in fear conditioning, the right amygdala is relatively more involved than the left amygdala in aversive emotional processing and expression of negative emotions (Adolphs et al. 1996; Bowers et al. 1991; Canli et al. 1998; Mandal et al. 1991; Makovac et al. 2016). Furthermore, growing evidence reported a functional dissociation

between amygdalae in terms of temporal dynamics (Sergeie et al. 2008). Particularly, it was suggested that the right amygdala is implicated in the rapid detection of emotional stimuli, whereas the left amygdala is involved in a more elaborate stimulus evaluation (Markowitsch 1998; Wright et al. 2003). Thus, the right amygdala is involved in the initial, automatic detection of emotional stimuli, whereas left amygdala performs subsequent and specific processing of changes of the levels of arousal associated with specific stimuli (Gläscher and Adolphs 2003; Sergeie et al. 2008). In contrast to the functional dissociation hypothesis, alternative theories posited that both right and left amygdalae receive equal inputs, but there are differences at neurotransmission level between them (Baker and Kim 2004). Specifically, a previous study reported a larger serotonin level in right amygdala, which was strongly correlated with anxiety behavior (Andersen and Teicher 1999). High levels of serotonin in the amygdala produce deficits in fear learning (Groenink et al. 2000). This could indicate that serotonin levels regulation, through fear processing, may help decrease anxiety. In line with this model, the initial reactivity of the right amygdala may be higher than the left amygdala.

Finally, we recognize that the ^1H -MRS voxel also contains regions surrounding the mPFC (Palomero-Gallagher et al. 2008, 2013). This is due to the limited spatial resolution of the current state-of-the-art technique for GABA measurement, MEGA-PRESS (Mullins et al. 2014). This sequence requires a ^1H -MRS voxel size equal to or larger than $2 \times 3 \times 3 \text{ cm}^3$ to guarantee a good signal-to-noise ratio (Edden and Barker 2007; Mullins et al. 2014). However, we underline that the ^1H -MRS voxel was well outside the dorsal portion of the mPFC which is deputed to appraisal (Robinson et al. 2012; Fullana et al. 2015). Particularly, it was placed on the ventral portion of the mPFC which is relevant for extinction and top-down control of the amygdala (Delli Pizzi et al. 2017).

Another aspect to consider is that the edited GABA signal contains contributions from homocarnosine and macromolecules. Homocarnosine is a dipeptide of GABA that is only found in the brain and is also a marker for GABA-ergic neurons. Thus, it does not greatly impact the interpretation of MRS GABA levels as a baseline marker of inhibitory capacity (Rae et al. 2014). The nature of the 3-ppm macromolecule is still poorly understood. One leading idea is that macromolecules are represented by lysine groups in mobile peptide side-chains (Henry et al. 2001). Although methods exist for suppressing the macromolecular component (Henry et al. 2001), they are more sensitive to experimental instability than the “GABA+” editing scheme we used (Edden et al. 2016). Hence, while a functional mechanism involving GABA-ergic inhibition motivated the experiment and seems the most parsimonious explanation of the findings, it remains possible that our findings might be influenced by macromolecules signals.

In conclusion, our findings suggest a close relationship between GABA content within the mPFC, effective connectivity within the amygdala-mPFC circuit and state anxiety, providing new insights on the physiology of the emotional brain and the underlying functional and neurochemical interactions. Particularly, we posit that the GABA-ergic inhibitory control regulates the top-down feedback control system on amygdala, defining the characteristics of the emotional response. Our findings could be an important starting point for further

investigations on neuropsychiatric diseases characterized by anxiety, to better clarify the symptoms pathophysiology and to propose new pharmacological targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

GABA	γ -Aminobutyric acid
GM	Grey matter
STAI	State-Trait Anxiety Inventory scale (Y1 = sub-scale-1, Y2 = subscale-2)

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Fig. 1.

Spectral dynamic causal model (sDCM). **a** Amygdalae and mPFC masks overlaid on the T₁-weighted image of a representative subject. **b** Schematic representation of the effective connectivity within the amygdala-mPFC loop. The *circles* around the mPFC and amygdalae represent the recurrent connectivity within node. The *arrows* represent extrinsic connectivity. The *red* and *blue* colours denote positive and negative values, respectively. The specific values of effective connectivity within node or between nodes are in Hertz (Hz) and they are reported near the *arrows* or *circle*, respectively. Regarding the effective connectivity between nodes, the positive values are interpreted as excitatory, whereas the negative values are interpreted as inhibitory. The effective connectivity “within node” (endogenous) is considered inhibitory. Specifically, the positive value means that the self-connections are highly inhibitory, whereas the negative value means that the self-connections are weakly inhibitory. Significant correlations were marked with an *asterisk*. **c** *Colour map* shows the correlation between the sDCM outcomes. The colours range from *red* to *yellow* for the positive correlations and from *blue* to *dark blue* for the negative correlations, respectively. FDR *p* values are reported for each correlation. Significant connections were marked with an *asterisk*. The wordings “mPFC-mPFC” and “AMG-AMG” refer to the intrinsic connectivity

within the mPFC and the amygdala, respectively; *RAMG* right amygdala, *LAMG* left amygdala, *mPFC* medial prefrontal cortex

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Fig. 2.

GABA-dependent circuit between the medial prefrontal cortex (mPFC) and the amygdalae. **a** Low GABA content within the mPFC leads to strong top-down control of mPFC on right amygdala and, thus, to a high efficiency of the feedback system. Therefore, right amygdala activity is well regulated, and anxiety is low. **b** High GABA content within the mPFC leads to weak top-down control of mPFC on right amygdala and, thus to low efficiency of the feedback system. Therefore, right amygdala activity is down-regulated, and anxiety is high. *Dark blue* high mPFC functioning, *blue* low mPFC functioning, *Red* high activity of amygdala which leads to weak autonomic response and low anxiety, *yellow* low activity of amygdala which leads to strong autonomic response and high anxiety

Table 1Demographic, psychometric, structural MRI, and ¹H-MRS outcomes

Outcomes	All participants (<i>n</i> = 22)	Male (<i>n</i> = 11)	Female (<i>n</i> = 11)	Female vs. male ^a
Age (years)	62.6 ± 11.0	64.2 ± 14.3	61.0 ± 6.6	<i>p</i> = 0.393
Educational level (years)	10.8 ± 4.5	10.0 ± 4.9	11.6 ± 4.1	<i>p</i> = 0.311
STAI-Y1	32.2 ± 6.7	30.9 ± 5.8	33.5 ± 7.6	<i>p</i> = 0.263
STAI-Y2	33.5 ± 6.4	31.5 ± 6.07229	35.5 ± 6.4	<i>p</i> = 0.130
GM (mm ³)	10,134 ± 931	9887 ± 975	10,381 ± 856	<i>p</i> = 0.308
GABA+/tCr	0.0615 ± 0.010	0.0585 ± 0.011	0.0645 ± 0.008	<i>p</i> = 0.158

Data are reported as mean ± standard deviation

^a *p* values were calculated using the Mann–Whitney Test

Table 2

Mean $\pm$ standard deviation for each test performed in participants

Domain	Test	All participants	Male	Female	Female vs. male *
Global cognitive function	MMSE	27.9 $\pm$ 1.0	28.1 $\pm$ 1.1	27.8 $\pm$ 0.8	$p = 0.699$
	DSF	6.3 $\pm$ 0.8	6.5 $\pm$ 0.8	6.2 $\pm$ 0.8	$p = 0.519$
Auditory working memory	DSB	4.3 $\pm$ 1.0	4.5 $\pm$ 1.2	4.1 $\pm$ 0.7	$p = 0.652$
	Attentional Matrices	48.2 $\pm$ 4.2	48.9 $\pm$ 5.2	47.6 $\pm$ 4.3	$p = 0.300$
Speed, attention and executive functions	TMT-A	40.6 $\pm$ 17.5	43.1 $\pm$ 23.0	38.2 $\pm$ 10.3	$p = 0.797$
	TMT-B	98.1 $\pm$ 53.2	85.5 $\pm$ 59.3	110.6 $\pm$ 45.6	$p = 0.088$
Language	FAB	16.5 $\pm$ 1.2	16.4 $\pm$ 1.3	16.7 $\pm$ 1.2	$p = 0.562$
	FAS	34.7 $\pm$ 8.4	33.6 $\pm$ 7.7	35.8 $\pm$ 9.3	$p = 0.562$
BSRT	Immediate recall	6.4 $\pm$ 1.2	6.2 $\pm$ 1.4	6.4 $\pm$ 1.0	$p = 0.300$
	Delayed recall	6.4 $\pm$ 1.2	6.2 $\pm$ 1.4	6.5 $\pm$ 1.0	$p = 0.748$

Data are reported as mean $\pm$ standard deviation

BSRT Babcock Story Recall Test, long-term, *DSF* Digit Span Forward, *DSB* Digit Span Backward, *FAB* Frontal Assessment Battery, *FAS* verbal fluency test, *MMSE* Mini-Mental State Examination, *TMT* Trail Making Test

* p values were calculated using the Mann–Whitney Test

Table 3

Spectral dynamic causal model (sDCM) outcomes

Connections	Mean $\pm$ SD ^a	<i>p</i> value [*]
mPFC–mPFC	−0.23 $\pm$ 0.25	0.0008
mPFC–RAMG	−0.12 $\pm$ 0.23	0.031
mPFC–LAMG	−0.10 $\pm$ 0.21	0.043
RAMG–mPFC	0.15 $\pm$ 0.32	0.043
RAMG–RAMG	−0.10 $\pm$ 0.22	0.043
RAMG–LAMG	0.20 $\pm$ 0.17	0.0002
LAMG–mPFC	0.19 $\pm$ 0.32	0.022
LAMG–RAMG	0.25 $\pm$ 0.23	0.0002
LAMG–LAMG	−0.12 $\pm$ 0.24	0.043

LAMG left amygdala, *RAMG* right amygdala, *mPFC* medial prefrontal cortex, *SD* standard deviation. The wordings “mPFC–mPFC” and “AMG–AMG” refer to the intrinsic connectivity within mPFC and amygdala, respectively

^{*} Significant *p* values after FDR correction are reported in bold

^a The connection strength was measured in Hz

Table 4

General regression model

Test of SS Whole Model vs. SS Residual	Multiple R	Multiple R ²	Adjusted R ²	MS model	SS model	F _{4,17}	p [*]	Power
mPFC-mPFC	0.519	0.269	0.097	0.085	0.342	1.566	0.343	0.46
mPFC-RAMG	0.770	0.592	0.497	0.159	0.637	6.179	0.026	0.96
mPFC-LAMG	0.524	0.275	0.104	0.066	0.264	1.609	0.343	0.47
RAMG-mPFC	0.373	0.139	-0.063	0.077	0.307	0.687	0.611	0.22
RAMG-RAMG	0.453	0.205	0.018	0.050	0.200	1.099	0.438	0.33
RAMG-LAMG	0.636	0.405	0.265	0.064	0.254	2.893	0.162	0.98
LAMG-mPFC	0.642	0.412	0.274	0.222	0.888	2.979	0.162	0.98
LAMG-RAMG	0.527	0.278	0.108	0.078	0.313	1.635	0.343	0.47
LAMG-LAMG	0.462	0.213	0.028	0.065	0.262	1.151	0.438	0.35

Multivariate tests of significance	Age		GABA+/iCr		GM		Gender	
	F _{1,17}	p [*]	F _{1,17}	p [*]	F _{1,17}	p [*]	F _{1,17}	p [*]
mPFC-mPFC	0.636	0.436	1.315	0.535	2.568	0.535	2.019	0.535
mPFC-RAMG	0.336	0.570	21.510	0.009	0.179	0.882	1.438	0.535
mPFC-LAMG	0.484	0.496	1.343	0.535	3.010	0.535	1.918	0.535
RAMG-mPFC	0.138	0.714	0.056	0.889	2.217	0.535	0.119	0.882
RAMG-RAMG	1.521	0.234	0.135	0.882	1.450	0.535	1.848	0.535
RAMG-LAMG	0.268	0.612	11.320	0.058	1.540	0.535	1.119	0.549
LAMG-mPFC	0.004	0.950	10.507	0.058	0.000	0.984	1.130	0.549
LAMG-RAMG	0.072	0.791	0.603	0.733	4.312	0.480	2.727	0.535
LAMG-LAMG	0.059	0.811	2.346	0.535	0.001	0.984	0.497	0.744

Dependent variables: strength of the connections between and within node; independent variables: GABA within mPFC, GM within the ¹H-MRS voxel and age; categorical predictor: gender

LAMG left amygdala; RAMG right amygdala; mPFC medial prefrontal cortex; SS sum of squares. The wordings “mPFC-mPFC” and “AMG-AMG” refer to the intrinsic connectivity within mPFC and amygdala, respectively

* Significant *p* values after FDR correction were reported in bold

Table 5

Linear regression (block-wise) assessing the relationship between the state anxiety (STAI-Y1) and the strength of connectivity between and within nodes

	mPFC-mPFC	mPFC-RAMG	mPFC-LAMG	RAMG-mPFC	RAMG-RAMG	RAMG-LAMG	LAMG-mPFC	LAMG-RAMG	LAMG-LAMG
$F_{(9,19)} = 3.28, p = 0.029$	β	-0.087	1.104	0.205	-0.597	0.263	0.880	0.148	0.105
	t	-0.240	2.214	0.501	-0.758	0.925	1.977	0.211	0.212
	p	0.814	0.047	0.626	0.463	0.373	0.072	0.836	0.836

LAMG left amygdala, RAMG right amygdala, mPFC medial prefrontal cortex. The wordings “mPFC-mPFC” and “AMG-AMG” refer to the intrinsic connectivity within mPFC and amygdala, respectively
Significant relationships were reported in bold