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Polysubstance and alcohol dependence: Unique abnormalities of magnetic resonance-derived brain metabolite levels

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ABSTRACT

Background: Although comorbid substance misuse is common in alcohol dependence, and polysubstance abusers (PSU) represent the largest group of individuals seeking treatment for drug abuse today, we know little about potential brain abnormalities in this population. Brain magnetic resonance spectroscopy studies of mono-substance use disorders (e.g., alcohol or cocaine) reveal abnormal levels of cortical metabolites (reflecting neuronal integrity, cell membrane turnover/synthesis, cellular bioenergetics, gliosis) and altered concentrations of glutamate and γ -aminobutyric acid (GABA). The concurrent misuse of several substances may have unique and different effects on brain biology and function compared to any mono-substance misuse.

Methods: High field brain magnetic resonance spectroscopy at 4 T and neurocognitive testing were performed at one month of abstinence in 40 alcohol dependent individuals (ALC), 28 alcohol dependent PSU and 16 drug-free controls. Absolute metabolite concentrations were calculated in anterior cingulate (ACC), parieto-occipital (POC) and dorso-lateral prefrontal cortices (DLPFC).

Results: Compared to ALC, PSU demonstrated significant metabolic abnormalities in the DLPFC and strong trends to lower GABA in the ACC. Metabolite levels in ALC and light drinking controls were statistically equivalent. Within PSU, lower DLPFC GABA levels are related to greater cocaine consumption. Several cortical metabolite concentrations were associated with cognitive performance.

Conclusions: While metabolite concentrations in ALC at one month of abstinence were largely normal, PSU showed persistent and functionally significant metabolic abnormalities, primarily in the DLPFC. Our results point to specific metabolic deficits as biomarkers in polysubstance misuse and as targets for pharmacological and behavioral PSU-specific treatment.

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1. Introduction

Magnetic resonance imaging (MRI) and proton MR spectroscopy (1H MRS) are invaluable tools in addiction research, as they permit non-invasive interrogation of the integrity of multiple aspects of neurobiology. MRS studies that investigate the neurobiological effects of cocaine, amphetamines, or marijuana (i.e., mono-substance dependence) have revealed abnormal levels of markers of neuronal integrity (N-acetylaspartate, NAA), cell membrane turnover/synthesis (choline-containing metabolites, Cho), cellular bioenergetics (Creatine, Cr), astroglia (myo-Inositol, mI) and alterations of glutamate (Glu) and

γ -aminobutyric acid (GABA), which are the primary excitatory and inhibitory neurotransmitter/neuromodulators in the human brain (for review see [Licata and Renshaw, 2010](#)). Alterations in brain metabolite concentrations have also been observed in alcohol dependent individuals (ALC), primarily in the frontal lobes ([Buhler and Mann, 2011](#); [Durazzo and Meyerhoff, 2007](#); [Mon et al., 2012](#); [Sullivan et al., 2000](#)). We showed recently that concentrations of Glu, NAA and Cr in the anterior cingulate cortex (ACC) of nine-days-abstinent ALC were significantly lower than in healthy controls; mI and γ -aminobutyric acid (GABA) in ACC as well as the other metabolite levels in the dorsolateral prefrontal cortex (DLPFC) and parieto-occipital cortex (POC) were normal ([Mon et al., 2012](#)). Over 30 days of abstinence from alcohol, the ACC metabolite concentrations largely normalized ([Mon et al., 2012](#)). Furthermore, mono-substance abuse/dependence is associated with neurocognitive dysfunction ([Abi-Saab et al., 2005](#); [Di Sclafani et al., 2002](#); [Hester and Garavan, 2004](#); [Lundqvist, 2005](#); [Moeller et al., 2005](#);

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Oscar-Berman, 2000; Salo et al., 2002; Simon et al., 2000; Verdejo-Garcia et al., 2011; Volkow et al., 2001), and improvements in functions such as learning, processing speed and working memory have been shown to relate to metabolic changes during abstinence (Meyerhoff et al., 2011).

Alcohol use disorders (AUD) are often accompanied by comorbid misuse of illicit drugs, such as cocaine and methamphetamine (Stinson et al., 2005), and their misuse, individual or combined, is associated with significant neurobiological, neurocognitive and psychiatric abnormalities (Licata and Renshaw, 2010). Individuals with concurrent abuse/dependence on more than one substance including alcohol (i.e., polysubstance abusers or PSU) represent the largest group of individuals seeking treatment for substance use disorders in the United States today (Kedia et al., 2007; Medina et al., 2004). Furthermore, more than 50% of patients treated for drug abuse relapse within one year of treatment (McLellan et al., 2000). Identification of the unique and common neurobiological abnormalities and related neurocognitive characteristics in PSU and ALC will facilitate the development of more efficacious pharmacological and behavioral interventions for these groups. Thus, measuring potentially unique neurobiological abnormalities and related neurocognitive characteristics in treatment seeking PSU and ALC is thought to be of high clinical importance.

Each class of substances alters neuronal integrity and neurotransmission via different mechanisms (Buttner, 2011; Licata and Renshaw, 2010). Their combined abuse may have unique, even different adverse effects on the brain than any mono-substance abuse. While there are numerous neuroimaging studies on effects of individual substances on brain biology, few studies have investigated drug effects in PSU. These can be summarized as follows: two-week-abstinent PSU had prefrontal gray matter (GM) atrophy (Liu et al., 1998). Cocaine use disordered ALC tended to have greater age-related white matter (WM) volume decreases (Bjork et al., 2003) and persistently lower NAA concentrations in the DLPFC than ALC (Meyerhoff et al., 1999). During early withdrawal, PSU demonstrated cerebral phosphorus metabolite alterations (Christensen et al., 1996). Actively using PSU had higher glucose metabolism rates in frontotemporal cortex than drug-free controls (Stapleton et al., 1995); those rates, however, were lower in the right orbitofrontal cortex of one-week-abstinent individuals abusing both methamphetamine and marijuana compared to “pure” methamphetamine abusers (Voytek et al., 2005). Non-abstinent cocaine dependent ALC showed lower frontal GABA levels than controls (Ke et al., 2004). Abstinent ALC with concurrent cocaine dependence had less WM in prefrontal brain regions than those dependent on only one substance (O'Neill et al., 2001). Consistent with these neurobiological abnormalities, studies in PSU have also indicated impaired cognition compared to controls (Di Sclafani et al., 2002; Ersche et al., 2011; Horner, 1997; Selby and Azrin, 1998; Verdejo-Garcia et al., 2004, 2007). These few reports illustrate that neurobiological correlates of polysubstance use disorders, as well as their associations with neurocognition, are complex and still unclear.

To better understand metabolic alterations in a well-characterized cohort of abstinent PSU, the specific goals of this study were to measure metabolite concentration differences between abstinent PSU and ALC using high-field MRS in brain regions with relevance to the development and maintenance of substance use disorders, and to measure potentially associated neurocognitive (dys)function. Based on the cited literature, we hypothesized unique and functionally significant regional metabolite concentration differences between one-month-abstinent PSU and ALC as well as light drinking controls (LD), and specifically lower cortical NAA (in the DLPFC) and GABA levels in PSU compared to both ALC and LD.

2. Materials and methods

2.1. Participants

All participants provided written informed consent prior to study according to the Declaration of Helsinki and underwent procedures approved by the University of California, San Francisco and the San Francisco VA Medical Center. Twenty eight treatment seeking PSU and 40 ALC were recruited from substance abuse treatment programs of the VA and Kaiser Permanente. All ALC and PSU participants met DSM-IV criteria for alcohol dependence. In addition, PSU participants met DSM-IV criteria for dependence on at least one psychostimulant, with and without marijuana use disorder: cocaine ($n=18$), methamphetamine ($n=4$), cocaine and methamphetamine ($n=4$); 2 PSU were dependent on other substances (opiates, marijuana, and/or ecstasy). Group demographics and relevant substance use characteristics are given in Table 1.

At study date, ALC and PSU were abstinent from alcohol and other substances, except nicotine, for approximately one month. Further inclusion and exclusion criteria are fully detailed elsewhere (Durazzo et al., 2004). Participants were excluded for neurological or psychiatric disorders known to affect neurobiology or neurocognition. Hepatitis C, type-2 diabetes, hypertension, and unipolar mood disorders were permitted given their high prevalence in substance use disorders (Hasin et al., 2007; Mertens et al., 2003, 2005; Parekh and Klag, 2001; Stinson et al., 2005). Sixteen light drinking controls (LD) without history of biomedical and/or psychiatric conditions known to influence the measures obtained in this study were recruited from the local community. Data from 85% of ALC and 94% of LD participants contributed to previous analyses (Mon et al., 2012).

2.2. Clinical assessment

ALC and PSU participants completed the Structured Clinical Interview for DSM-IV Axis I Disorder Patient Edition, Version 2.0 (First et al., 1998), and LD participants were administered the accompanying screening module. Within one day of the MR study, all participants filled out questionnaires that assessed depression (Beck Depression Inventory; Beck, 1978) and anxiety symptomatology (State-Trait Anxiety Inventory, Y-2; Spielberger et al., 1977). Alcohol consumption was assessed with the lifetime drinking history semi-structured interview (Skinner and Sheu, 1982; Sobell and Sobell, 1990; Sobell et al., 1988), which yielded estimates of the average number of alcoholic drinks consumed per month over 1 year and 3 years, before enrollment and over lifetime. For PSU, lifetime substance use history (other than alcohol) was assessed with an in-house interview questionnaire based on the Addiction Severity Index (McLellan et al., 1992), NIDA Addictive Drug Survey (Smith, 1991), drinking history, and Axis I disorders Patient Edition, Version 2.0 (SCID-I/P; First et al., 1998). This instrument gathers information relevant to phases of drug use for each substance a participant has a current or past disorder diagnosis on; including age of first and last use, number of total lifetime phases, duration of individual and total lifetime phases (including phases of abstinence), frequency and quantity of use during each phase, and route of administration. It includes conversion of money spent per day to one metric, using catchment area-specific conversion norms. Thus, monthly averages for grams of cocaine and/or methamphetamine over 1 year prior to enrollment and over lifetime were estimated. Level of nicotine dependence was assessed via the Fagerstrom Tolerance Test for Nicotine Dependence (Fagerstrom et al., 1991), and total numbers of years of smoking and average daily cigarettes currently smoked were recorded. To evaluate basic nutritional and erythrocyte status and hepatocellular injury, we obtained laboratory tests for serum

Table 1
Demographics, laboratory and substance consumption variables for ALC, PSU and LD (mean $\pm$ standard deviation). NS: not significant ($p > 0.05$). FTND: Fagerstrom Tolerance Test for Nicotine Dependence; BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: γ -glutamyltransferase; WBC: white blood cell counts; RBC: red blood cell counts.

Variable	LD	ALC	PSU	$p(\text{LD-ALC})$	$p(\text{LD-PSU})$	$p(\text{ALC-PSU})$
<i>n</i> (male, female)	16 (15, 1)	40 (37, 3)	28 (26, 2)	–	–	–
Age [years]	49.0 $\pm$ 10.1	52.1 $\pm$ 9.1	45.3 $\pm$ 9.6	NS	NS	0.004
Education [years]	15.4 $\pm$ 2.7	13.6 $\pm$ 1.8	12.8 $\pm$ 1.4	0.001	0.000	NS
Smoker, <i>n</i>	6	26	18	–	–	–
NART	116.4 $\pm$ 8.0	114.8 $\pm$ 8.3	106.4 $\pm$ 9.2	NS	0.001	0.000
Onset heavy drinking [age]	–	25.0 $\pm$ 9.1	20.7 $\pm$ 7.8	–	–	NS
Months heavy drinking	–	269 $\pm$ 119	238 $\pm$ 132	–	–	NS
Sober days (alcohol)	–	32.7 $\pm$ 7.5	28.6 $\pm$ 11.8	–	–	NS
Sober days (any drug)	–	32.7 $\pm$ 7.5	27.0 $\pm$ 11.9	–	–	0.021
1 year avg. (alcohol) [drinks/month]	22 $\pm$ 24	377 $\pm$ 258	302 $\pm$ 326	0.000	0.001	NS
3 years avg. (alcohol) [drinks/month]	22 $\pm$ 24	340 $\pm$ 206	300 $\pm$ 318	0.000	0.000	NS
Life time avg. (alcohol) [drinks/month]	23 $\pm$ 17	211 $\pm$ 109	261 $\pm$ 261	0.000	0.000	NS
1 year avg. (cocaine) [g/month]	–	–	82.2 $\pm$ 106.2	–	–	–
Life time avg. (cocaine) [g/month]	–	–	75.2 $\pm$ 96.2	–	–	–
1 year avg. (methamphetamine) [g/month]	–	–	21.2 $\pm$ 29.1	–	–	–
Life time avg. (methamphetamine) [g/month]	–	–	14.1 $\pm$ 13.6	–	–	–
FTND total (smokers)	5.7 $\pm$ 2.4	4.7 $\pm$ 1.7	4.0 $\pm$ 1.6	NS	0.049	NS
Cigarettes/day	21.2 $\pm$ 9.7	16.8 $\pm$ 7.9	10.0 $\pm$ 7.3	NS	0.004	0.005
BMI	25.5 $\pm$ 1.1	26.3 $\pm$ 0.7	27.9 $\pm$ 0.9	NS	0.085	NS
Prealbumin [mg/dl]	29.2 $\pm$ 5.8	25.8 $\pm$ 5.7	30.2 $\pm$ 7.0	NS	NS	0.009
GGT [U/l]	42.9 $\pm$ 78.5	72.1 $\pm$ 117.4	39.9 $\pm$ 71.1	NS	NS	NS
Albumin [mg/dl]	4.2 $\pm$ 0.3	4.1 $\pm$ 0.3	4.2 $\pm$ 0.2	NS	NS	NS
AST [U/l]	49.2 $\pm$ 82.1	41.2 $\pm$ 52.4	39.9 $\pm$ 60.9	NS	NS	NS
ALT [U/l]	51.0 $\pm$ 93.2	44.2 $\pm$ 67.6	41.0 $\pm$ 51.1	NS	NS	NS
WBC [K/cmm]	6.1 $\pm$ 1.6	7.2 $\pm$ 2.1	6.2 $\pm$ 1.7	0.10	NS	0.039
RBC [M/cmm]	4.7 $\pm$ 0.4	4.5 $\pm$ 0.5	4.6 $\pm$ 0.4	NS	NS	NS
Hemoglobin [g/dl]	15.0 $\pm$ 1.0	14.4 $\pm$ 1.3	14.4 $\pm$ 1.0	NS	NS	NS
Hematocrit [%]	43.3 $\pm$ 3.3	42.0 $\pm$ 3.9	42.6 $\pm$ 3.1	NS	NS	NS
MCV [fl]	92.0 $\pm$ 4.1	93.7 $\pm$ 4.5	91.8 $\pm$ 4.9	NS	NS	NS
BDI	5.8 $\pm$ 2.2	12.2 $\pm$ 1.4	11.7 $\pm$ 1.5	0.017	0.031	NS
State t anxiety y2	35.0 $\pm$ 3.0	44.3 $\pm$ 1.8	43.0 $\pm$ 2.0	0.010	0.030	NS
State t anxiety y1	26.3 $\pm$ 2.8	33.9 $\pm$ 1.6	34.2 $\pm$ 1.8	0.020	0.019	NS

albumin, pre-albumin, alanine aminotransferase, aspartate aminotransferase and γ -glutamyltransferase, white and red blood cell counts, hemoglobin, and hematocrit.

2.3. Neurocognitive assessment

Within 3 days of the MR study, all participants completed a neurocognitive battery focusing on working memory (Wechsler Adult Intelligence Scale-III (WAIS-III) Digit Span), processing speed (WAIS-III Digit Symbol Coding, Symbol Search; [Wechsler, 1997](#)), visuospatial learning and memory (Brief Visual memory Test-Revised, BVMT-R; [Benedict, 1997](#)), as well as auditory-verbal learning and memory (California Verbal Learning Test-II, CVLT-II: immediate recall trials 1–5 (learning), average of short and long delay free recall, and average of short and long delay cued recall (memory); [Delis et al., 2000](#)). (For details see [Durazzo et al., 2007](#).) Premorbid verbal intelligence was assessed using the American National Adult Reading Test (NART; [Grober and Sliwinski, 1991](#)). Group comparisons on these measures will be detailed in a separate report.

2.4. MR acquisition and processing

MR data were acquired on a 4T Bruker MedSpec system with a Siemens Trio console (Siemens, Erlangen, Germany) using an 8-channel transmit-receive head coil. 3D sagittal T1-weighted and 2D axial T2-weighted images were acquired using Magnetization Prepared Rapid Gradient imaging (1 mm $\times$ 1 mm $\times$ 1 mm resolution) and turbo spin-echo (0.9 mm $\times$ 0.9 mm $\times$ 3 mm resolution) sequences respectively. Volumes-of-interest (VOIs) for MRS were placed over the ACC (35 mm $\times$ 25 mm $\times$ 20 mm), POC (20 mm $\times$ 40 mm $\times$ 20 mm) and right DLPFC (40 mm $\times$ 20 mm $\times$ 20 mm) ([Fig. 1](#)), maximizing GM

Fig. 1. Representative VOI placements (left: ACC; center: DLPFC; right: POC), STEAM and J-edited GABA spectra obtained from PSU (note: intensity scale differs between spectra). Spectra shown as fitted (after DC-correction and apodization). Glx: glutamate + glutamine.

Table 2

DLPFC, ACC and POC metabolite concentrations (mean $\pm$ standard deviation) in institutional units [i.u.]. NS: Not significant; *p*-values that are significant after Bonferroni adjustment are bolded. Bonferroni adjusted alpha levels: DLPFC: *p* = 0.022, ACC: *p* = 0.017, POC: *p* = 0.020.

Region	Metabolite	<i>p</i> -value			Metabolite concentration [i.u.]		
		LD-ALC	LD-PSU	ALC-PSU	LD	ALC	PSU
DLPFC	NAA	NS	0.018	0.002	5.10 $\pm$ 0.84	5.12 $\pm$ 0.84	4.41 $\pm$ 0.84
	Cr	NS	0.053	0.006	4.28 $\pm$ 0.71	4.34 $\pm$ 0.70	3.80 $\pm$ 0.72
	Cho	NS	0.056	0.008	1.06 $\pm$ 0.17	1.07 $\pm$ 0.17	0.95 $\pm$ 0.17
	Glu	NS	NS	NS	3.06 $\pm$ 0.69	2.90 $\pm$ 0.69	2.80 $\pm$ 0.69
	mI	NS	0.029	0.010	3.60 $\pm$ 0.81	3.56 $\pm$ 0.81	2.99 $\pm$ 0.81
	GABA	NS	NS	NS	2.10 $\pm$ 0.61	2.09 $\pm$ 0.61	1.86 $\pm$ 0.61
ACC	NAA	NS	NS	NS	5.49 $\pm$ 1.02	4.92 $\pm$ 1.02	4.96 $\pm$ 1.02
	Cr	NS	NS	NS	4.60 $\pm$ 0.97	3.99 $\pm$ 0.97	4.05 $\pm$ 0.97
	Cho	NS	NS	NS	1.31 $\pm$ 0.33	1.25 $\pm$ 0.33	1.17 $\pm$ 0.34
	Glu	NS	NS	NS	4.03 $\pm$ 0.94	3.73 $\pm$ 0.95	3.44 $\pm$ 0.99
	mI	NS	NS	NS	4.24 $\pm$ 1.01	4.15 $\pm$ 1.02	4.28 $\pm$ 1.04
	GABA	NS	0.095	0.028	1.32 $\pm$ 0.39	1.36 $\pm$ 0.40	1.09 $\pm$ 0.35
POC	NAA	NS	NS	NS	5.11 $\pm$ 0.88	5.44 $\pm$ 0.88	5.10 $\pm$ 0.88
	Cr	NS	NS	NS	4.24 $\pm$ 0.73	4.50 $\pm$ 0.74	4.27 $\pm$ 0.75
	Cho	0.058	NS	0.029	0.73 $\pm$ 0.19	0.85 $\pm$ 0.20	0.74 $\pm$ 0.19
	Glu	NS	NS	NS	3.89 $\pm$ 0.72	4.02 $\pm$ 0.72	3.74 $\pm$ 0.72
	mI	NS	NS	NS	3.30 $\pm$ 0.74	3.25 $\pm$ 0.74	3.06 $\pm$ 0.74
	GABA	NS	NS	NS	1.42 $\pm$ 0.47	1.53 $\pm$ 0.47	1.47 $\pm$ 0.47

content as displayed on the structural MR images. NAA, Cr, Cho, mI and Glu signals were acquired with a Stimulated Echo Acquisition Mode (STEAM) sequence (Frahm et al., 1987). GABA signal was acquired from the same VOIs with a modified J-editing sequence (MEGA PRESS; Kaiser et al., 2008). MR images were segmented into GM, WM and cerebrospinal fluid (CSF; Van Leemput et al., 1999) to estimate tissue fraction and CSF contributions to each VOI. (A full description of the spectral processing methods can be found in Mon et al., 2012.) ALC and LD were scanned fully contemporaneously, while PSU were scanned contemporaneously and in random order with the latter half of the cohort. Data acquisition and processing conditions were the same for all participants, and data processing was done by operators blind to participant diagnosis.

2.5. Statistical analyses

Separate univariate analyses of covariance (ANCOVA) were performed for each VOI (DLPFC, ACC, and POC) and each metabolite, followed up with planned pairwise comparisons to test for group differences among PSU, ALC and LD in metabolite concentrations using SPSS, v20. Since age (e.g., Schuff et al., 2001) and differences in brain tissue contributions to the VOIs (GM, WM, CSF; Jansen et al., 2006) affect observed brain metabolite levels, we corrected for age and GM-tissue contribution, the target of our VOI placement. The number of acquired J-edited (GABA) spectra was often lower than for STEAM spectra (DLPFC: PSU = 20 vs. 27, ALC = 24 vs. 30, LD = 13 in both; ACC: PSU = 21 vs. 20, ALC = 27 vs. 37, LD = 14 in both; POC: PSU = 20 vs. 23, ALC = 30 vs. 35, LD = 9 vs. 12). ANCOVA was also used to test for differences in participants' characterization measures. In pairwise group comparisons of metabolite levels, we corrected alpha levels (0.05) to account for the multiplicity of metabolites in each VOI via a modified Bonferroni procedure (Sankoh et al., 1997). This approach yields adjusted alpha levels for each VOI separately using the number of metabolites under investigation (six) and their average inter-correlation coefficients (DLPFC: *r* = .54, ACC: *r* = .39, POC: *r* = .53); corresponding adjusted alpha levels for pairwise group comparisons were 0.022 (DLPFC), 0.017 (ACC) and 0.020 (POC). Effect sizes were calculated via Cohen's *d* (Cohen, 1988). For each VOI within PSU, correlations (Spearman's Rho) between metabolite concentrations and neurocognitive measures (raw scores), drug consumption variables, age and days of

abstinence were calculated. Where necessary, relationships between metabolite levels and other measures were corrected for age (partial correlations).

3. Results

3.1. Participants characterization

Characteristics for ALC, PSU and LD are shown in Table 1. PSU were younger than ALC and had lower NART scores than ALC or LD. PSU and ALC had fewer years of education than LD. Although PSU had a higher prealbumin concentration than ALC and LD, and ALC had higher white blood cell counts than PSU and LD, these and other clinical laboratory measures were within the normal range for all groups. PSU and ALC did not differ on any alcohol drinking measure. The number of days abstinent from alcohol at time of study (sober days) was equivalent at about 30 days; PSU were abstinent from any drug approximately 6 fewer days than from alcohol. PSU and ALC were equivalent on measures of depressive and anxiety symptomatology, both had higher measures than LD. Among smokers, the Fagerstrom test indicated moderate nicotine dependence in PSU and moderate to high dependence in ALC and LD; PSU smoked significantly fewer cigarettes per day than ALC and LD. The ALC and PSU group each contained four participants diagnosed with hepatitis C. Within each group, however, outcome measures of those with and without hepatitis C were equivalent. The same applied to participants with (10 ALC, 5 PSU) and without controlled hypertension. Finally, total brain tissue, GM, WM and CSF fractions in the three VOIs did not differ significantly between groups (mean values: DLPFC: GM = 0.38, WM = 0.55, CSF = 0.06; ACC: GM = 0.46, WM = 0.33, CSF = 0.20; POC: GM = 0.61, WM = 0.29, CSF = 0.09; unassigned 1%), and omitting GM-tissue contribution as covariate did not appreciably change the results of observed group differences for any of the metabolites and VOIs.

3.2. Group comparison of metabolite concentrations

Univariate tests were significant for group differences in the DLPFC: NAA (*p* = 0.004), Cr (*p* = 0.015), Cho (*p* = 0.020) and mI (0.018). Table 2 shows mean metabolite concentrations, and pairwise group statistics. In planned pairwise comparisons of

Table 3

Age corrected partial correlations (r^*) between brain metabolite concentrations and neurocognitive measures within PSU.

Region	Metabolite concentration and cognitive measure	r^*	p
DLPFC	NAA and visuospatial memory	0.42	0.045
	NAA and working memory	0.53	0.019
ACC	NAA and visuospatial learning	0.51	0.039
	Cho and processing speed	0.55	0.023
	GABA and auditory-verbal learning	−0.48	0.049
POC	GABA and visuospatial memory	0.51	0.031

metabolite levels in the DLPFC, PSU had significantly lower NAA, Cho and ml concentrations than both ALC and LD ($p \leq .022$). Cr was significantly lower in PSU compared to ALC and tended to be lower compared to LD. Effect sizes for differences between PSU and ALC were moderate for Cho, Cr, ml and strong for NAA. Similar patterns were observed between PSU and LD. In the ACC, no significant group differences were found. However, PSU showed a strong trend to lower GABA concentrations compared to ALC and LD (moderate effect sizes: $0.64 < ES < 0.73$). Effect sizes for all ACC metabolites but ml in comparisons of PSU to LD were moderate to strong. In the POC, ALC showed a trend to elevated Cho levels compared to both PSU and LD, whereas Cho was similar in PSU and LD. The statistically weaker differences between PSU/ALC and LD were likely related to the smaller LD sample.

Considering that cigarette smoking affected brain metabolite concentrations in individuals with AUD (Durazzo et al., 2004, 2006), in secondary analysis we covaried for smoking status (non-smoker and smoker) in the three-group-comparisons. This did not significantly alter the aforementioned results. Similarly, controlling for years of education, days of sobriety, body mass index, NART, depression, anxiety and drinking severity measures did not alter the above group differences. Therefore, those variables were not used as covariates in our final analyses.

3.3. Correlations among main outcome measures within PSU

3.3.1. Metabolite concentrations and neurocognition. Metabolite concentrations and cognitive performance are typically robustly associated with age. In the combined cohort, age-metabolite relationships were found in DLPFC and POC, primarily with Cho and Cr; in PSU only, DLPFC ml was significantly related to age ($p = 0.017$, $r = .46$), whereas statistical trends for age-metabolite relationships were seen in ALC and LD only. Therefore, we controlled for the effects of age when correlating other outcome measures. In the DLPFC, NAA was positively related to visuospatial and working memory raw scores (see Table 3), in the ACC, NAA and Cho were positively related to visuospatial learning and processing speed raw scores of the BVMT-R and WAIS-III, respectively. GABA in the ACC showed an inverse relationship with auditory-verbal learning (CVLT-II raw scores), whereas in the POC, GABA was positively correlated with the visuospatial memory raw score of the BVMT-R. Different associations were observed in ALC, who showed inverse relationships between DLPFC ml and processing speed and between POC ml and visuospatial learning (both: $p < 0.014$, $r > -.48$). No significant metabolite level-function relationships were found in LD.

3.3.2. Metabolite levels and substance consumption. Within 13 PSU participants, lower DLPFC GABA was related to greater monthly cocaine consumption measures, averaged over both life time ($p < 0.019$, $r = -.64$) and one year ($p < 0.016$, $r = -.65$) (Fig. 2), and independent of average life time alcoholic drinks/month. Methamphetamine consumption in eight PSU was not related to any metabolite concentrations in any VOI. Moreover, in PSU, which

Fig. 2. DLPFC GABA concentrations of cocaine dependent PSU in relation to their monthly cocaine consumption (averaged over last year before abstinence).

showed significantly lower ml than ALC and LD, more days of abstinence from any drug were related to higher DLPFC ml levels ($p = 0.01$, $r = 0.50$).

4. Discussion

We employed high-field ^1H MRS and neuropsychological assessments to compare regional brain metabolite levels in the ACC, DLPFC and POC and examine their functional relevance in alcohol dependent individuals with and without comorbid illicit substance dependence after one month of abstinence. The ACC and DLPFC are important components of the brain reward/executive oversight system (BREOS), a network that is critically involved in the development and maintenance of all forms of addictive disorders (Goldstein et al., 2009; Volkow et al., 2011).

In the DLPFC, PSU demonstrated significantly lower NAA, Cho, ml and Cr concentrations than ALC. DLPFC GABA levels were inversely related to cocaine use. In the ACC, GABA concentrations tended to be lower in PSU compared to both ALC and LD. Moreover, cortical metabolite levels were significantly related to neurocognitive measures, affirming the functional relevance of absolute MRS measures in PSU. Finally, metabolite levels in ALC and LD were statistically equivalent, presumably due to longitudinal recovery of metabolite levels found low in ALC earlier in sobriety (Mon et al., 2012).

Our results suggest that PSU, when compared to both LD and ALC, had abnormal neuronal integrity (lower NAA), abnormalities in glial or cell membrane synthesis/turnover (lower Cho), altered glial content or osmoregulation (lower ml), and trends to abnormal cellular bioenergetics (lower Cr; Licata and Renshaw, 2010). These abnormalities at one month of abstinence were specific to the DLPFC, a region critical for such integrating activities as planning and organization, response inhibition, working memory, reasoning, problem solving and set shifting (Goldstein and Volkow, 2011; Petrides, 2005). There were strong trends to GABAergic abnormalities in the ACC, a region critical to decision making and response inhibition. Since the detected metabolic differences were shown to be unrelated to age, alcohol consumption, body mass index, smoking status, depression and anxiety symptomatology, the misuse of illicit drugs in PSU is likely related to the observed metabolite group

differences. Considering research in both ALC (reviewed in [Licata and Renshaw, 2010](#); [Meyerhoff et al., 2011](#)) and cocaine dependent ALC ([Meyerhoff et al., 1999](#)), we further conclude that neurobiological abnormalities in alcohol dependent individuals with comorbid illicit substance dependence are qualitatively and regionally different at one month of abstinence and longer lasting than those related to alcohol dependence alone.

Although not statistically significant, we observed differences of moderate effect sizes in ACC metabolite levels between PSU and LD (e.g., NAA, Glu, GABA). This may indicate greater metabolic abnormalities in the ACC of PSU earlier in sobriety, similar to what is observed in one-week abstinent ALC ([Mon et al., 2012](#)) and opiate-dependent individuals ([Yucel et al., 2007](#)). This data therefore suggests partial metabolic recovery in PSU with extended abstinence, reminiscent of ALC ([Durazzo et al., 2006](#); [Mon et al., 2012](#)). Longitudinal metabolic changes in abstinent PSU are also suggested by the association of DLPFC mI with days of abstinence.

4.1. Myo-Inositol

Other reports showed lower NAA, Cho and Cr in anterior brain regions associated with mono-substance use disorders (reviewed in [Licata and Renshaw, 2010](#)). However, whereas most MRS studies on mono-substance dependence reported *higher* mI in frontal brain, we found *lower* mI concentrations in the DLPFC of PSU compared to both ALC and LD. Although the precise function(s) of cerebral mI is still debated ([Griffin et al., 2002](#); [Moore et al., 1999](#)), it is suggested to be a marker of astroglial content, with elevations mostly interpreted as astroglial hypertrophy and/or proliferation adversely affecting brain function ([Licata and Renshaw, 2010](#); [Meyerhoff et al., 2011](#); [Mon et al., 2012](#); [Schweinsburg et al., 2001](#)). Since the DLPFC VOI in this study contains a majority of WM tissue, lower mI may indicate glial content (e.g., astrocytes) loss and/or damage related to polydrug consumption. Reductions in mI could also occur through inhibition of its synthesis or uptake ([Coupland et al., 2005](#)). The interaction of two or more different drugs used concurrently leads to formation of adducts of the primary compounds and their metabolites (e.g., cocaethylene, benzoylecgonine, norcocaine, norcocaethylene ([Cardona et al., 2006](#); [Toennes et al., 2003](#))). They may have additional toxic and/or inflammatory effects ([Farooq et al., 2009](#)) and/or might also inhibit mI synthesis or uptake.

4.2. GABA

The trends to low GABA concentration in the ACC of PSU (17–20% lower than in LD or ALC) indicate that the GABA system is altered in PSU. Our GABA findings are consistent with [Ke et al. \(2004\)](#), who showed low GABA levels in the prefrontal cortex of a cohort concurrently dependent on alcohol and cocaine. It is therefore likely that our GABA findings were driven by the 18 PSU individuals dependent on these two substances. However, their ACC GABA levels were equivalent to those in the 10 PSU individuals dependent on substances other than cocaine. This, together with our finding of largely normal GABA levels in ALC after both one week and one month of abstinence ([Mon et al., 2012](#)), suggest that GABA abnormalities in the ACC are associated with concurrent use of alcohol and illicit drugs in PSU, but not with alcohol dependence alone.

Our interpretation of lower metabolite levels (GABA, NAA, Cr, Cho, mI) relating to cell injury or dysfunction associated with polydrug use is further supported by three observations: First, we observed worse neurocognitive performance of PSU compared to ALC and LD (results will be published elsewhere), and neurocognitive deficits have been associated frequently with metabolic abnormalities ([Meyerhoff and Durazzo, 2008](#)). Secondly, the positive correlation in PSU between days of abstinence and DLPFC

mI levels suggests an increase of mI into the normal range (some degree of metabolic recovery) during the first 10–45 days of abstinence. Lastly, the inverse relationship between DLPFC GABA levels and cocaine consumption in PSU, together with lower ACC GABA in PSU vs. LD and ALC, suggest that the prefrontal GABA metabolic pool is impacted by chronic cocaine consumption. On these grounds, we share the view put forth by Johnson and colleagues, who suggest that efforts, which increase GABA levels/activity in PSU (e.g., topiramate treatment), may offer fruitful treatment possibilities ([Johnson, 2005](#)). However, our observation of a simultaneous decrease of four different metabolites (incl. mI) in the DLPFC of PSU might also be explained by possible substance use associated neuronal/glial edema, leading to higher water, thus lower overall metabolite concentrations. We suggest that both cell injury and/or swelling (in GM and/or WM) might occur in the DLPFC of PSU.

4.3. Study limitations and outlook

Given our PSU cohort was abstinent for about one month, greater or different metabolic abnormalities may have been present earlier in abstinence, as observed in the ACC-specific metabolite abnormalities in one-week-abstinent ALC ([Mon et al., 2012](#)). We suggest metabolic recovery occurring in PSU. Longitudinal follow-up will assist in clarifying if the observed abnormalities are reversible, or if our observations are influenced by premorbid and/or comorbid factors not assessed in this study. Few metabolite levels correlated with substance and alcohol consumption quantities, and notwithstanding the known limitations of self-report, this suggests that metabolite level abnormalities may be (in part) premorbid. If this were the case, brain metabolite concentrations could serve as biomarkers or risk factors for the development of PSD. Larger study samples will help identify the degree to which comorbid factors such as cigarette smoking may have influenced our findings. Another observation in need for further investigation is the age-related DLPFC mI increase in PSU, not observed in ALC or LD: polydrug consumption may be associated with alterations of the brain ageing process, similar to what was shown in cocaine dependent individuals ([Ersche et al., 2012](#)). Furthermore, neurobiological abnormalities in the ACC and DLPFC are known to be associated with increased risk of relapse to substance use ([Baler and Volkow, 2006](#); [Goldstein et al., 2009](#); [Heatherton and Wagner, 2011](#); [Volkow et al., 2011](#)). Hence, it is also critical to investigate if the abnormal regional brain metabolite levels detected in this study are related to relapse risk, as observed in those with AUD ([Durazzo et al., 2008, 2010](#)). Different relapse rates in PSU and ALC may be related to their unique neurobiological and cognitive differences demanding differently designed treatment approaches for these disorders.

4.4. Conclusions

MRS-derived metabolite concentrations show that PSU are uniquely different from ALC at one month of abstinence. PSU showed persistent metabolic abnormalities, primarily in the DLPFC, whereas metabolite levels in ALC are normal, after having recovered from abnormal levels in the ACC ([Mon et al., 2012](#)). These abnormalities reflect neuronal and glial injury/dysfunction and some are also related to neurocognition. Our results point to specific metabolic abnormalities as polydrug abuse biomarkers and as potential targets for pharmacological and behavioral PSU-specific treatment aimed at decreasing high relapse rates in PSU.

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Contributors

Drs. Dieter Meyerhoff and Timothy Durazzo designed the research, wrote the study protocol and took part in manuscript preparation. Dr. Meyerhoff had central oversight and overall responsibility for this research. Dr. David Pennington, Thomas Schmidt and Dr. Timothy Durazzo were responsible for assessment and preparation of demographic, behavioral and cognitive data. Dr. David Pennington developed the in-house questionnaire for the assessment of substance use (LSUH). Timothy Durazzo also mentored data analyses. Dr. Anderson Mon and Dr. Christoph Abé were responsible for MR data acquisition, processing, preparation and pre-analysis. Dr. Anderson Mon also mentored processing methods and took part in manuscript preparation. Dr. Christoph Abé performed the final data analyses, reviewed literature of related work, prepared figures and wrote the first draft of the manuscript. All authors have approved the submission of the manuscript.

Conflict of interest

All authors reported no biomedical financial interests or potential conflicts of interest.

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