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This Section of *Epidemiology and Psychiatric Sciences* appears in each issue of the Journal to stress the relevance of epidemiology for behavioral neurosciences, reporting the results of studies that explore the use of an epidemiological approach to provide a better understanding of the neural basis of major psychiatric disorders and, in turn, the utilisation of the behavioural neurosciences for promoting innovative epidemiological research.

The ultimate aim is to help the translation of most relevant research findings into every-day clinical practice. These contributions are written in house by the journal's editorial team or commissioned by the Section Editor (no more than 1000 words, short unstructured abstract, 4 key-words, one Table or Figure and up to ten references).

Paolo Brambilla, *Section Editor*

Metabolic alterations in generalised anxiety disorder: a review of proton magnetic resonance spectroscopic studies

G. Delvecchio[1], J. A. Stanley[2], A. C. Altamura[1] and P. Brambilla[1,3]*

[1] *Department of Neurosciences and Mental Health, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Milan, Italy*
[2] *Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, USA*
[3] *Department of Psychiatry and Behavioural Neurosciences, University of Texas at Houston, TX, USA*

Generalised anxiety disorder (GAD) is a common psychiatric illness characterised by selective morpho-functional brain alterations. The breath of neuroimaging studies investigating the neural basis of GAD is extensive; however, its pathophysiology is still largely unknown. Specifically for proton Magnetic Resonance Spectroscopy (^{1}H MRS) investigations, which have the aim of identifying differences in metabolite levels between conditions in key brain areas, often showed contrasting results. Indeed, there are selected ^{1}H MRS studies reporting deficits of key metabolites in GAD patients; however, collectively the literature remains mixed with respect to consistency of major findings. In this review, we evaluate published ^{1}H MRS studies on GAD with the final aim of providing a comprehensive overview of the extent of neurometabolic dysfunctions associated with GAD. Interestingly, the majority of the studies reviewed showed altered metabolite levels in the dorsolateral prefrontal cortex and hippocampus suggesting regional specificity. These results also provide evidence of the utility of ^{1}H MRS not only for elucidating the pathophysiology of neuropsychiatric diseases, but also for the identification of more beneficial and targeted pharmacological interventions. Additionally, future studies are warranted to overcome methodological differences observed across the studies.

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Generalised anxiety disorder (GAD) is a common psychiatric disease characterised by specific physical and psychological symptoms, including persisting worry, irritability and fatigue (DSM5, American Psychiatry Association, 2013; Diwadkar *et al*. 2017). GAD causes high human suffering, which is poorly understood. With respect to neuroimaging studies, the exploration of putative biomarkers of this disease is still at an early stage (Terlevic *et al*. 2012). This is true especially for the application of proton Magnetic Resonance Spectroscopy (^{1}H MRS), which has the unique ability of providing important quantitative biochemical information in localised brain areas (Stanley, 2002). This can lead to identifying possible and more effective pharmacological treatments for GAD. The prominent ^{1}H metabolites include N-acetyl-aspartate (NAA), a marker for neuronal density and functioning,

* Address for correspondence: P. Brambilla, Department of Neurosciences and Mental Health, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, via F. Sforza 35, 20122 Milan, Italy.
(Email: paolo.brambilla1@unimi.it)

glycerophosphocholine plus phosphocholine (GPC + PC), membrane phospholipid metabolites, and phosphocreatine and creatine (PCr + Cr), involved in energetic processes (Brambilla *et al*. 2002, 2012; Stanley *et al*. 2007).

In this review, we aimed at providing an overview of ^{1}H MRS studies carried out in GAD with the final goal of shedding light on the significance of the reported altered metabolite levels in this disorder. A bibliographic search on PUBMED on ^{1}H MRS studies in GAD was performed and the research terms used were 'MRS', 'spectroscopy', and 'generalised anxiety disorder'. Studies were excluded if the publications: (a) included twin samples, (b) investigated GAD not in relation to healthy controls (HC) or (c) explored only white matter structures. In total, eleven papers met the inclusion criteria and are summarised in Table 1. Briefly, among the 11 studies retrieved, the majority employed a multi-voxel ($N = 7$) instead of a single-voxel ($N = 4$) ^{1}H MRS technique and a 1.5 T scanner ($N = 6$) instead of a 3 T ($N = 4$) or a 4 T ($N = 1$) scanner. Interestingly, all the ^{1}H MRS studies on GAD, except for two studies Abdallah *et al*. 2012*a*; Strawn *et al*. 2013), focused on brain regions within the hippocampus and dorsolateral prefrontal cortex (DLPFC).

Regarding the DLPFC, four studies of which three of them are from the same group, reported multiple contrasts including higher ratios of NAA/PCr + Cr (Mathew *et al*. 2004) and lower ratios of GPC + PC/PCr + Cr and GPC + PC/NAA (Moon *et al*. 2015; 2016*b*; Moon & Jeong, 2016*a*) ratios in GAD patients compared with HC. Additionally, Raparia *et al*. (2016) found higher NAA, PCr + Cr and GPC + PC levels in DLPFC, premotor cortex (PC) and secondary somatosensory cortex (SSC) bilaterally in GAD patients compared with HC. Interestingly, Mathew *et al*. (2004) reported that GAD patients with childhood abuse had higher NAA/PCr + Cr ratios compared with GAD patients without childhood abuse. In contrast, Raparia *et al*. (2016) found that GAD patients had no significant associations between emotional abuse scores and NAA, PCr + Cr and GPC + PC levels in DLPFC, PC and SSC bilaterally, but were significant in HC. Additionally, the three studies carried out by Moon *et al*. found that GPC + PC/PCr + Cr and GPC + PC/NAA ratios positively correlated with right DLPFC volumes (Moon & Jeong, 2016*a*; Moon *et al*. 2016*b*) and blood oxygenation level-dependent signal change in right DLPFC (Moon *et al*. 2016*b*). In contrast, a negative correlation was observed between GPC + PC/NAA ratio and anxiety symptom severity (Moon *et al*. 2015). Collectively, these studies suggest DLPFC neuronal deficits, which may in turn explain the neurocognitive deficits often observed in GAD patients. Indeed DLPFC is a key brain area regulating cognition and emotion, and plays a prominent role in working memory and executive brain functions (Brambilla *et al*. 2005).

Regarding the hippocampus, the study by Mathew *et al*. (2008) showed increased hippocampal NAA levels after 8 weeks of treatment with the glutamate-antagonist riluzole in GAD responder patients, whereas hippocampal NAA levels decreased over time in non-responders. Moreover, the change over time (post-minus pre treatment) in hippocampal volume was positively associated with change over time in NAA (especially in the right side) and with the improvement in anxiety symptoms (Abdallah *et al*. 2013). In contrast, lower ratios of NAA/PCr + Cr in bilateral hippocampus of nine GAD patients were not reversed after 12 weeks of paroxetine, despite marked symptoms improvement (Mathew *et al*. 2010). Additionally, Abdallah *et al*. (2012*a*) observed a negative correlation between right occipital NAA and symptoms improvement after riluzole treatment. Riluzole has been demonstrated to modulate extracellular glutamate through glial reuptake mechanisms regulating neural plasticity in the hippocampus (Frizzo *et al*. 2004). SSRIs have also been linked to enhance neural plasticity in hippocampal cells (Wang *et al*. 2008). Therefore, hippocampal NAA may reflect non-neuronal activity (Mathew *et al*. 2008) being a possible biomarker of GAD, and NAA change might be differently related to disparate mechanisms of drug action. Additionally, Coplan *et al*. (2014) also reported significant metabolites alterations associated with weight, with overweight GAD patients showing lower NAA in hippocampus compared with HC. Moreover, an inverse correlation was observed between hippocampal NAA and body mass index as well as higher worry predicted low hippocampal NAA and PCr + Cr. Lastly, Strawn *et al*. (2013) recently reported no significant alterations in glutamate/PCr + Cr ratios in the anterior cingulate of adolescents with GAD; however, a positive correlations between glutamate/PCr + Cr and anxiety symptoms severity.

In conclusions, these findings together suggest that GAD is associated with metabolic dysfunctions in selective brain regions, including the DLPFC and hippocampus. However, these results require further independent replications. Indeed, although the majority of the studies employed absolute metabolite values, some others used metabolite ratios, which might have therefore limited the interpretations of the results. Additionally, most of the studies were characterised by relatively small sample sizes and were carried out by the same research group, further decreasing the generalisability of their findings. Despite these limitations, these findings illustrate that alterations in specific metabolites, especially NAA, PCr + Cr and GPC + PC, might be considered putative biomarkers of

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Table 1. *Selection of studies on generalised anxiety disorder exploring metabolic alterations with 1-H magnetic resonance spectroscopy*

Study	Sample (age, mean ± S.D.)	Gender F/M	Study design	Psychotropic medications	Method	Location (voxel size)	Quantification and reported ^{1}H metabolites	^{1}H MRS findings
Mathew *et al.* (2004)	GAD patients = 15 (39.3 ± 13.3) HC = 15 (39.1 ± 13.5)	GAD patients = 8/7 HC = 8/7	Cross-sectional	Six medication-naïve. No psychotropic drugs within at least 4 weeks	Multi-voxel ^{1}H MRSI with TE = 280 ms at 1.5 T	Left and right hippocampus and DLPFC (1.5×0.75 × 0.75 cm^3)	Metabolite ratio NAA/PCr+Cr GPC+PC	– Higher NAA\Cr ratio in right DLPFC in GAD patients compared with HC. – GAD patients with childhood abuse had higher NAA\creatine ratio in right DLPFC compared with GAD patients without childhood abuse
Mathew *et al.* (2008)	GAD patients = 14 (31.7 ± 9.6) HC = 7 (27.4 ± 4.2)	GAD patients = 8/6 HC = 5/2	Longitudinal	Six medication- naïve. No current psychotropic drugs	Multi-voxel ^{1}H MRSI with TE = 280 ms at 1.5 T. 8 weeks of therapy with Riluzole	Left and right hippocampus (1.13 cm^3)	Absolute relative to water NAA PCr+Cr GPC+PC	– Increased NAA in hippocampus bilaterally, in responder patients over time. – Decrease NAA in hippocampus bilaterally over time in non-responders patients. – No differences in NAA concentrations between responders and non-responders patients in any time point. – In GAD patients, hippocampal NAA concentration and proportional increase in NAA from baseline were positively associated with improvements in worry and clinician-rated anxiety
Abdallah et al. (2012*a*)	GAD patients = 14 (33.9 ± 2.7) HC = 10 (30.3 ± 2.4)	GAD patients = 8/6 HC = 6/4	Longitudinal	Fourteen medication-free	Multi-voxel ^{1}H MRSI with TE = 280 ms	Left and right lateral	Absolute relative to water NAA	– Negative correlation between right occipital NAA and symptoms

Continued

Table 1. *Continued*

Study	Sample (age, mean ± S.D.)	Gender F/M	Study design	Psychotropic medications	Method	Location (voxel size)	Quantification and reported ^{1}H metabolites	^{1}H MRS findings
					and MRI at 1.5 T. 8 weeks of therapy with Riluzole	occipital (1.13 cm^3)		improvement after treatment
Abdallah *et al.* (2012*b*)	GAD patients = 18 (33.9 ± 2.7) HC = 10 (30.3 ± 2.4)	GAD patients = 8/10 HC = 6/4	Longitudinal	Eighteen medication-free	Multi-voxel ^{1}H MRSI with TE = 280 ms and MRI at 1.5 T. 8 weeks of therapy with Riluzole	Left and right hippocampus (1.13 cm^3)	Absolute relative to water NAA	– Reduction in total hippocampal volume at baseline in GAD patients (more pronounced in remitters). – Delta (final-baseline) ippocampal volume positively correlate with delta NAA (especially on the right side) in GAD.
Mathew *et al.* (2010)	GAD patients = 9 (41.7 ± 15.8) HC = 10 (37.1 ± 14.8)	GAD patients = 4/5 HC = 4/10	Longitudinal	Four medication-naïve. No psychotropic drugs within at least 4 weeks	Multi-voxel ^{1}H MRSI with TE = 280 ms at 1.5 T. 12 weeks of therapy with Paroxetine	Left and right hippocampus (1.5 × 0.75 × 0.75 cm^3 or 0.84 cc)	Metabolite ratio NAA/PCr+Cr	– Lower levels of NAA/Cr ratio in bilateral hippocampus in GAD patients compared with HC, before and after therapy. – Hippocampal NAA/Cr ratios were positively correlated with PSWQ
Strawn *et al.* (2013)	GAD patients = 10 (14 ± 2.2) HC = 10 (14.5 ± 2.3)	GAD patients = 6/4 HC = 6/4	Cross-sectional	No psychotropic drugs within at least five half-lives	Single-voxel ^{1}H-MRS with TE = 30 ms at 4 T	ACC (2.2 × 2.2 × 2.2cm^3)	Metabolite ratio Glu/PCr + Cr	– No differences in NAA, creatine or myo-inositol between GAD and HC. – Positive correlations between Glu/PCr + Cr and anxiety symptom severity
Raparia *et al.* (2016)	GAD patients = 16 (37.9 ± 14.2) HC = 16 (35.3 ± 10.3)	GAD patients = 11/5 HC = 10/6	Cross-sectional	No medication for at least 2 weeks prior the MRSI scan	Multi-voxel ^{1}H-MRSI with TE = 280 ms at 3 T	mPFC PMC SCC (7.5 × 7.5 × 15 mm^3)	Absolute relative to water NAA PCr + Cr GPC + PC	– In GAD patients, emotional abuse scores did not correlate with either NAA, Cr or Cho levels for the

Continued

Table 1. *Continued*

Study	Sample (age, mean ± S.D.)	Gender F/M	Study design	Psychotropic medications	Method	Location (voxel size)	Quantification and reported ^{1}H metabolites	^{1}H MRS findings
								mPFC, PMC and SCC bilaterally. – GAD patients exhibited greater NAA, Cr and Cho than HC. – CTQ emotional abuse effect was inversely predicting NAA, Cr and Cho only in HC
Moon & Jeong (2016*a*)	GAD patients = 14 (36.6 ± 8.8) HC = 14 (37.8 ± 7.8)	GAD patients = 8/6 HC = 8/6	Cross-sectional	Eleven patients with Anxiolytics and/or antidepressants. Three patients with single psychiatric medication comprising escitalopram or bupropion	Single-voxel ^{1}H-MRS with TE = 30 ms and MRI at 3 T	DLPFC (20 × 20 × 20 or 8 cm^{3})	Metabolite ratio NAA PCr + Cr GPC + PC MI Lactate Lip α-Glx/ PCr + Cr β,γ-Glx/NAA	– GAD patients had significantly lower Cho/Cr and Cho/NAA ratios in the DLPFC compared with HC. – DLPFC volume was positively correlated with the ratios of Cho/Cr and Cho/NAA in GAD patients
Moon *et al.* (2016*b*)	GAD patients = 13 (37.8 ± 7.6) HC = 13 (35.9 ± 3.5)	GAD patients = 7/6 HC = 7/6	Cross-sectional	Seven patients with Anxiolytics and/or antidepressants. Six patients each were taking one psychotropic medication	Single-voxel ^{1}H-MRS with TE = 30 ms and fMRI at 3 T	DLPFC (20 × 20 × 20 or 8 cm^{3})	Metabolite ratio α-Glx/ PCr + Cr mI/ PCr + Cr GPC + PC/PCr + Cr β,γ-Glx/Cr NAA/Cr Lac/ PCr + Cr Lip/ PCr + Cr α-Glx/NAA mI/NAA GPC + PC/NAA β,γ-Glx/NAA PCr + Cr/NAA	– GAD patients had significantly lower Cho/Cr and Cho/NAA ratios in the DLPFC compared with HC – Cho ratios were positively correlated with the brain activities based on blood oxygenation level-dependent signal change in the DLPFC – DLPFC volume was positively correlated with the ratios of Cho/Cr and Cho/NAA in GAD patients

Continued

Table 1. *Continued*

Study	Sample (age, mean ± S.D.)	Gender F/M	Study design	Psychotropic medications	Method	Location (voxel size)	Quantification and reported ^{1}H metabolites	^{1}H MRS findings
Moon *et al.* (2015)	GAD patients = 15 (35.4 ± 9.6) HC = 15 (38.8 ± 8.9)	GAD patients = 9/6 HC = 9/6	Cross-sectional	Ten patients with Anxiolytics and/or antidepressants. Five patients each were taking one psychotropic medication	Single-voxel ^{1}H-MRS with TE = 30 ms and MRI at 3 T	DLPFC (20 × 20 × 20 or 8 cm^{3})	Lac/NAA Lip/NAA Metabolite ratio NAA GPC + PC PCr + Cr MI Lactate Lip α-Glx/ PCr + Cr β,γ-Glx/NAA	– GAD patients had significantly lower Cho/Cr and Cho/NAA ratios in the DLPFC compared with HC. – No significant differences in other metabolite ratios between the two groups – Cho/NAA ratio in GAD patients was negatively correlated with the scores of HAMA and GAD-7
Coplan *et al.* (2014)	GAD patients = 29 (35.1 ± 11.9) HC = 22 (33.7 ± 10.4)	GAD patients = 18/11 HC = 14/8	Cross-sectional	No medication	Multi-voxel ^{1}H-MRSI with TE = 280 ms at 1.5 T	Left and right hippocampus (1.13 cm^{3})	Absolute relative to water NAA PCr + Cr GPC + PC	– Overweight subjects exhibited lower NAA levels in the hippocampus than normal-weight subjects in both GAD patients and HC. – Women overall exhibited relative elevations of hippocampal NAA concentration, compared with males. – Lower Cho in the left hippocampus and higher Cho in the right hippocampus in GAD subjects compared with HC. – Reduction in Cr concentration in GAD

Continued

Table 1. *Continued*

Study	Sample (age, mean ± S.D.)	Gender F/M	Study design	Psychotropic medications	Method	Location (voxel size)	Quantification and reported ^{1}H metabolites	^{1}H MRS findings
								subjects compared with HC in the left hippocampus. – Subjects with BMI ≥25 exhibited lower Cr than subjects with BMI ≤25 in the hippocampus. – An inverse correlation was noted in all subjects between the right hippocampal NAA and BMI. – An inverse linear correlation was noted in all subjects between right hippocampal NAA and BMI. – High scores on the PSWQ predicted low hippocampal NAA and Cr. – Both BMI and worry were independent inverse predictors of hippocampal NAA. – High scores on the PSWQ predicted low hippocampal NAA and Cr. Both BMI and worry were independent inverse predictors of hippocampal NAA

GAD, Generalised anxiety disorder; MRI, Magnetic Resonance Imaging; fMRI, Functional Magnetic Resonance Imaging; MRS, Magnetic Resonance Spectroscopy; MRSI, Magnetic Resonance Spectroscopy Imaging; NAA, N-Acetyl-Aspartate; GPC + PC, Glycerophosphocholine plus Phosphocholine; PCr + Cr, Phosphocreatine plus Creatine; HC, Healthy controls; DLPFC, Dorsolateral prefrontal cortex; ACC, Anterior Cingulate Cortex; SSC, Somatosensory cortex; mPFC, medial prefrontal cortex; BMI, Body mass index; PSWQ, Penn State Worry Questionnaire.

GAD. Additionally, from the ^{1}H MRS studies here described emerged that pharmacological treatments positively interact with specific metabolites, especially NAA, within selective brain regions. Therefore, the investigation of brain metabolites could be very effective not only for elucidating the pathophysiology of neuropsychiatric diseases, but also for the identification of more beneficial and targeted pharmacological interventions. Finally, although ^{1}H MRS has been combined with other neuroimaging methods in recent studies (Abdallah *et al*. 2012*a*, *b*, 2013; Moon *et al*. 2015, 2016*b*; Moon & Jeong, 2016*a*), the evidence are still scarce. However, it is important to point out that the combination of more MRI methods allows the integration of different measures, which might increase the information and consequently the reliability of the findings.

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Conflict of interest

None.

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