Drug and Alcohol Dependence 179 (2017) 117–123

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Full length article

Prefrontal Glx and GABA concentrations and impulsivity in cigarette smokers and smoking polysubstance users

Mieke H.J. Schulte[a,b,1,*], Anne Marije Kaag[a,b,1], Reinout W. Wiers[a], Lianne Schmaal[c], Wim van den Brink[b], Liesbeth Reneman[d,g], Judith R. Homberg[e], Guido A. van Wingen[b,f,g,2], Anna E. Goudriaan[b,h,2]

[a] Addiction, Development, and Psychopathology (ADAPT) lab, Department of Psychology, University of Amsterdam, The Netherlands
[b] Department of Psychiatry, Academic Medical Center, University of Amsterdam, The Netherlands
[c] Department of Psychiatry and Neuroscience Campus Amsterdam, VU University Medical Center, Amsterdam, The Netherlands
[d] Department of Radiology and Nuclear Medicine, Academic Medical Center, University of Amsterdam, The Netherlands
[e] Donders Institute for Brain, Cognition and Behavior, Radboud University Medical Centre, The Netherlands
[f] Spinoza center for Neuroimaging, Institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, The Netherlands
[g] Amsterdam Brain and Cognition, University of Amsterdam, Amsterdam, The Netherlands
[h] Arkin Mental Health, Amsterdam, The Netherlands

ARTICLE INFO

Keywords:
Cocaine
GABA
Glutamate
Impulsivity
Smoking
Substance dependence

ABSTRACT

Glutamate and GABA play an important role in substance dependence. However, it remains unclear whether this holds true for different substance use disorders and how this is related to risk-related traits such as impulsivity. We, therefore, compared Glx (as a proxy measure for glutamate) and GABA concentrations in the dorsal anterior cingulate cortex (dACC) of 48 male cigarette smokers, 61 male smoking polysubstance users, and 90 male healthy controls, and investigated the relationship with self-reported impulsivity and substance use. Glx and GABA concentrations were measured using proton Magnetic Resonance Spectroscopy. Impulsivity, smoking, alcohol and cocaine use severity and cannabis use were measured using self-report instruments. Results indicate a trend towards group differences in Glx. Post-hoc analyses showed a difference between smokers and healthy controls ($p = 0.04$) and a trend towards higher concentrations in smoking polysubstance users and healthy controls ($p = 0.09$), but no differences between smokers and smoking polysubstance users. dACC GABA concentrations were not significantly different between groups. Smoking polysubstance users were more impulsive than smokers, and both groups were more impulsive than controls. No significant associations were observed between dACC neurotransmitter concentrations and impulsivity and level and severity of smoking, alcohol or cocaine use or the presence of cannabis use. The results indicate that differences in dACC Glx are unrelated to type and level of substance use. No final conclusion can be drawn on the lack of GABA differences due to assessment difficulties. The relationship between dACC neurotransmitter concentrations and cognitive impairments other than self-reported impulsivity should be further investigated.

1. Introduction

Neurobiological models of substance use disorders (SUDs) have mainly focused on the dopaminergic system (Berridge and Robinson, 1998; but see Nutt et al., 2015 for a critical appraisal). Glutamatergic and GABAergic mechanisms in substance dependence have recently become more prominent in models of addiction (e.g., Li et al., 2013). Glutamate and GABA play a key role in personality traits associated with addiction, such as impulsivity (Schmaal et al., 2012a; Silveri et al., 2014). Several Magnetic Resonance Spectroscopy (^{1}H-MRS) studies in humans have shown that glutamate is affected in substance dependence (e.g., Schmaal et al., 2012b; Yücel et al., 2007). However, the role of GABA in human addiction is less well studied and it remains to be investigated whether glutamate and GABA deviate between different substances of abuse. Moreover, very little is known about the relationship between these neurotransmitters and risk-related traits, including impulsivity, in people with (SUDs). However, glutamate levels in

* Corresponding author at: University of Amsterdam, Department of Psychology, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands.
E-mail address: m.h.j.schulte@uva.nl (M.H.J. Schulte).
[1] Shared first authorship.
[2] Shared last authorship.

http://dx.doi.org/10.1016/j.drugalcdep.2017.06.025
Received 10 February 2017; Received in revised form 12 June 2017; Accepted 12 June 2017
Available online 25 July 2017

the Anterior Cingulate Cortex (ACC) have been related to functions that are affected in SUDs such as delay discounting (e.g., Schmaal et al., 2012a).

The ACC has been a main region of interest for imaging studies on SUDs, as this brain region is suggested to play a key role in craving and relapse to drug-seeking, and impulsivity (Brand et al., 2014; Garavan and Hester, 2007; Myers and Carlezon, 2010). This region is highly suitable for ^{1}H MRS studies due to its homogeneous structure, resulting in several human ^{1}H MRS studies on ACC glutamate concentrations in SUDs. However, the results are inconsistent with regard to the existence and direction of ACC glutamate and GABA alterations in SUDs. That is, both increased (Bauer et al., 2013; Lee et al., 2007; Mon et al., 2012; Schmaal et al., 2012b; Thoma et al., 2011), decreased (Durazzo et al., 2016; Ende et al., 2013; Hermann et al., 2012; Mashhoon et al., 2011; Prescot et al., 2013; Yang et al., 2009; Yücel et al., 2007), as well as no differences in ACC glutamate concentrations in substance users compared to non-substance using controls have been reported (Chang et al., 1999; Gallinat and Schubert, 2007). Similar to the latter findings, the literature on ACC GABA concentrations in substance dependent individuals is also inconsistent. Both lower (Abé et al., 2013; Prescot et al., 2013; Silveri et al., 2014), as well as no differences in substance users compared to non-using controls have been reported (Mon et al., 2012). These contradictory findings may be the result of methodological differences, such as the location of the MRS voxel (e.g., dorsal (Schmaal et al., 2012) or rostral ACC (Yang et al., 2009)) It should be noted that ^{1}H MRS studies are not always able to differentiate between glutamate, glutamine, and other components due to overlap in spectral assignment, for which the term Glx (a composite measure) is often used (Ramadan et al., 2013). Therefore, the term Glx is used henceforth as a proxy measure of glutamate when referring to data acquired in the current study.

GABA and glutamate concentrations may depend on the number of substances used. For instance, lower ACC GABA concentrations may be more pronounced in cocaine dependent subjects with a history of alcohol dependence compared to cocaine dependent subjects without such a history (Ke et al., 2004). Similarly, GABA concentrations in the dorsolateral prefrontal cortex seem to be more pronounced in polysubstance users compared to alcohol dependent patients (Abé et al., 2013). Furthermore, Mason et al. (2006) did not find differences in glutamate and GABA concentrations between alcohol dependent subjects and healthy controls. However, they did find higher glutamate and lower GABA concentrations when comparing smoking with non-smoking alcohol dependent subjects. Therefore, we included a group of cigarette smokers as well as a group of smoking polysubstance users to investigate group differences in ACC Glx and GABA concentrations. This has been shown to be a sensitive method to detect the relation between the types of substances used and microstructural changes in the brain (Kaag et al., 2016).

SUDs have also been associated with impaired impulse control and deviant ACC functioning (Garavan and Hester, 2007; Hester and Garavan, 2004; Li et al., 2008; Moeller et al., 2001). Furthermore, higher impulsivity has been positively associated with deviating glutamate concentrations in rodents (see e.g., Pattij and Vanderschuren, 2008), and this association has also been found in cocaine dependent subjects (Schmaal et al., 2012a). However, no such data are available on other SUDs and therefore the relationship between neurotransmitter concentrations and cognitive abnormalities remains unknown.

To address these issues, we compared Glx and GABA concentrations in the dorsal Anterior Cingulate Cortex (dACC) of cigarette smokers, smoking polysubstance users and healthy controls. We chose the dACC as a region of interest since it has been associated with impaired impulse inhibition in cocaine dependence (Garavan and Hester, 2007; Hester and Garavan, 2004). Furthermore, we examined the relationship of these neurotransmitter concentrations with self-reported impulsivity and the level of substance use. Our first hypothesis is that there will be group differences with regard to Glx and GABA concentrations. More specifically, we expect that each substance-using group shows higher Glx concentrations and lower GABA concentrations compared to healthy controls. Furthermore, since the use of multiple substances has been shown to be associated with larger group differences in neurotransmitter concentrations (Abé et al., 2013; Ke et al., 2004; Mason et al., 2006), our second hypothesis is that smoking polysubstance users will show larger neurotransmitter deviations from the healthy controls than smokers. In addition, neurotransmitter concentrations are hypothesized to be associated with self-reported substance use (reported as the level of substance use severity and the amount of substance use). Our fourth hypothesis is that there will be group differences with regard to self-reported impulsivity. More specifically, we expect that both smokers and smoking polysubstance users show higher impulsivity than healthy controls, and that smoking polysubstance users will show higher impulsivity compared to smokers. Finally, we hypothesize that higher self-reported impulsivity levels are associated with higher Glx and lower GABA concentrations across all three groups.

2. Materials and methods

2.1. Participants

Data (unpublished at time of submission) from two different studies were combined, hereafter referred to as Study A and Study B. Study A focused on the effects of N-acetylcysteine on dACC neurotransmitter concentrations of smokers, of which the baseline measures of smokers (S, n = 48) were included in the current analyses. Study B focused on neurocognitive measures in cocaine users, who reported the use of multiple substances and are therefore referred to as polysubstance users. Those who also smoked cigarettes were included in the current analyses (SP, n = 61). Healthy controls (HC, n = 90) who do not smoke or use any substances besides recreational alcohol use were included from both studies.

In study A, inclusion criteria were: male; 18–55 years old; smoking at least 15 cigarettes per day; a desire to quit smoking; a Fagerström Test Nicotine Dependence (FTND; Heatherton et al., 1991) score of at least 3, indicating at least a low degree of smoking dependence (Heatherton et al., 1991), and an Alcohol Use Disorder Identification (AUDIT) score lower than 13, indicating the absence of heavy alcohol use and/or an alcohol use disorder. In study B, inclusion criteria were: male; 18–50 years old; non-treatment seeking; and snorting cocaine at least once per week in the last 6 months. Healthy controls were also excluded if they reported a history of substance (ab)use or dependence including nicotine use, and in case of any psychotropic medication use.

Exclusion criteria for all participants were: major medical or neurological disease; lifetime history of psychotic or bipolar disorder; the presence of contraindication to MRI scanning; and the use of antidepressants or antipsychotics. Participants in study B were instructed not to use alcohol or cocaine ten hours before the session, but participants from both studies were allowed to smoke ad libitum before the start of the studies. In addition, participants in study B were excluded if they ever used heroin, but were not excluded on the use of other substances. All participants were recruited by means of advertisements and word of mouth. Written informed consent was acquired before the beginning of the session. Study A was approved by the Ethics Committee of the Psychology Department of the University of Amsterdam, and registered with the Netherlands Trial Registry (number: NTR3576). Study B was approved by the Ethical Review Board of the Academic Medical Centre of the University of Amsterdam, the Netherlands.

2.2. Clinical assessments

For the substance-using groups, the severity of smoking dependence was assessed using the FTND. For the SP group, the Drug Use Disorder Identification Test (DUDIT; Berman et al., 2005) was used to assess the

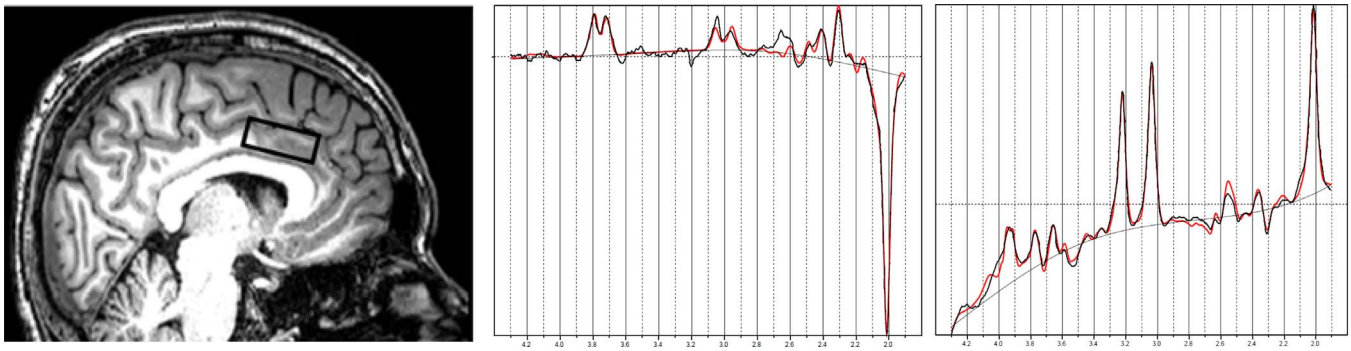

Fig. 1. Spectroscopy details. Left: placement of the MRS voxel in the dorsal Anterior Cingulate Cortex (dACC). Middle: Example spectrum for the difference acquisition from the dACC voxel. Right: Example spectrum for the even acquisition from the dACC voxel.

level of cocaine use and related problems. For all participants, the premorbid level of intellectual functioning was measured using the Dutch version of the National Adult Reading Test (NART; Schmand et al., 1991), the AUDIT (Babor et al., 1989) was used to assess the level of alcohol use and related problems, the Time Line Follow Back method (TLFB; Sobell and Sobell, 1992) was used to assess cannabis use in the preceding 6 months, and the Barratt Impulsiveness Scale (BIS-11; Patton et al., 1995) was administered to assess self-reported impulsivity.

2.3. Magnetic resonance spectroscopy acquisition and processing

Participants of study A and B were scanned on the same scanner. First, a structural three-dimensional T1-weighted volume (TR = 8.2 ms; TE = 3.7 ms; 220 slices; voxel size 1 × 1 × 1 mm; matrix size 240 × 187) was acquired in the transverse plane using a SENSE 32-channel receiver head coil on an Achieva XT 3T head-only MRI scanner (Philips Healthcare, Best, The Netherlands) at the Spinoza Centrum of the University of Amsterdam. This structural image was used to place the MRS voxel in the dACC (see Fig. 1), based on each individual's anatomical landmarks. The voxel was placed over the midline covering both hemispheres, since there was no prior expectation of differences in Glx or GABA concentrations between hemispheres. GABA-edited ^{1}H J-difference spectra were acquired using a MEGA-PRESS sequence (Waddell et al., 2007; TR = 2000 ms; TE = 73 ms; voxel size 35 mm × 20 mm × 15 mm, 384 transients). During the odd transients, a 15.64 ms sinc-center editing pulse (64 Hz full width at half maximum) was applied at 1.9 ppm and 4.6 ppm in an interleaved manner to specifically excite GABA and suppress water, respectively.

In the current study, Glx concentrations are reported as the best available proxy measure of glutamate, due to overlap in spectral assignment of glutamate, glutamine, and other components. The applied ^{1}H MRS measurements allowed us to quantify the concentration of Glx and GABA in the dACC. The even and the J-difference (odd-even) acquisitions were analyzed using LCModel (Linear Combination of Model spectra; Provencher, 1993). Total creatine (Cre + PCr) and total N-acetyl aspartate (NAA + NAAG) were quantified from the even acquisitions, and Glx, GABA, and again NAA were quantified from the difference acquisitions. Glx and GABA were normalized to the difference-spectra NAA, and the even-spectra NAA was normalized to creatine. This procedure calibrated signal amplitude across even and difference acquisitions within each subject, enabling Glx and GABA concentrations to be expressed in units of creatine (see for details Waddell et al., 2011, 2007). Cramer–Rao Lower Bounds (CRLBs) of 20% were used for each individual peak as a quality criterion (Provencher, 1993). All spectra were also visually inspected. Scans that did not meet de CRLB criterion or passed the visual inspection were excluded from analyses. CRLBs for all metabolites in all subjects were between 3 and 9%. Additional indicators for the quality of the spectra were mean (± SD) signal to noise ratio and mean (± SD) full width half maximum (FWHM). The SNR for the heavy smokers was 4.20 (0.89), for smoking polysubstance users it was 4.16(1.13), and 4.32 (1.04) for the healthy controls. The FWHM for the heavy smokers was 0.05 (0.02), for the smoking polysubstance users it was 0.06 (0.02), and 0.05 (0.02) for the healthy controls.

2.4. Statistical analyses

Data were checked for outliers and normal distribution. Non-parametric tests were used if a variable did not meet the criterion of normal distribution after log-transformation. Differences between groups were analyzed using one-way ANOVAs, followed by planned pairwise comparisons to test for possible group differences. To account for multiple testing, a Bonferroni correction of 2 was applied for the analyses on neurotransmitter concentrations (Glx and GABA). Since the results on Glx were trend significant, the post-hoc analyses were exploratory and therefore performed without correction for multiple comparisons. Eta squared is reported as an index of effect size. Bivariate correlation analyses were used to investigate associations between neurotransmitters and impulsivity. For exploratory purposes, multiple regression analyses were used to investigate, per group, associations between neurotransmitter concentrations and the level of substance use, as well as the severity of substance use disorders for alcohol, tobacco and cocaine, and cannabis used, as measured with the AUDIT, FTND, DUDIT, and TLFB, respectively. For each analysis, only the substances that were used by that particular group were entered in the regression analyses.

3. Results

3.1. Sample characteristics

After excluding participants due to unreliable MRS spectra, 30/48 (62.5%) from the S group, 38/61 (62.3%) from the SP group, and 61/90 (67.7%) from the HC group were included in the analyses. Demographic, clinical, and neurotransmitter information is displayed in Table 1. The groups were of similar age (H_2 = 3.00, p = 0.22), but differed in IQ ($F_{2,126}$ = 4.20, p = 0.017). Post-hoc analyses showed that the S group did not differ from the HC group (p = 0.981), but that the SP group had lower IQ compared to the HC group (p = 0.008) and the S group (p = 0.023). Since IQ was not a matching criterion and represents a group characteristic, IQ was not entered as a covariate in the analyses on group differences in Glx and GABA. Moreover, IQ was not correlated with Glx (p = 0.71) or GABA (p = 0.69). There was no difference between the S and SP groups with regard to nicotine dependence as measured with the FTND (t_{66} = 0.539, p = 0.59). There were significant group differences in alcohol use and related problems as measured with the AUDIT ($F_{2,126}$ = 51.87, p < 0.001). Post-hoc

Table 1
Between group differences in demographic, clinical and neurobiological outcomes.

	HC N = 61		S N = 30		SP N = 38		F[a]/H(df)[b]/U[c]	P-value	Effect size (η^2)
	Mean	SD	Mean	SD	Mean	SD			
Age	32.10	9.69	35.87	10.48	32.66	7.45	3.00(2)[b]	0.22	0.02
IQ (NART)	104.9	8.60	105.00	8.90	99.90	9.60	4.196[a]	0.017ee	0.06
Alcohol (units/week)	4.87	6.36	7.23	6.39	25.98	25.49	47.34(2)[b]	< 0.0001	0.29
AUDIT scores	5.67	3.75	6.10	2.91	13.37	4.58	51.87[a]	< 0.0001	0.45
Cigarettes/week	–	–	155.24	67.79	115.22	61.35	6.504[a]	0.013	0.09
FTND scores	–	–	6.07	1.96	5.79	2.21	0.291[a]	0.59	0.004
DUDIT scores	–	–	–	–	18.19	5.00	–	–	–
Cocaine/week (g)	–	–	–	–	2.29	1.32	–	–	–
Cannabis/week (joints)	–	–	0.09	0.19	4.73	7.19	67.00[c]	0.01	0.18
BIS-11	59.27	7.92	64.89	9.85	72.94	9.96	26.83[a]	< 0.001	0.31
Glx	0.62	0.11	0.68	0.13	0.67	0.13	2.71[a]	0.07	0.04
GABA	0.20	0.04	0.20	0.09	0.20	0.04	0.04[a]	0.96	0.001

Abbreviations: AUDIT, Alcohol Use Disorder Identification Test; BIS-11, Barratt Impulsiveness Scale version 11; DUDIT, Drug Use Disorder Identification Test; FTND, Fagerström Test for Nicotine Dependence; GABA, gamma-aminobutyric acid, referenced to creatine; Glx, composite measure of glutamate and glutamine, referenced to creatine; HC, Healthy Controls; NART, National Adult Reading Test; S, Smokers; SD, Standard Deviation; SP, Smoking Polysubstance Users.
[a] p-values represent univariate ANOVAs.
[b] p-values represent non-parametric univariate ANOVAs.
[c] p-values represent non-parametric *t*-test.

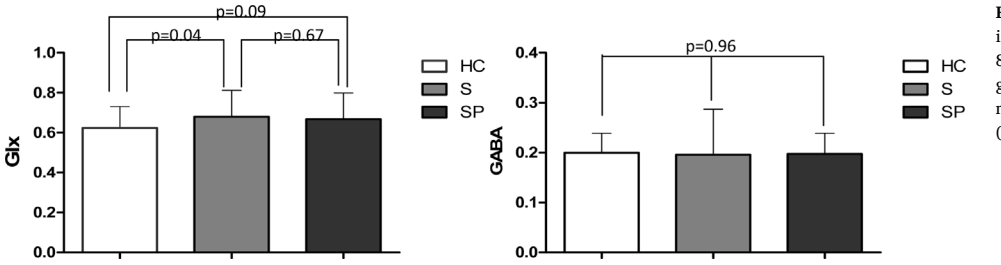

Fig. 2. Concentration of Glx (left) and GABA (right) in the dorsal Anterior Cingulate Cortex per group 85(M ± SD). There was a trend significant between group difference with regard to Glx (p = 0.07), but no between group difference with regard to GABA (p = 0.96).

analyses showed that the SP group had higher AUDIT scores compared to the HC (p < 0.001) and the S group (p < 0.001), but that there was no difference in AUDIT scores between the S and HC groups. However, AUDIT scores were not correlated with Glx (p = 0.45) or GABA (p = 0.72). Moreover, since cocaine use is highly related to alcohol use and therefore high AUDIT scores are inherently connected to cocaine use (EMCDDA, 2009; Connor et al., 2014; Tang et al., 2007), AUDIT scores were not included as a covariate in the analyses when comparing the S and SP groups to the HC group on brain Glx and GABA measures to avoid the risk of overcorrection resulting in a serious reduction of variance in Glx and GABA measures (Miller and Chapman, 2001). Also see section 'substance use and neurotransmitter levels' for the association between neurotransmitter concentrations and AUDIT scores.

3.2. Glx and GABA concentrations

To account for multiple testing, a Bonferroni correction of 2 was applied for the analyses on neurotransmitter concentrations (Glx and GABA), resulting in an adjusted alpha level of 0.025. With regard to Glx concentrations, there was a trend significant group difference ($F_{2,126}$ = 2.71, p = 0.07; see Fig. 2). Since this suggests the presence of individual group differences in Glx, planned group comparisons were performed to explore these potential differences. Since these analyses were exploratory, no correction for multiple comparisons was applied. In these post-hoc group comparisons the S group had higher dACC Glx concentrations compared to the HC group (p = 0.04; η^2 = 0.05) and the SP group tended to have higher dACC Glx concentration than the HC group (p = 0.09; η^2 = 0.03). No difference in dACC Glx concentrations was found between the S and SP groups (p = 0.67). There were no between group differences in GABA concentrations ($F_{2,105}$ = 0.041, p = 0.96; see Fig. 2).[3] Additionally, there were no between group differences with respect to total N-Acetyl Aspartate (NAA + NAAG; $F_{2,126}$ = 0.334, p = 0.72, η^2 = 0.005) and total creatine (Cre + PCr; $F_{2,126}$ = 1.456, p = 0.24, η^2 = 0.023). Also, there were no group differences in regional grey matter ($F_{2,126}$ = 1.625, p = 0.20, η^2 < 0.001) or white matter ($F_{2,126}$ = 0.849, p = 0.43, η^2 < 0.001) volume within the dACC voxel for those individuals included in the analyses on Glx differences. In addition, there were no group differences in grey matter (p = $F_{2,126}$ = 0.346, p = 0.71, η^2 < 0.001) or white matter ($F_{2,126}$ = 0.172, p = 0.84, η^2 < 0.001) for those included in the analyses on GABA differences.

3.3. Impulsivity and neurotransmitter levels

The groups were significantly different from each other with regard to BIS-11 score ($F_{2,122}$ = 26.83, p < 0.001; see Table 1). Planned comparisons showed that the HC group had the lowest BIS-11 scores, and that their score was significantly different from the S (p = 0.008) the SP group (p < 0.001). In addition, the SP group scored significantly higher on the BIS-11 than the S group (p = 0.001). However, there was no association between BIS-11 score and Glx (p = 0.85) or GABA concentrations (p = 0.34; see Fig. 3).

[3] There appeared to be a subtraction error at 3.2 ppm in a considerable proportion of the spectra (smokers: 80%, smoking polysubstance users: 69%, healthy controls: 60%), which might have led to a distortion in the quantification of GABA. However, there was no difference in proportion of subtraction error between groups ($\chi^2(2)$ = 1.553, p = 0.460).

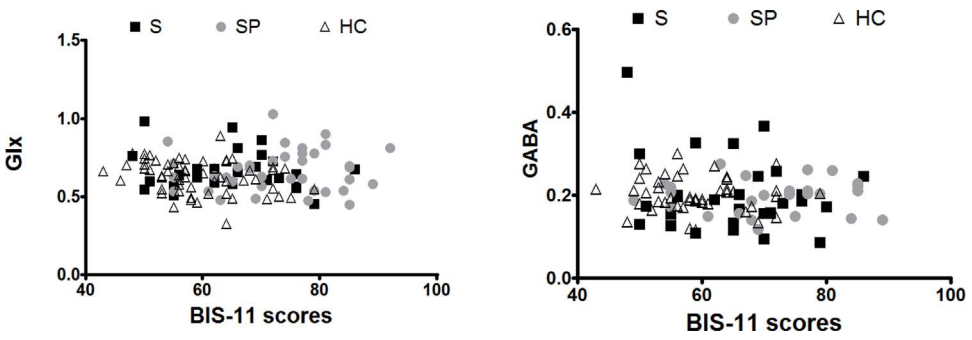

Fig. 3. Correlation between Glx (left) and GABA (right) with BIS-scores per group. There was no association between BIS-11 score and Glx ($p = 0.85$) or GABA ($p = 0.34$).

3.4. Substance use and neurotransmitter levels

Additional exploratory analyses were performed to investigate possible associations between substance use levels and substance use disorder severity with dACC neurotransmitter concentrations.

In the HC group, multiple regression did not show a significant relation between AUDIT scores and Glx ($F_{1,60} = 0.001$, $p = 0.97$) or GABA concentrations ($F_{1,47} = 0.166$, $p = 0.69$), and no significant relations between the amount of alcohol used and Glx ($F_{1,56} = 0.084$, $p = 0.77$) or GABA concentrations ($F_{1,44} = 0.303$, $p = 0.59$).

In the S group, there also was no significant relation between AUDIT and FTND scores and Glx ($F_{2,29} = 0.120$, $p = 0.89$) or GABA concentrations ($F_{2,33} = 0.203$, $p = 0.82$), and no significant relation between the amount of alcohol and the number of cigarettes and Glx ($F_{2,29} = 0.186$, $p = 0.83$) or GABA concentrations ($F_{2,33} = 0.112$, $p = 0.90$).

Finally, in the SP group there were no significant relations of AUDIT score, FTND score, DUDIT score, and cannabis use with Glx ($F_{4,36} = 0.044$, $p = 0.99$) or GABA concentrations ($F_{4,24} = 0.86$, $p = 0.51$), and no significant relations of alcohol, cigarette, cocaine and cannabis use with Glx ($F_{4,37} = 0.712$, $p = 0.59$) or GABA concentrations ($F_{4,25} = 1.021$, $p = 0.42$).

4. Discussion

This study investigated differences in dACC Glx and GABA concentrations and impulsivity between cigarette smokers, smoking polysubstance users, and healthy controls. In line with our first hypothesis, there was a trend towards group differences in dACC Glx concentrations. Furthermore, in contrast to our first hypothesis, there were no between group differences in dACC GABA concentrations. Post hoc analyses showed that both substance using groups tended to have higher Glx concentrations compared to healthy controls, but that there was no difference between both substance using groups, which refutes our second hypothesis. It should be noted that no correction for multiple comparison was applied since these comparisons were performed exploratory. In contrast to our third hypothesis, there were no significant correlations between dACC neurotransmitter concentrations and substance use severity, or the amount of substance use. In line with our fourth hypothesis, there were group differences with regard to impulsivity. Post hoc analyses showed that smokers and smoking polysubstance users had higher BIS-11 scores than healthy controls, and smoking polysubstance users showed higher scores than smokers. However, in contrast to our fifth hypothesis, there were no significant correlations between dACC neurotransmitter concentrations and self-reported impulsivity.

The trend towards increased Glx concentrations in smokers and smoking polysubstance users versus healthy controls are in line with previous research showing higher dACC glutamate concentrations (Bauer et al., 2013; Lee et al., 2007; Schmaal et al., 2012b; Thoma et al., 2011). However, no difference in dACC Glx concentrations between smokers and smoking polysubstance users were found, which contradicts previous findings of larger differences in multiple versus single substance users (Abé et al., 2013; Ke et al., 2004; Mason et al., 2006). Since the substance using groups did not differ in the level of nicotine dependence, this may indicate that alterations in Glx concentrations are possibly more related to smoking dependence than to other types of substance use. However, since there were no significant correlations between glutamate concentrations and nicotine use severity (or alcohol and cocaine use severity), higher glutamate concentrations may be associated with the presence of (underlying processes related to) substance dependence in general, unspecific to the type of substance. Other explanations could be that there is a 'threshold effect' resulting in affected glutamate concentrations after someone has used for a certain period of time of with a specific intensity of substance use, or the cardiovascular effect of the used substances that cause a circulatory compromise in the dACC. More extensive studies should be performed to further disentangle this differential effect.

As expected, we found between group differences in self-reported impulsivity with the highest impulsivity scores in smoking polysubstance users followed by smokers and the lowest in healthy controls. However, impulsivity was not significantly correlated to dACC Glx or GABA concentrations. This is not in line with previous studies showing a significant correlation between impulsivity and glutamate concentrations (Pattij and Vanderschuren, 2008; Schmaal et al., 2012b). However, the review by Pattij and Vanderschuren (2008) is based on animal studies using different indicators for impulsivity. The study of Schmaal et al. (2012b) tested in only 8 treatment seeking cocaine dependent patients with similar BIS-11 scores but much higher AUDIT scores than the cocaine users in the current study (21.3 vs. 13.4).

In contrast to previous studies (Abé et al., 2013; Durazzo et al., 2016; Ke et al., 2004; Prescot et al., 2013; Silveri et al., 2014), we did not find differences in dACC GABA concentrations between the substance using groups and healthy controls, or between smokers and smoking polysubstance users. Possible explanations include differences in methodology between the previous studies and the current study. For instance, previous studies recruited mainly non-smokers (Ke et al., 2004; Prescot et al., 2013; Silveri et al., 2014), whereas both substance using groups in the current study were smokers. Another explanation could be the duration of abstinence at the moment of testing since the aforementioned studies all tested GABA concentrations after at least one week of abstinence. In the current study, the substance using participants were allowed to smoke ad libitum until the session. However, Mon et al. (2012) also found no differences in GABA concentrations despite a period of abstinence.

Although at trend level, our findings support the literature on deviating glutamate concentrations and thus point to the potential of (anti)glutamatergic agents for the treatment of substance dependence. For instance, acamprosate (commonly used in the treatment of alcohol dependence) and N-acetylcysteine (commonly used as a mucolytic agent in cough medicine) have both been found to reduce glutamate concentrations in alcohol and cocaine dependent individuals, respectively (Schmaal et al., 2012b; Umhau et al., 2010). In addition, several studies indicate that N-acetylcysteine is effective in the treatment of

other substance use disorders (for reviews, see Deepmala et al., 2015; McClure et al., 2014). More research is needed to investigate the exact mechanisms underlying the efficacy of such glutamatergic agents.

Major strengths of this study are the relatively large samples and the direct comparison of neurotransmitter concentrations and impulsivity between smokers with or without concurrent polysubstance use and non-substance using healthy controls. Another strength of this study is the simultaneous assessment of neurobiological and behavioral measures. The current study also has limitations. First, only male participants were recruited in both studies. Although this may lead to more homogeneous groups and thereby strengthens the results of the study, this also limits the generalizability of the results to the general population. Second, as mentioned before, the subjects were recruited as part of two individual studies to answer study-specific research questions. However, all participants were recruited in the same way and scanned in the same period with the same MRI scanner. Third, there is the issue of the use of multiple substances, especially in the group of smoking polysubstance users. Even though this resembles most cocaine users in the community and in treatment (EMCDDA, 2009; Connor et al., 2014), who are suggested to be also dependent on alcohol and/or tobacco (Tang et al., 2007), it could be that different substances have a different or even opposite effect on glutamate concentrations. This may also play an important role in the treatment of polysubstance users. In addition, for cannabis only the effect of the amount of cannabis use was analyzed, due to the lack of a questionnaire assessing problematic cannabis use in the original studies. Even though our sample size was fairly large for an imaging study, even larger (possibly multi-site) studies would be needed to study the neurobiological effects of polysubstance use. Fourth, to ensure overall good quality of the MRS data some participants had to be excluded from the analyses on Glx and GABA concentrations, due to unreliable MRS spectra. However, similar proportions had to be excluded in all three groups and there was no difference in sociodemographic and clinical characteristics between those excluded and those included in the current analyses. In addition, we were unable to verify if potential head movement during scanning affected scan quality. This should be taken into account in future studies. Furthermore, the results concerning GABA should be interpreted with caution, due to the presence of a choline peak at 3.2 ppm that might have distorted the quantification of GABA. This did not influence the quantification of Glx. Fifth, even though the BIS-11 is widely used to assess impulsivity, additional measures of behavioral impulsivity may capture different aspects of impulsivity unrelated to self-reported impulsivity (Broos et al., 2012; Kräplin et al., 2014). Finally, the ^{1}H MRS sequences that we applied in the current study were not optimized to differentiate between glutamate, glutamine and glutathione because they largely overlap in their chemical shift range (Ramadan et al., 2013). Therefore, the composite measure Glx was used as a proxy measure of glutamate, but some contribution of glutamine and glutathione cannot be ruled out.

In conclusion, the current study found indications of dACC Glx differences between substance using groups and healthy controls, but not between the substance using groups. Moreover, Glx concentrations were not associated with the level of substance use and therefore the observed increase in Glx in substance users is not just a consequence of substance use itself. Furthermore, no differences in dACC GABA concentrations were found. There were group differences in self-reported impulsivity, but self-reported impulsivity was not significantly correlated with dACC Glx or GABA concentrations. More research is needed to investigate the relationship between dACC neurotransmitter concentrations and aspects of cognitive impairment other than self-reported impulsivity.

Role of funding source

Nothing declared.

Contributors

MHJS and AMK collected and analyzed the data, and wrote the first draft of the paper. RWS, LS, WvdB, LR, JRH, GAvW, and AEG were involved in the design of the studies and actively participated in writing and revising the manuscript for publication. All authors critically reviewed the manuscript for content and approve the final version for publication.

Conflict of interest

No conflict declared.

Acknowledgements

Study A was supported by a grant from the NWO (Dutch National Science Foundation), awarded to the Dutch-Flemish Research School Experimental Psychopathology (grant number 022.003.038). Study B was supported by a grant provided by ZonMW (grant number 91211002).

References

Abé, C., Mon, A., Durazzo, T.C., Pennington, D.L., Schmidt, T.P., Meyerhoff, D.J., 2013. Polysubstance and alcohol dependence: unique abnormalities of magnetic resonance-derived brain metabolite levels. Drug Alcohol Depend. 130, 30–37. http://dx.doi.org/10.1016/j.drugalcdep.2012.10.004.

Babor, T.F., Kranzler, H.R., Lauerman, R.J., 1989. Early detection of harmful alcohol consumption: comparison of clinical, laboratory, and self-report screening procedures. Addict. Behav. 14, 139–157. http://dx.doi.org/10.1016/0306-4603(89)90043-9.

Bauer, J., Pedersen, A., Scherbaum, N., Bening, J., Patschke, J., Kugel, H., Heindel, W., Arolt, V., Ohrmann, P., 2013. Craving in alcohol-dependent patients after detoxification is related to glutamatergic dysfunction in the nucleus accumbens and the anterior cingulate cortex. Neuropsychopharmacology 38, 1401–1408. http://dx.doi.org/10.1038/npp.2013.45.

Berman, A.H., Bergman, H., Palmstierna, T., Schlyter, F., 2005. Evaluation of the Drug Use Disorders Identification Test (DUDIT) in criminal justice and detoxification settings and in a Swedish population sample. Eur. Addict. Res. 11, 22–31. http://dx.doi.org/10.1159/000081413.

Berridge, K.C., Robinson, T.E., 1998. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res. Rev. 28, 309–369. http://dx.doi.org/10.1016/S0165-0173(98)00019-8.

Brand, M., Young, K.S., Laier, C., 2014. Prefrontal control and internet addiction: a theoretical model and review of neuropsychological and neuroimaging findings. Front. Hum. Neurosci. 8, 1–13. http://dx.doi.org/10.3389/fnhum.2014.00375.

Broos, N., Schmaal, L., Wiskerke, J., Kostelijk, L., Lam, T., Stoop, N., Weierink, L., Ham, J., de Geus, E.J.C., Schoffelmeer, A.N.M., van den Brink, W., Veltman, D.J., de Vries, T.J., Pattij, T., Goudriaan, A.E., 2012. The relationship between impulsive choice and impulsive action: a cross-species translational study. PLoS One 7, 1–9. http://dx.doi.org/10.1371/journal.pone.0036781.

Chang, L., Ernst, T., Strickland, T., Mehringer, C.M., 1999. Gender effects on persistent cerebral metabolite changes in the frontal lobes of abstinent cocaine users. Am. J. Psychiatry 156, 716–722.

Connor, J.P., Gullo, M.J., White, A., Kelly, A.B., 2014. Polysubstance use: diagnostic challenges, patterns of use and health. Curr. Opin. Psychiatry 27, 269–275. http://dx.doi.org/10.1097/YCO.0000000000000069.

Deepmala, Slattery, J., Kumar, N., Delhey, L., Berk, M., Dean, O., Spielholz, C., Frye, R., 2015. Clinical trials of N-acetylcysteine in psychiatry and neurology: a systematic review. Neurosci. Biobehav. Rev. 55, 294–321. http://dx.doi.org/10.1016/j.neubiorev.2015.04.015.

Durazzo, T.C., Meyerhoff, D.J., Mon, A., Abé, C., Gazdzinski, S., Murray, D.E., 2016. Chronic cigarette smoking in healthy middle-aged individuals is associated with decreased regional brain N-acetylaspartate and glutamate levels. Biol. Psychiatry 79, 481–488. http://dx.doi.org/10.1016/j.biopsych.2015.03.029.

Ende, G., Hermann, D., Demirakca, T., Hoerst, M., Tunc-Skarka, N., Weber-Fahr, W., Wichert, S., Rabinstein, J., Frischknecht, U., Mann, K., Vollstädt-Klein, S., 2013. Loss of control of alcohol use and severity of alcohol dependence in non-treatment-seeking heavy drinkers are related to lower glutamate in frontal white matter. Alcohol. Clin. Exp. Res. 37, 1643–1649. http://dx.doi.org/10.1111/acer.12149.

European Monitoring Centre for Drugs and Drug Addiction. Polydrug use: Patterns and Responses (2009). Available at http://www.emcdda.europa.eu.

Gallinat, J., Schubert, F., 2007. Regional cerebral glutamate concentrations and chronic tobacco consumption. Pharmacopsychiatry 40, 64–67. http://dx.doi.org/10.1055/s-2007-970144.

Garavan, H., Hester, R., 2007. The role of cognitive control in cocaine dependence. Neuropsychol. Rev. 17, 337–345. http://dx.doi.org/10.1007/s11065-007-9034-x.

Heatherton, T.F., Kozlowski, L.T., Frecker, R.C., Fagerstrom, K., 1991. The fagerstrom test for nicotine dependence: a revision of the fagerstrom tolerance questionnaire.

Addiction 86, 1119–1127. http://dx.doi.org/10.1111/j.1360-0443.1991.tb01879.x.
Hermann, D., Frischknecht, U., Heinrich, M., Hoerst, M., Vollmert, C., Vollst??dt-Klein, S., Tunc-Skarka, N., Kiefer, F., Mann, K., Ende, G., 2012. MR spectroscopy in opiate maintenance therapy: association of glutamate with the number of previous withdrawals in the anterior cingulate cortex. Addict. Biol. 17, 659–667. http://dx.doi.org/10.1111/j.1369-1600.2010.00290.x.
Hester, R., Garavan, H., 2004. Executive dysfunction in cocaine addiction: evidence for discordant frontal, cingulate, and cerebellar activity. J. Neurosci. 24, 11017–11022. http://dx.doi.org/10.1523/JNEUROSCI.3321-04.2004.
Kaag, A.M., Wingen, G.A., Caan, Van, Homberg, M.W.A., Brink, J.R., Van Den, W., Reneman, L., 2016. White matter alterations in cocaine users are negatively related to the number of additionally (ab) used substances. Addict. Biol. 22 (4), 1048–1056. http://dx.doi.org/10.1111/adb.12375.
Ke, Y., Streeter, C.C., Nassar, L.E., Sarid-Segal, O., Hennen, J., Yurgelun-Todd, D.A., Awad, L.A., Rendall, M.J., Gruber, S.A., Nason, A., Mudrick, M.J., Blank, S.R., Meyer, A.A., Knapp, C., Ciraulo, D.A., Renshaw, P.F., 2004. Frontal lobe GABA levels in cocaine dependence: a two-dimensional, J-resolved magnetic resonance spectroscopy study. Psychiatry Res. Neuroimaging 130, 283–293. http://dx.doi.org/10.1016/j.pscychresns.2003.12.001.
Kräplin, A., Bühringer, G., Oosterlaan, J., Van den Brink, W., Goschke, T., Goudriaan, A.E., 2014. Dimensions and disorder specificity of impulsivity in pathological gambling. Addict. Behav. 39, 1646–1651. http://dx.doi.org/10.1016/j.addbeh.2014.05.021.
Lee, E., Jang, D., Kim, J., An, S.K., Park, S., Kim, I., Kim, S.I., Yoon, K., Namkoong, K., 2007. Alteration of brain metabolites in young alcoholics without structural changes. Neuroreport 18, 1511–1514. http://dx.doi.org/10.1097/WNR.0b013e3282ef7625.
Li, C.R., Huang, C., Yan, P., Bhagwagar, Z., Milivojevic, V., Sinha, R., 2008. Neural correlates of impulse control during stop signal inhibition in cocaine-dependent men. Neuropsychopharmacology 33, 1798–1806. http://dx.doi.org/10.1038/sj.npp.1301568.
Li, X., Semenova, S., D'Souza, M.S., Stoker, A.K., Markou, A., 2013. Involvement of glutamatergic and GABAergic systems in nicotine dependence: implications for novel pharmacotherapies for smoking cessation. Neuropharmacology 76, 554–565. http://dx.doi.org/10.1016/j.neuropharm.2013.05.042.
Mashhoon, Y., Janes, A.C., Jensen, J.E., Prescot, A.P., Pachas, G., Renshaw, P.F., Fava, M., Evins, A.E., Kaufman, M.J., 2011. Anterior cingulate proton spectroscopy glutamate levels differ as a function of smoking cessation outcome. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1709–1713. http://dx.doi.org/10.1016/j.pnpbp.2011.05.006.Anterior.
Mason, G.F., Petrakis, I.L., de Graaf, R.A., Gueorguieva, R., Guidone, E., Coric, V., Epperson, C.N., Rothman, D.L., Krystal, J.H., 2006. Cortical gamma-aminobutyric acid levels and the recovery from ethanol dependence: preliminary evidence of modification by cigarette smoking. Biol. Psychiatry 59, 85–93. http://dx.doi.org/10.1016/j.biopsych.2005.06.009.
McClure, E.A., Gipson, C.D., Malcolm, R.J., Kalivas, P.W., Gray, K.M., 2014. Potential role of N-acetylcysteine in the management of substance use disorders. CNS Drugs 28, 95–106. http://dx.doi.org/10.1007/s40263-014-0142-x.
Miller, G.A., Chapman, J.P., 2001. Misunderstanding analysis of covariance. J. Abnorm. Psychol. 110, 40–48. http://dx.doi.org/10.1037/0021-843X.110.1.40.
Moeller, F.G., Dougherty, D.M., Barratt, E.S., Schmitz, J.M., Swann, A.C., Grabowski, J., 2001. The impact of impulsivity on cocaine use and retention in treatment. J. Subst. Abuse Treat. 21, 193–198.
Mon, A., Durazzo, T.C., Meyerhoff, D.J., 2012. Glutamate, GABA, and other cortical metabolite concentrations during early abstinence from alcohol and their associations with neurocognitive changes. Drug Alcohol Depend. 125, 27–36. http://dx.doi.org/10.1016/j.drugalcdep.2012.03.012.
Myers, K.M., Carlezon, W.A., 2010. Extinction of drug- and withdrawal-paired cues in animal models: relevance to the treatment of addiction. Neurosci. Biobehav. Rev. 35, 285–302. http://dx.doi.org/10.1016/j.neubiorev.2010.01.011.
Nutt, D.J., Lingford-Hughes, A., Erritzoe, D., Stokes, P.R.A., 2015. The dopamine theory of addiction: 40 years of highs and lows. Nat. Rev. Neurosci. 16, 305–312. http://dx.doi.org/10.1038/nrn3939.
Pattij, T., Vanderschuren, L.J.M.J., 2008. The neuropharmacology of impulsive behaviour. Trends Pharmacol. Sci. 29, 192–199. http://dx.doi.org/10.1016/j.tips.2008.01.002.
Patton, J.H., Stanford, M.S., Barrat, E.S., 1995. Factor structure of the barrat impulsiveness scale. J. Clin. Psychol. 51, 768–774. http://dx.doi.org/10.1177/017084068800900203.
Prescot, A.P., Renshaw, P.F., Yurgelun-Todd, D.A., 2013. γ-Amino butyric acid and glutamate abnormalities in adolescent chronic marijuana smokers. Drug Alcohol Depend. 129, 232–239. http://dx.doi.org/10.1016/j.drugalcdep.2013.02.028.
Provencher, S.W., 1993. Estimation of metabolite concentrations from localizedin vivo proton NMR spectra. Magn. Reson. Med. 30, 672–679. http://dx.doi.org/10.1002/mrm.1910300604.
Ramadan, S., Lin, A., Stanwell, P., 2013. Glutamate and glutamine: a review of in vivo MRS in the human brain. NMR Biomed. 26, 1630–1646. http://dx.doi.org/10.1002/nbm.3045.
Schmaal, L., Goudriaan, A.E., van der Meer, J., van den Brink, W., Veltman, D.J., 2012a. The association between cingulate cortex glutamate concentration and delay discounting is mediated by resting state functional connectivity. Brain Behav. 2, 553–562. http://dx.doi.org/10.1002/brb3.74.
Schmaal, L., Veltman, D.J., Nederveen, A., van den Brink, W., Goudriaan, A.E., 2012b. N-acetylcysteine normalizes glutamate levels in cocaine-dependent patients: a randomized crossover magnetic resonance spectroscopy study. Neuropsychopharmacology 37, 2143–2152. http://dx.doi.org/10.1038/npp.2012.66.
Schmand, B., Bakker, D., Saan, R., Louman, J., 1991. The dutch reading test for adults: a measure of premorbid intelligence level. Tijdschr. Gerontol. Geriatr. 22, 15–19.
Silveri, M.M., Cohen-Gilbert, J., Crowley, D.J., Rosso, I.M., Jensen, J.E., Sneider, J.T., 2014. Altered anterior cingulate neurochemistry in emerging adult binge drinkers with a history of alcohol-induced blackouts. Alcohol. Clin. Exp. Res. 38, 969–979. http://dx.doi.org/10.1111/acer.12346.
Sobell, L.C., Sobell, M.B., 1992. Timeline follow-back. A technique for assessing self-reported alcohol consumption, Measuring Alcohol Consumption. Psychosocial and Biochemical Methods. Springer.
Tang, Y., Kranzler, H.R., Gelernter, J., Farrer, L.A., Cubells, J.F., 2007. Comorbid psychiatric diagnoses and their association with cocaine-induced psychosis in cocaine-dependent subjects. Am. J. Addict. 16, 343–351. http://dx.doi.org/10.1080/10550490701525723.
Thoma, R., Mullins, P., Ruhl, D., Monnig, M., Yeo, R.A., Caprihan, A., Bogenschutz, M., Lysne, P., Tonigan, S., Kalyanam, R., Gasparovic, C., 2011. Perturbation of the glutamate-glutamine system in alcohol dependence and remission. Neuropsychopharmacology 36, 1359–1365. http://dx.doi.org/10.1038/npp.2011.20.
Umhau, J.C., Momenan, R., Schwandt, M.L., Singley, E., Lifshitz, M., Doty, L., Adams, L.J., Vengeliene, V., Spanagel, R., Zhang, Y., Shen, J., George, D.T., Hommer, D., Heilig, M., 2010. Effect of acamprosate on magnetic resonance spectroscopy measures of central glutamate in detoxified alcohol-dependent individuals: a randomized controlled experimental medicine study. Arch. Gen. Psychiatry 67, 1069–1077. http://dx.doi.org/10.1001/archgenpsychiatry.2010.125.
Waddell, K.W., Avison, M.J., Joers, J.M., Gore, J.C., 2007. A practical guide to robust detection of GABA in human brain by J-difference spectroscopy at 3 T using a standard volume coil. Magn. Reson. Imaging 25, 1032–1038. http://dx.doi.org/10.1016/j.mri.2006.11.026.
Waddell, K.W., Zanjanipour, P., Pradhan, S., Xu, L., Welch, E.B., Joers, J.M., Martin, P.R., Avison, M.J., Gore, J.C., 2011. Anterior cingulate and cerebellar GABA and Glu correlations measured by ^{1}H J-difference spectroscopy. Magn. Reson. Imaging 29, 19–24. http://dx.doi.org/10.1016/j.mri.2010.07.005.
Yücel, M., Lubman, D.I., Harrison, B.J., Fornito, A., Allen, N.B., Wellard, R.M., Roffel, K., Clarke, K., Wood, S.J., Forman, S.D., Pantelis, C., 2007. A combined spectroscopic and functional MRI investigation of the dorsal anterior cingulate region in opiate addiction. Mol.
Yang, S., Salmeron, B.J., Ross, T.J., Xi, Z., Stein, E.A., Yang, Y., 2009. Lower glutamate levels in rostral anterior cingulate of chronic cocaine users − A (1)H-MRS study using TE-averaged PRESS at 3 T with an optimized quantification strategy. Psychiatry Res. 174, 171–176. http://dx.doi.org/10.1016/j.pscychresns.2009.05.004.