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Cortical GABA levels are reduced in young adult binge drinkers: Association with recent alcohol consumption and sex

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ABSTRACT

Binge drinking refers to a pattern of alcohol intake that raises blood alcohol concentration to or above legal intoxication levels. It is common among young adults and is associated with health risks that scale up with alcohol intake. Acute intoxication depresses neural activity via complex signaling mechanisms by enhancing inhibition mediated by gamma-amino butyric acid (GABA), and by decreasing excitatory glutamatergic effects. Evidence primarily rooted in animal research indicates that the brain compensates for the acute depressant effects under the conditions of habitual heavy use. These neuroadaptive changes are reflected in neural hyperexcitability via downregulated inhibitory signaling, which becomes apparent as withdrawal symptoms. However, human evidence on the compensatory reduction in GABA signaling is scant. The neurochemical aspect of this mechanistic model was evaluated in the present study with proton magnetic resonance spectroscopy (^{1}H-MRS) which is sensitive to GABA plus macromolecule signal (GABA +). Furthermore, we examined sex differences in GABA + levels as a function of a recent history of binge drinking, given interactions between endogenous neurosteroids, GABA signaling, and alcohol.

The study recruited young adult women and men (22.2 ± 2.8 years of age) who were classified as binge drinkers (BDs, N = 52) if they reported ≥ 5 binge episodes in the previous six months. Light drinkers (LDs, N = 49) reported drinking regularly, but not exceeding ≤ 2 binge episodes in the past six months. GABA-edited ^{1}H-MR spectra were acquired from the occipital cortex at 3 T with the MEGA-PRESS sequence. GABA + signal was analyzed relative to water and total creatine (Cr) levels as a function of binge drinking history and sex. Controlling for within-voxel tissue composition, both GABA + indices showed decreased GABA + levels in BDs relative to LDs. The reduced GABA + concentration was associated with occasional high-intensity drinking in the BD group.

This evidence is consistent with compensatory GABA downregulation that accompanies alcohol misuse, tipping the excitation/inhibition balance towards hyperexcitability. Analysis of the time course of GABA + neuroplasticity indicated that GABA + was lowest when measured one day after the last drinking occasion in BDs. While the BD vs LD differences were primarily driven by LD women, there was no interaction between Sex and a history of binge drinking. GABA + was higher in LD women compared to LD men.

Aligned with the allostasis model, the mechanistic compensatory GABA downregulation observed in young emerging adults engaging in occasional binge drinking complements direct neural measures of hyperexcitability in BDs. Notably, these results suggest that neuroadaptation to alcohol is detectable at the levels of consumption that are within a normative range, and may contribute to adverse health outcomes.

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1. Introduction

Binge drinking, also termed heavy episodic drinking, is associated with a range of health problems (Hingson et al., 2017; White and Hingson, 2013) that scale up with drinking levels (Esser et al., 2012; Haber et al., 2016), and incur a high cost to society (Bouchery et al., 2011; Sacks et al., 2015). A binge episode is defined as drinking enough alcohol to reach blood alcohol concentration (BAC) of 0.08 g/dl which is approximated with an intake of at least 4/5 drinks in about 2 hr for women/men, respectively (NIAAA, 2018, 2021). Peaking in their early twenties, many young adults exceed this level of intake and engage in high-intensity drinking, defined as consuming alcohol at twice the binge threshold or higher (Linden-Carmichael et al., 2017; Patrick et al., 2016). In large sample studies, approximately 40% (22%) of men and 26% (6%) of women in their early twenties reported consuming 5+ (10 +) drinks on the same occasion in the past two weeks (Patrick et al., 2017; Schulenberg et al., 2020; White et al., 2006). Depending on drinking quantity, frequency, and duration, binge drinkers (BDs) are likely to experience long-term neural changes that extend beyond a binge episode (Almeida-Antunes et al., 2020; Correas et al., 2020; Cservenka and Brumback, 2017; Petit et al., 2014). Evidence of the neurobiological sequelae of binge drinking is primarily rooted in animal research (Burnett et al., 2016; Crabbe et al., 2011; Crews et al., 2016; Cui et al., 2013; Ehlers and Criado, 2010; Koob, 2014; Olsen and Liang, 2017; Roberto and Varodayan, 2017), with human studies additionally revealing persistent neuroadaptive alterations across multiple neural systems (Affan et al., 2018; Alderson Myers et al., 2021; Almeida-Antunes et al., 2020; Arienzo et al., 2020; Courtney and Polich, 2009; Cservenka and Brumback, 2017; Holcomb et al., 2019; Jacobus and Tapert, 2013; Petit et al., 2014).

Acute alcohol intoxication affects neural activity via complex signaling mechanisms. It increases inhibition mediated by gamma-amino butyric acid (GABA) and decreases excitatory glutamatergic effects, in addition to affecting other neurotransmitter systems (Kumar et al., 2009; Most et al., 2014; Roberto and Varodayan, 2017; Spanagel, 2009; Vengeliene et al., 2008). Consequently, the excitation/inhibition (E/I) balance is tipped towards neural inhibition during acute intoxication. Indeed, human studies indicate that acute alcohol intoxication decreases cortical excitability (Beaton et al., 2018; Campbell et al., 2014; Correas et al., 2020; Kovacevic et al., 2012; Marinkovic et al., 2012; Rosen et al., 2016), and it dysregulates neural synchrony (Amodeo et al., 2017; Beaton et al., 2018; Marinkovic et al., 2019).

When subjected to frequent binge episodes, the brain compensates for the acute depressant effects of alcohol to restore the E/I homeostasis. The neuroplasticity mechanisms have been outlined in the model of allostasis (i.e. "stability through change"), which is anchored in a highly influential *neurobiological opponent-process theory* (Koob and Le Moal, 2005, 2008). The countervailing effects include down-regulation of $GABA_A$-mediated inhibitory signaling, and a hyperresponsive (i.e. upregulated) excitatory glutamatergic function (Finn and Crabbe, 1997; Most et al., 2014; Roberto and Varodayan, 2017). The resulting hyperexcitability becomes apparent upon cessation of prolonged misuse in alcohol use disorder (AUD), and is reflected in withdrawal symptoms including insomnia, irritability, anxiety, autonomic hyperactivity, and even seizures (Becker, 2008; Finn and Crabbe, 1997). These neuroadaptive changes in neurotransmission underlie tolerance and physiological dependence on alcohol, as well as increased risk of relapse (Becker and Mulholland, 2014; Koob and Le Moal, 2005, 2008). However, direct measures of neuroplasticity in humans in the absence of frank withdrawal symptoms are lacking. The allostasis model predicts that alcohol misuse is associated with increased neural excitability (Koob and Le Moal, 2005, 2008), which has been confirmed in young adult BDs using magnetoencephalography, a direct measure of synaptic currents (Correas et al., 2020). The model further predicts that the hyperexcitability derives mechanistically from a compensatory reduction in inhibitory (GABA) function. This prediction was evaluated in the present study with an MRI-based imaging method that is sensitive to the neurochemical environment in the brain.

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a noninvasive method that can provide *in vivo* insights into the chemical composition of brain tissues with an emphasis on low-molecular-weight metabolites (Bluml, 2012; Cox, 1996; Ende, 2015; Govindaraju et al., 2000; Harris et al., 2017; Licata and Renshaw, 2010; Meyerhoff, 2014; Prichard and Shulman, 1986; Radda et al., 1989; Scheau et al., 2012). As a primary inhibitory neurotransmitter, GABA plays an important role in a range of neuropsychiatric and neurologic disorders, as well as substance abuse including AUD (Levy and Degnan, 2013; Mason et al., 2005; Mason and Krystal, 2006; Schur et al., 2016). Reliable detection of GABA in the neural tissue is hindered by its low concentration and overlapping resonances of other metabolites that attenuate the strength of the signal (Ende, 2015). Dedicated spectral editing methods are used to mitigate these issues (Rothman et al., 1984; Rothman et al., 1992), with the MEscher-GArwood Point RESolved Spectroscopy (MEGA-PRESS) technique (Mescher et al., 1998) considered to be the standard in the field (Mullins et al., 2014). It takes advantage of the J-coupling (indirect spin–spin interactions) between groups of protons that give rise to peaks at 1.9 and 3 ppm, as it suppresses unwanted resonance by applying frequency-selective pulses during "on" and "off" acquisitions (Mullins et al., 2014). The resulting GABA + measure includes contributions from co-edited macromolecules and homocarnosine with overlapping resonances, and is commonly expressed as a ratio of GABA + relative to water (GABA+/water) and total creatine (GABA+/Cr) (Ende, 2015; Mikkelsen et al., 2017; Mikkelsen et al., 2019). Studies of epilepsy have shown that low levels of MRS-detected GABA are associated with poor seizure control, and that enhancement of the levels improves seizure control, strongly suggesting a functional link between tissue GABA levels and functional inhibition (Hammen and Kuzniecky, 2012; Pan et al., 2013; Petroff et al., 1999; Petroff et al., 2000; Petroff et al., 1996).

Human ^{1}H-MRS studies have reported alterations in a range of metabolites as a function of alcohol misuse (Hillmer et al., 2015; Licata and Renshaw, 2010; Meyerhoff, 2014; Moeller et al., 2016; Silveri, 2014; Zahr et al., 2016). Even though creatine is the most commonly used reference metabolite, lower levels have been reported in individuals with AUD (Durazzo et al., 2010; Lee et al., 2007; Mon et al., 2012; Zahr et al., 2016) calling into question its stability (Rae, 2014). Therefore, the present study has employed both, water and total creatine as reference signals to quantify GABA + levels (Saleh et al., 2016). Studies focusing on GABA have reported decreased levels in young emerging adult BDs in comparison to light drinkers (LDs) in the anterior cingulate cortex (ACC) (Silveri et al., 2014). Evidence in people with AUD is mixed. Small-scale early studies reported lower GABA in abstinent participants diagnosed with AUD in the occipital cortex (Behar et al., 1999; Mason et al., 2005) which was modulated by smoking status (Mason et al., 2006). A voxel placed in the ACC also revealed lower GABA concentration in participants with AUD (Prisciandaro et al., 2019; Prisciandaro et al., 2017), but other studies observed no group differences in the ACC, dorsolateral prefrontal, or posterior cortical areas (Abe et al., 2013; Mon et al., 2012). Notably, the reported changes in GABA levels exhibit temporal dynamics and are especially sensitive to recent drinking (Prisciandaro et al., 2020), which was investigated in the present study.

Additionally, it is important to consider the impact of biological sex, since endogenous neurosteroids act as positive allosteric modulators at $GABA_A$ receptors (Belelli and Lambert, 2005; Puia et al., 1990). Indeed, some ^{1}H-MRS studies have observed higher GABA levels in healthy women compared to men (Sanacora et al., 1999; Spurny-Dworak et al., 2021), although the opposite results (O'Gorman et al., 2011), or no sex-based differences (Gao et al., 2013) have been reported as well. Furthermore, GABA concentration fluctuates across the phases of the menstrual cycle, with higher levels reported during the follicular compared to the luteal phase (Epperson et al., 2002; Silveri et al., 2013). In general, our current knowledge of brain-based indices of possible interactions between drinking patterns and sex is rudimentary (Ewing

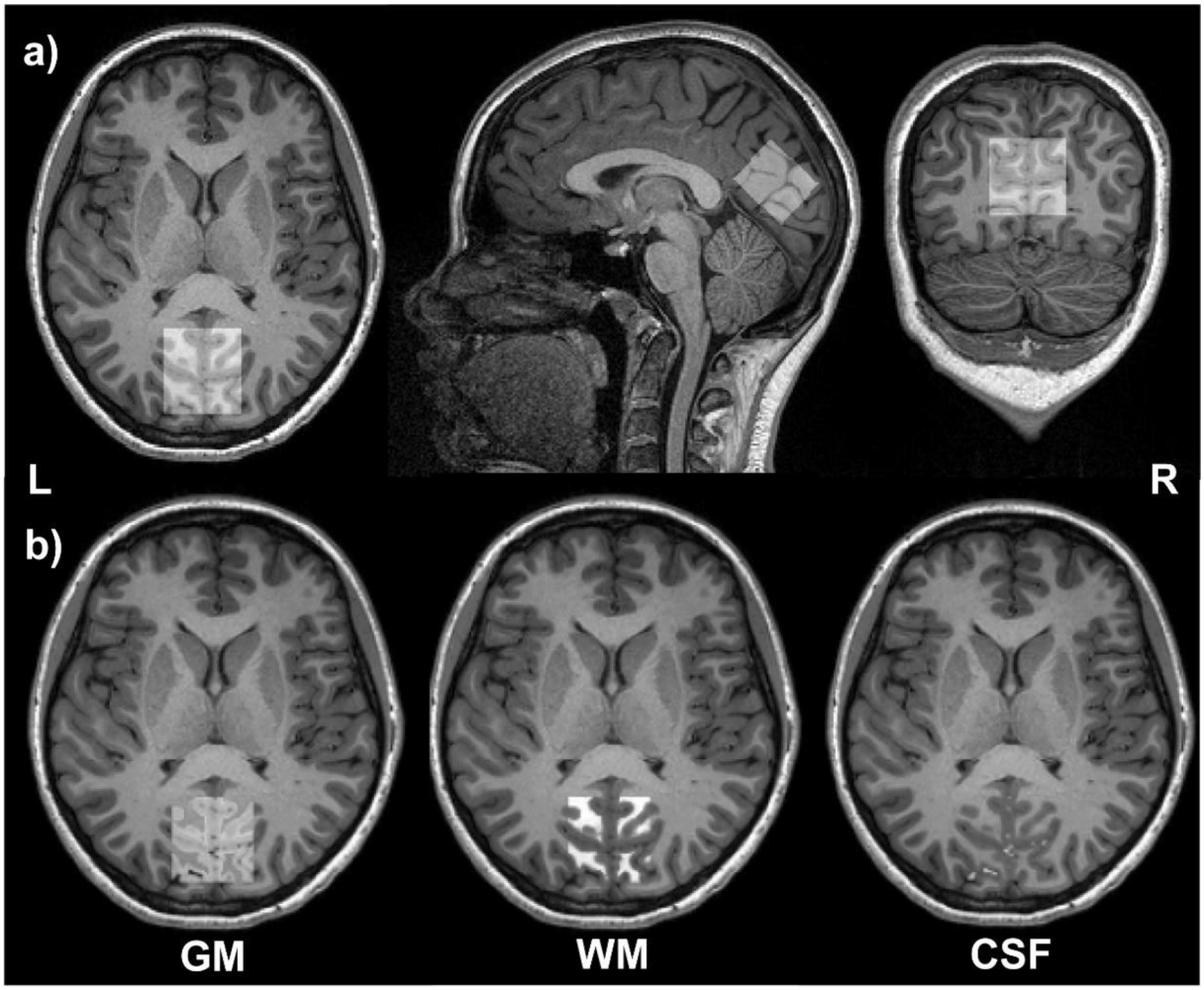

Fig. 1. Examples of a) ^{1}H-MRS voxel placement in the occipital lobe centered on the median and aligned with the tentorium in the sagittal plane; b) segmentation of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) in the occipital lobe for a single participant.

et al., 2014; Nixon et al., 2014; Peltier et al., 2019; Ruiz et al., 2013; Sawyer et al., 2018; Verplaetse et al., 2021). High rates of heavy drinking among young women (Keyes et al., 2011; Patrick et al., 2019), and their susceptibility to health risks including neuroadaptation to alcohol (Lewis and Nixon, 2014; Sharrett-Field et al., 2013), provide compelling arguments for recruiting large samples and including the factor of sex in studies of alcohol use and misuse.

Clearly, evidence on the impact of alcohol consumption on GABA levels in young emerging adults is very limited (Silveri et al., 2014), especially as regards the E/I balance as a function of engaging in binge drinking patterns (Cohen-Gilbert et al., 2014; Correas et al., 2020; Hillmer et al., 2015). To gain a mechanistic insight into compensatory changes in neurochemistry as a function of binge drinking, the present ^{1}H-MRS study was designed to examine GABA + levels normalized by water and total creatine in a large sample of young adult men and women. Preliminary analyses were conducted to investigate the time course of the GABA + changes with respect to the time elapsed since last drink, and to evaluate the impact of the menstrual cycle phases on GABA + levels.

Methods.

2. Research participants

The study included data from 101 participants, all right-handed young adults (50 women and 51 men, age 22.3 ± 2.9), recruited from the local community through approved postings and ads. Flyers were posted on San Diego State University campus, and ads were placed in classrooms and on Craigslist. They contained the following description: "The study will use magnetic resonance imaging to examine structural and functional changes as a function of alcohol drinking levels and behavioral traits". Participants reported no history of seizures, traumatic brain injury, hearing or vision problems, or neuropsychiatric disorders, and all complied with MRI safety criteria. Participants were medication-free, reported no nicotine or illicit drug use at least one month prior to scanning, and none sought treatment or had been previously enrolled in an alcohol abuse treatment program. A screening questionnaire assessing the frequency, quantity, and rate of alcohol consumption was used to determine BD and LD group assignment. Binge episodes were defined as drinking occasions on which 5+/4 + drinks (men/women, respectively) were consumed within a 2-hour time span (NIAAA, 2017). Participants who reported ≥ 5 binge episodes in the previous six months and at least one in the past month were classified as BDs. The LD group consisted of individuals who reported drinking regularly, but at low levels, with 43% of LDs reporting one or two binge episodes in the past six months, but no more than one within the past month (24%). Groups were equated for age, sex, intelligence, and family history of alcoholism. The study's procedures were approved by the Institutional Review Board at San Diego State University (HS-2019–0135) and were conducted following the human subjects research ethics in accordance with the Declaration of Helsinki. Written informed consent was obtained prior to administration of any experimental procedures and participants were monetarily compensated for their time. Four additional LD participants were

recruited for the study, but their data were not included due to poor data quality based on the criteria described in the Methods section.

2.1. Experimental protocol

Participants filled out a battery of questionnaires evaluating details of frequency, quantity, and the pattern of alcohol consumption (modified from Cahalan et al., 1969), presence of behaviors associated with alcohol use disorder (Alcohol Use Disorder Identification Test, AUDIT, Saunders et al., 1993), DSM-5 (O'Brien, 2011), a self-reported checklist that tallies the number of diagnostic criteria, alcohol intake over the past thirty days (Time Line Follow Back, TLFB, Sobell and Sobell, 1996), severity of craving (The Penn Alcohol Craving Scale, PACS, Flannery et al., 1999), motivations guiding drinking behaviors (Drinking Motive Questionnaire Revised Short Form, DMQ-R SF, Kuntsche and Kuntsche, 2009), and the frequency of negative consequences brought about through drinking (Brief Young Adult Consequences Questionnaire, B-YAACQ, Kahler et al., 2005). No clinical interview (e.g. the Structured Clinical Interview - SCID-5) was administered. Participants were also asked to rate the existence of depressive symptoms (Patient Health Questionnaire, PHQ-9, Kroenke and Spitzer, 2002), anxiety (Generalized Anxiety Disorder, GAD-7, Spitzer et al., 2006), degree of impulsive qualities linked with motor, non-planning, and attentional characteristics (Abbreviated Brief Impulsivity Scale, ABIS, Coutlee et al., 2014), propensity for risk taking and/or sensation seeking behaviors (Brief Sensation Seeking Scale, BSSS, Hoyle et al., 2002). Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI, Buysse et al., 1989) as well as perceived levels of stress (Perceived Stress Scale, PSS, Cohen et al., 1983). Cognitive abilities were assessed using the Full Scale Intelligence Quotient two-subtest form (FSIQ-2) of the Wechsler Abbreviated Scale of Intelligence (WASI-II, Wechsler, 1999). An abbreviated version of the Family History Assessment Module was used to screen for family history of alcoholism (FHAM, Rice et al., 1995). Participants that reported having at least one immediate family member (father, siblings) and one immediate or second-degree relative (grandparents, aunts, uncles) or 3 or more second- or third-degree family members with a prior diagnosis for AUD were considered positive for family history of alcoholism (FH +). Participants who reported maternal FH + were excluded to avoid possible fetal alcohol exposure confounds. Screening for the presence of illegal substances was performed on the day of scanning with a 12-panel urine multidrug test (Discover, American Screening Corporation). Additionally, women were screened for pregnancy and all tests returned negative.

2.2. ^{1}H-MRS and structural MRI acquisition

The scanning was carried out with a 3-T Siemens Prisma scanner equipped with a 32-channel head coil at the San Diego State University (SDSU) Imaging Center. For each participant, a localizer image was followed by a structural scan. A T_1-weighted three-dimensional Magnetization-Prepared Rapid-acquisition Gradient Echo (MPRAGE) sequence used the following parameters: TR = 7.2 ms, TE = 3.01 ms, flip angle = 9°, TI = 900 ms, inversion repeat time = 2300 ms, bandwidth = 320 Hz/pix, FOV = 256 mm, matrix = 256 × 256, 176 axial slices, GRAPPA = 2, isotropic resolution of 1 mm.-

^{1}H-MRS spectra were acquired from a volume in the occipital lobe to examine the impact of binge drinking on basic neurotransmission in an area that is less likely to be confounded with higher-level cognitive effects. Guided by the anatomical image, the volume was placed in the occipital lobe centered on the median and aligned with the tentorium in the sagittal plane (Fig. 1a). Axial and coronal images were used to adjust the voxel placement and ensure that the voxel did not include the skull. The GABA-edited ^{1}H-MRS data were acquired with the Siemens MEGA-PRESS sequence (Mescher et al., 1998; Mullins et al., 2008) configured as follows: 30 × 35 × 25 mm (26.3 mL) single voxel of interest (VOI), TR = 1500 ms, TE = 68 ms, bandwidth = 1670 Hz, 1024 datapoints. A total of 256 averages were obtained including 128 ON and 128 OFF transients, 90° excitation/180° refocusing pulses. The number of acquired signal averages is well within a high SNR range (Mikkelsen et al., 2018). The bandwidth (full-width half-maximum) of the Gaussian-shaped editing pulses was set to 80 Hz, set after empirical verification in a phantom of 50 mM GABA and 50 mM choline, pH adjusted to 7.0. The pulses were applied at 1.9 ppm ('ON') and 7.5 ppm ('OFF') for 128 trials each, their difference resulting in a J-edited spectrum. However, the difference signal at 3.0 ppm generated from the ON/OFF acquisitions contains co-edited contributions from homocarnosine and macromolecules (Harris et al., 2017; Petroff et al., 2001), which is referred to as GABA +. Water suppression was achieved using the Siemens VAPOR full water suppress option. Participants were scanned with this protocol while keeping their eyes closed.

2.3. ^{1}H-MRS data analysis and voxel of interest (VOI) tissue segmentation

Modeling and quantification of the ^{1}H-MRS data were conducted with the MATLAB-based (Mathworks, Natick, MA, USA) toolkit Gannet 3.1.3, which was developed and optimized for the analysis of GABA-edited ^{1}H-MRS data (Edden et al., 2014). Gannet Load software component gathers subject-specific variables from data headers and applies spectral registration, frequency and phase correction, 3-Hz exponential line broadening, and rejection of outlier data points. Individual ON and OFF spectra were subtracted to provide the edited difference spectrum. To optimize post alignment, the total-choline (tCho) peak was used as the reference signal for aligning ON and OFF spectra with the SubspectralAlign script. Of note, the total-choline "peak" reflects contributions from several choline-containing and other compounds (Haddadin et al., 2009). GannetFit implements a single Gaussian model to fit the edited GABA + signal, which is then considered relative to water or total creatine (Cr) levels. Implementation of GannetFit divides standard deviations of fitting residuals by fitted GABA + peak amplitudes, thereby producing overall fit errors that reflect signal-to-noise-ratios and are most often impacted by subject motion or scanner drift (Edden et al., 2014). To mitigate such influence, only the spectra of the participants that showed GABA+/water and GABA+/Cr fit errors of $\leq$ 12% were used for statistical analysis (Cuypers et al., 2021; Peek et al., 2021; Puts et al., 2018). This threshold placed the excluded four data sets at z-scores = 3.11 and 3.31 for GABA+/water and GABA/Cr respectively, representing the 99.9th percentile. In the present study, fit errors equaled to 7.34 ± 1.5% for GABA+/water and 7.36 ± 1.4% for GABA+/Cr. Due to an invalid water reference for 7 participants, GABA+/water analyses were conducted for N = 94 (50 men), whereas the GABA+/Cr ratio was analyzed for N = 101 (51 men).

The concentration of GABA + is expressed as a ratio to a reference, commonly total Cr or water. While some studies report no difference in cortical creatine levels as a function of a history of binge drinking (Silveri et al., 2014) or AUD (Abe et al., 2013; Bauer et al., 2013; Pennington et al., 2014), others have reported mostly lower levels in individual with AUD (Durazzo et al., 2010; Lee et al., 2007; Mon et al., 2012). Given that potential group differences in the denominator can confound GABA + measurement, we have normalized GABA + with both, water and total Cr to verify the results. If both types of analyses yield analogous group differences, they could be interpreted as being due to differences in GABA + concentration. CSF-corrected values for GABA+/water concentration were obtained with GannetQuantify. This module incorporates modeled peak areas from GannetFit and within-voxel tissue fractions from GannetSegment, to generate concentration values corrected for CSF-fraction and tissue-dependent relaxation, as recommended by previous studies (Gasparovic et al., 2006; Mullins et al., 2014).

Because the spectral quantification can be influenced by tissue composition, each participant's VOI was co-registered to their anatomical image using the GannetCoRegister analysis module. Once aligned

Table 1
Tissue Segmentation.

Occ VOI	BDm (n = 25)	BDw (n = 27)	LDm (n = 26)	LDw (n = 23)	Group $F_{1,97}$	Sex $F_{1,97}$	Group × Sex $F_{1,97}$
GM ratio	67.7 ± 3.5	68.1 ± 3.2	67.9 ± 4.7	67.9 ± 4.5	.005, p =.95	.063, p =.80	.046, p =.83
GM	61.6 ± 3.7	62.3 ± 3.8	61.8 ± 4.4	62.2 ± 4.5	.003, p =.96	.421, p =.52	.061, p =.81
WM	29.3 ± 3.2	29.1 ± 2.9	29.3 ± 4.3	29.4 ± 4.1	.025, p =.88	.004, p =.95	.03, p =.86
CSF	9.0 ± 2.4	8.6 ± 2.7	8.8 ± 2.4	8.4 ± 2.2	.159, p =.69	.58, p =.45	.006, p =.94

Mean values ± standard deviations for binge drinking (BD) and light drinking (LD) groups stratified by Sex (men: m and women: w). There were no main effects of Group or Sex nor Group × Sex interaction for the calculated GM ratio and all tissue types.

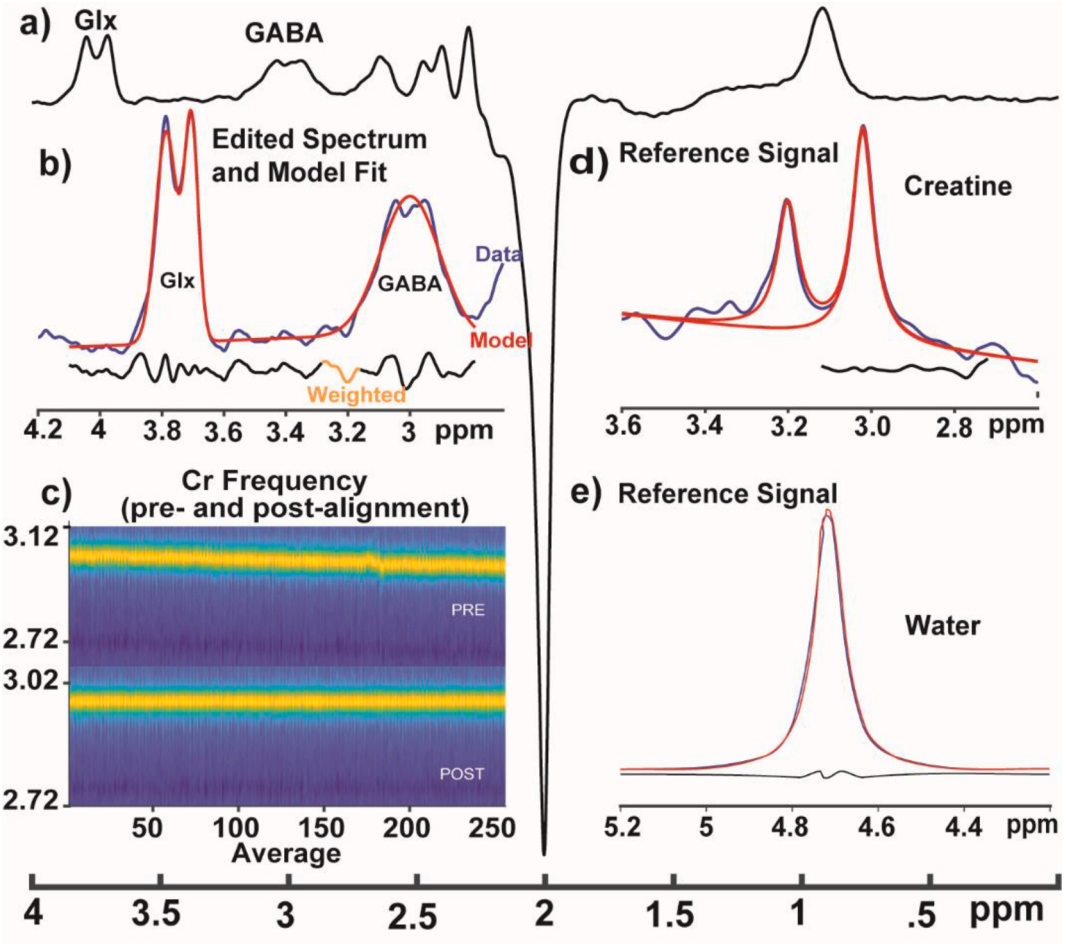

Fig. 2. Representative spectra for a single participant: a) Edited spectra from 0 to 4 ppm; b) Edited spectra for 2.8 – 4.2 ppm range (blue line) and fitted peaks representing GABA + and Glx model (red line) and the residuals (black line). c) the pre- and post-alignment Cr signal is shown as a yellow stripe over the duration of the experiment. d) Reference signal depicting the modeling of creatine (Cr) from the OFF spectrum. e) reference signal showing the modeling of water from the OFF spectrum. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and checked for accuracy, GannetSegment (Fig. 1b) was used to calculate the percentage of gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) within the VOI. The tissue segmentation percentages were used to calculate the GM ratio for each participant. It was expressed as GM / (GM + WM) and used as a covariate in all analyses. As shown in Table 1, there were no interactions, Group or Sex main effects for the GM ratio or all other tissue types.

2.4. Statistical analysis

To account for individual variation in tissue content within the VOI, GM ratio was used as a covariate in all analyses of GABA+/water and GABA+/Cr levels. Univariate 2 × 2 ANCOVAs with factors of Group (BD, LD) and Sex (m, w) were used to test for main and interaction effects (Fig. 3), followed by the analyses evaluating simple effects of Group (i.e. BDm vs LDm and BDw vs LDw) and Sex (i.e. BDm vs BDw and LDm vs LDw).

To evaluate the sensitivity of GABA+/water to the time elapsed since the last drink (Prisciandaro et al., 2020), we analyzed the data from a subset of participants whose last drinking occasion occurred 1 (N = 16), 2 (N = 13) or 3 (N = 12) days prior to scanning. For GABA+/Cr comparisons, the participant subset is as follows: 1 day delay (N = 17), 2

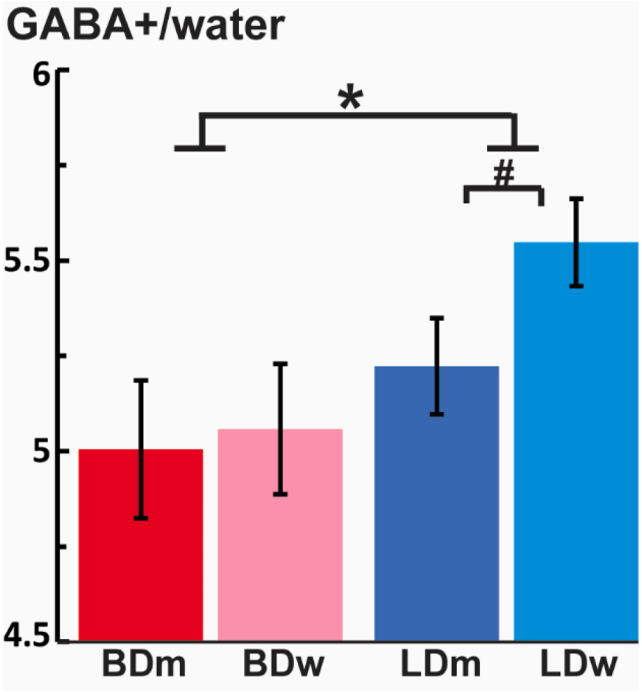

Fig. 3. Means (±standard errors) of GABA+/water levels across the factors of Group and Sex. Controlling for tissue composition, BDs have a lower GABA+/water concentration than LDs overall, which is confirmed for women only. Light drinking men (LDm) tend to have lower GABA+/water levels relative to women (LDw), while binge drinking men (BDm) and women (BDw) do not differ. #p =.07, **p <.01.

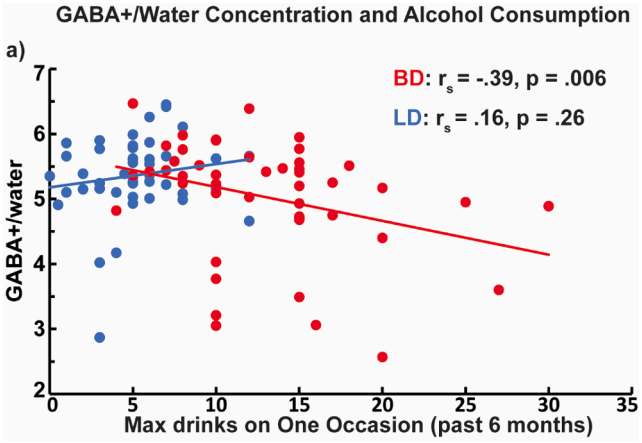

Fig. 4. Scatter plots depicting correlations between GABA+/water concentrations and a maximum number of drinks consumed on a single occasion in past six months for BDs and LDs separately. Spearman's rank correlations and p-values are given for each group.

days (N = 13), and 3 days (N = 13). A 2 × 3 ANCOVA with factors of Group and Days was used to test for main effects and interactions (Fig. 5a). For each Group, GABA+/water or GABA+/Cr changes were tested as a function of the number of days elapsed since the last drink. In those analyses, we used alcohol-related variables as covariates to control for possible differences in consumption levels. They included weekly consumption, the maximum number of drinks consumed on a single occasion, and the number of binge episodes in the previous six months. Additionally, we analyzed the quantity and frequency of alcohol intake on the last drinking day (Fig. 5b), to examine whether GABA+/water and GABA+/Cr changes were possibly related to alterations in drinking patterns.

Previous studies revealed that GABA+/Cr is sensitive to the phases of the menstrual cycle, with higher levels characterizing the follicular, compared to the luteal phase (Epperson et al., 2005; Silveri et al., 2013). Controlling for tissue composition, we compared GABA+/water and GABA+/Cr levels measured in men to those observed in women scanned in the follicular and luteal phases. Please see Supplementary Material for more details on this analysis.

Non-parametric Spearman's rank correlations were used to investigate associations between GABA+/water and GABA+/Cr ratios, and variables related to alcohol intake and personality/disposition. To correct for multiple correlations, the false discovery rate-based Benjamini-Hochberg procedure was used with FDR = 0.1 (Benjamini and Hochberg, 1995). For Group and Sex comparisons in Table 2 and Table 3, categorical variables were assessed using χ^2 test, while all other variables were analyzed with a non-parametric Mann-Whitney *U* test. Statistical analyses were carried out with SPSS 27 software.

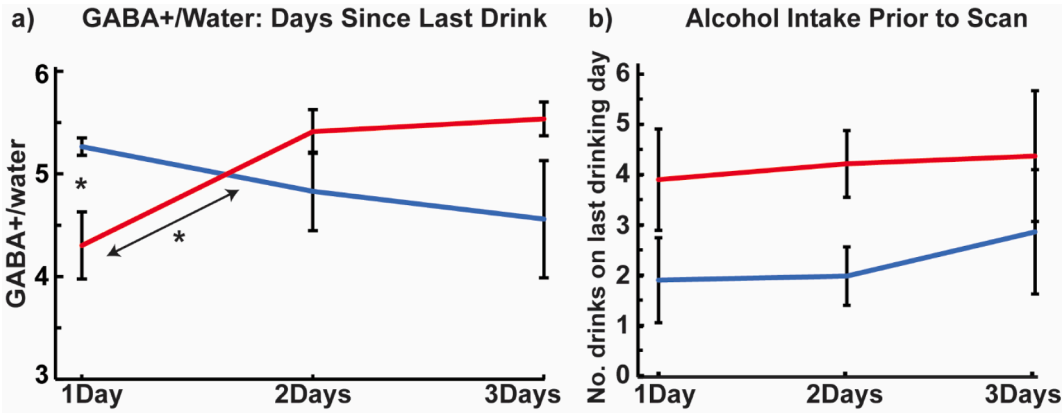

Fig 5. a) Means (±standard errors) of GABA+/water levels for BDs and LDs as a function of the number of days that elapsed since they last consumed alcohol prior to scanning. BDs had lower GABA+/water compared to LDs one day after drinking, which normalized by Day 2. b) Means (±SEM) of alcohol intake on the last day prior to scanning did not change as a function of the time elapsed for either group. *p ≤ 0.05.

3. Results

3.1. Participant characteristics

As shown in Table 2, BD and LD groups did not differ by age, sex, ethnicity, FH+, or intelligence. As expected, BDs reported greater levels of alcohol consumption, alcohol craving, motivation, earlier onset of alcohol use, and a greater likelihood of having experienced negative consequences because of alcohol use compared to LDs. BDs also reported greater levels of disinhibition and boredom on the Sensation Seeking BSSS subscales. Conversely, groups did not differ on measures of anxiety, depression, impulsivity, perceived levels of stress, or sleep quality. Table 3 includes descriptive group parameters and statistical comparisons for men and women within BD and LD groups. With the exception of the age of drinking onset, there were no Sex differences in the LD group. In the BD group, men reported drinking more frequently and at higher weekly levels, as well as consuming more on high-intensity drinking occasions compared to women. They also had higher scores on Thrill and Disinhibition Sensation Seeking subscales. Conversely, binge drinking women reported higher levels of stress compared to BDm.

3.2. ^{1}H-MRS: GABA+/water concentration as a function of group and Sex

Controlling for tissue composition, GABA+/water in the occipital VOI was lower in BDs than in LDs, as indicated by a main effect of Group, $F_{1,89} = 5.24$, p =.02 (Fig. 3), but there was no Group × Sex interaction, $F_{1,89} = 0.78$, p =.38, nor a main effect of Sex, $F_{1,89} = 1.32$, p =.26. Group comparisons for each sex revealed lower GABA+/water in BDw than LDw, $F_{1,41} = 4.53$, p =.03, but no differences in GABA+/water levels in BDm relative to LDm, $F_{1,47} = 1.10$, p =.29. Sex comparisons for each Group showed a trend toward lower GABA+/water concentrations in LDm compared to LDw, $F_{1,43} = 3.23$, p =.07, whereas BDm did not differ from BDw, $F_{1,45} = 0.02$, p =.89. These findings are very similar to GABA+/Cr measures (please see Supplementary Material and Fig. 1S for details).

FDR-corrected Spearman's rank correlations computed separately for each group indicated that GABA+/water levels were negatively associated with drinking for BDs only (Fig. 4). More specifically, the lower GABA+/water, the greater maximum number of drinks BDs consumed on a drinking occasion in the last six months, $r_s = -0.39$, p <.01. In contrast, for LDs, there was no association between GABA+/water and the maximum number of drinks, $r_s = 0.16$, p =.26. Very similar correlations were observed for GABA+/Cr levels as well (see Supplementary Material and Fig. 2S for full details). The average number of weekly drinks consumed was positively correlated with GABA+/water levels in BDs ($r_s = 0.29$, p =.05) but not LDs ($r_s = -0.02$, p =.87), but this association did not survive the FDR correction (Benjamini and Hochberg, 1995).

3.3. Time course of GABA+/water changes as a function of the number of days since last drink

Given the evidence that GABA + is sensitive to the recent history of alcohol intake (Prisciandaro et al., 2020), we compared it in the subsets of participants who consumed alcohol 1, 2, or 3 days prior to scanning (Fig. 5a). Controlling for tissue composition, the analysis revealed a Group × Day interaction, $F_{2,33} = 6.63$, p <.01, but no main effects of Group $F_{1,33} = 0.63$, p =.44, or Day, $F_{2,33} = 0.40$, p =.67. BDs had lower GABA+/water levels compared to LDs when scanning occurred one day after the last drink, $F_{1,13} = 5.45$, p =.04. This group difference was no longer present after a 2-day, $F_{1,9,} = 1.57$, p =.24 or 3-day delay, $F_{1,9} = 4.52$, p =.07. Within-group comparisons were conducted after controlling for alcohol consumption to mitigate possible group differences. The covariates included the number of binge episodes and maximum number of drinks consumed on a single occasion within last 6 months, and average drinks consumed per week. Analyzing each group separately, we observed a main effect of Day on GABA+/water for BDs only, $F_{2,20} = 6.35$, p <.01, with no change in LDs, $F_{2,6} = 2.77$, p =.12. The lowest BD GABA+/water was again observed on Day 1, with an increase on Day 2, $F_{1,13} = 6.42$, p =.02, and Day 3, $F_{1,12} = 8.41$, p =.01, relative to Day 1. Fig. 5b shows the number of drinks consumed by BDs and LD on the last drinking day which happened 1, 2, or 3 days before the scan. Alcohol consumption did not differ as a function of the interval length prior to scan for BD, $F_{2,26} = 0.06$, p =.95, or LD groups, $F_{2,12} = 0.16$, p =.85. The analysis of GABA+/Cr levels provides converging results (please see Supplementary Material and Fig. 3S).

3.4. Impact of the phase of the menstrual cycle on GABA + levels

GABA+/Cr levels tended to be higher in women compared to men when the females were scanned in the follicular phase, in agreement with previous findings (Epperson et al., 2002; Epperson et al., 2005; Harada et al., 2011; Silveri et al., 2013). Please see Supplementary Material and Fig. 4Sfor further details.

4. Discussion

^{1}H-MRS is sensitive to regional neurochemical profiles of the brain tissue. Despite its low signal, GABA + concentration can be quantified with spectral editing methods, providing insight into the inhibitory

Table 2
Participant characteristics for BD and LD groups.

Participants	Binge (n = 52)	Light (n = 49)	U/χ^2	p-value
Age	21.8 ± 2.8	22.8 ± 3.1	1045	.12
% Women[a]	52	47	0.25	.62
Ethnicity (white, non-Hispanic)[a]	55%	49%	1.03	.79
Family History of Alcoholism[a]	15%	14%	0.02	.87
WASI-II % rank (FSIQ-2)	70.7 ± 17.4	72.5 ± 19.2	1154	.51
In the past six months				
Drinking days per week	2.2 ± 1.1	0.9 ± 0.8	423	**<.001**
Drinks per occasion	4.8 ± 2.8	1.7 ± 1.4	310	**<0.001**
Drinks consumed per week	9.8 ± 5.9	2.0 ± 2.1	146	**<.001**
Binge episodes	18.9 ±	1.2 ± 1.2	000	**<0.001**
Alcohol-related blackouts	15.6	0.2 ± 0.6	507	**<.001**
Max No. of drinks in 24 h	3.5 ± 5.5 12.8 ± 5.5	5.0 ± 2.7	212	**<.001**
Age onset of alcohol use	16.2 ± 2.1	17.5 ± 1.7	799	**.001**
Alc. Use Disorder Ident. Test (AUDIT)	10.2 ± 4.2	4.6 ± 2.9	325	**<.001**
Alcohol cravings (PACS)	5.2 ± 4.1	2.4 ± 2.8	701	**<.001**
DSM-5 checklist	2.8 ± 1.9	1.7 ± 1.9	753	**<.001**
Alcohol consequences (B-YAACQ)	7.0 ± 4.9	2.7 ± 4.3	470	**<.001**
Motivations for drinking (DMQ-R)	7.5 ± 1.3	6.3 ± 1.9	771	**.001**
Social	4.5 ± 1.7	3.9 ± 1.2	960	**.03**
Coping	4.3 ± 1.3	4.1 ± 1.3	1099	.28
Conformity	6.7 ± 1.3	5.3 ± 1.7	667	**<.001**
Enhancement				
Anxiety (GAD-7)	5.2 ± 4.7	4.9 ± 5.3	1176	.50
Depression (PHQ-9)	4.5 ± 4.5	4.3 ± 4.6	1213	.67
Impulsivity (ABIS)				
Motor	8.0 ± 2.1	7.3 ± 2.0	1000	.08
Attention	9.2 ± 1.8	9.0 ± 2.2	1166	.56
Non-planning	7.4 ± 2.4	7.0 ± 2.6	1131	.41
Sensation Seeking (BSSS)				
Experience	7.8 ± 1.6	7.2 ± 1.7	1020	.11
Boredom	7.5 ± 1.4	6.5 ± 1.7	860	**.006**
Thrill	6.5 ± 2.1	6.3 ± 2.1	1191	.68
Disinhibition	6.9 ± 1.5	5.7 ± 2.0	814	**.002**
Perceived stress (PSS)	20.4 ± 3.7	19.8 ± 5.1	1204	.63
Sleep quality (PSQI)	4.7 ± 2.8	4.4 ± 2.6	1215	.81

Group means ± standard deviations for all continuous variables. [a]Group differences tested with chi-square; all other comparisons performed using non-parametric Mann-Whitney *U* test. Significant p-values are reported with boldface font. WASI-II: Wechsler Abbreviated Scale of Intelligence; AUDIT: Alcohol Use Disorder Identification Test; PACS: Penn Alcohol Craving Scale; DSM-5: Diagnostic Criteria for Alcohol Use Disorder (self-reported checklist that tallies the number of endorsed diagnostic criteria); B-YAACQ: Brief Young Adult Alcohol Consequences Questionnaire; DMQ-R: Drinking Motivations Questionnaire Revised; GAD-7: Generalized Anxiety Disorder; PHQ-9: Patient Health Questionnaire; ABIS: Abbreviated Impulsiveness Scale; BSSS: Brief Sensation Seeking Scale; PSS: Perceived Stress Scale; PSQI: Pittsburgh Sleep Quality Index.

aspects of neurotransmission. In the present study, we examined GABA + levels in emerging adult men and women as a function of their recent history of binge drinking. GABA + was quantified relative to water and total Cr references and both measures yielded highly analogous results, providing converging validity to the findings, and suggesting that GABA + measures are stable across both denominators. Lower GABA + levels were observed in BDs compared to LDs, after accounting for tissue composition in the occipital voxel. This evidence supports the principal tenet of the allostasis model (Koob and Le Moal, 2005, 2008), which predicts that compensatory GABA downregulation accompanies alcohol misuse (Kumar et al., 2009; Most et al., 2014; Roberto and Varodayan, 2017). Indeed, in the present study, lower GABA + was associated with high-intensity drinking among BDs. We investigated the time course of GABA + neuroplasticity by comparing GABA + levels after 1-, 2-, or 3-day delay since the last drinking occasion. BDs had lower GABA + levels than LDs when the scan took place with a 1-day delay since drinking. Furthermore, only BDs showed a significant GABA + increase from Day 1 to Day 2, consistent with a previously reported finding in individuals with less severe AUD (Prisciandaro et al., 2020). While the BD vs LD differences were primarily driven by LD women, there was no interaction between Sex and a recent history of binge drinking. The effect of Sex was explored further as a function of the phase of the menstrual cycle. Though based on small samples, the explorative findings indicate that GABA + Cr tended to be higher in women who were scanned in the follicular phase relative to men.

Overall, the observed GABA + downregulation in young adult BDs extends previous ^{1}H-MRS findings (Silveri et al., 2014), and complements direct measures of neural hyperexcitability in BDs (Correas et al., 2020). This evidence aligns with compensatory neuroadaptive changes manifesting as downregulated inhibitory signaling associated with alcohol misuse (Kumar et al., 2009). As a dynamic, interactive system (Bullmore and Sporns, 2012), the brain relies on an optimal coaction between synaptic excitation and inhibition which is tightly regulated by homeostatic mechanisms, and which underlies local neural activity and long-range communication. Even though neural activity is modulated by non-glutamatergic afferents primarily arising from the nuclei in the brainstem, the overall network stability is predominantly determined by the two principal neurotransmitters (Amzica and Lopes da Silva, 2011; Buzsaki, 2006; Haider et al., 2006; Knight, 2007; Tatti et al., 2017). Notably, E/I imbalance has been associated with a range of brain-based disorders (Gao and Penzes, 2015; Hammen and Kuzniecky, 2012; Marin, 2012; Petroff et al., 1996; Rubenstein and Merzenich, 2003; Selten et al., 2018; Tatti et al., 2017). Clinical features and neural measures clearly indicate that alcohol dependence reflects shifting the balance towards elevated excitation (Enoch, 2008; Krystal et al., 2006; Porjesz and Rangaswamy, 2007). This compensatory increase in excitability has been described in a mechanistic model of allostasis contrasting acute and persistent effects of alcohol (Koob and Le Moal, 2008). At a level of cell signaling, acute alcohol exerts its primary effects on GABA and glutamate, resulting in enhanced inhibition during an episode of acute intoxication. For individuals who drink habitually, this frequent state of alcohol-induced inhibition promotes compensatory, countervailing changes in neurotransmission. The resulting allostatic E/I imbalance is reflected in downregulated inhibition and excitatory facilitation, which can be observed as hyperexcitability during sober state in young binge drinkers (Correas et al., 2020) and withdrawal symptoms in individuals with AUD (Becker and Mulholland, 2014; Enoch, 2008; Krystal et al., 2006; Roberto and Varodayan, 2017).

^{1}H-MRS is a noninvasive imaging method that is capable of measuring GABA levels *in vivo*. It has been suggested that it is primarily sensitive to the tonic inhibitory properties of the neural tissue (Stagg, 2014). Indeed, GABA-expressing inhibitory interneurons are essential for stability of the neural circuitry as they modulate excitation, gain, timing, and other properties of synaptic transmission (Ferguson and Gao, 2018; Hu et al., 2014; Isaacson and Scanziani, 2011; Markram et al., 2004; Roux and Buzsaki, 2015; Sadeh and Clopath, 2021). The overall aim of the present study was to measure GABA + levels to evaluate the neural underpinnings of compensatory changes associated with binge drinking in a mechanistic framework. The principal finding indicated lower GABA + concentration in BDs compared to LDs in the occipital cortex. Lower GABA + was associated with higher levels of high-intensity drinking, which is indicative of compensatory down-regulation of inhibitory signaling. In a smaller sample of emerging adults with comparable characteristics, Silveri and colleagues (Silveri et al., 2014) observed no group differences in the parieto-occipital cortex, but found lower GABA+/Cr levels in a voxel placed in the anterior cingulate cortex (ACC), which was associated with greater alcohol use consequences. The effect was strengthened by limiting the comparison to the binge drinkers who experienced alcohol-induced blackouts. The present study extended those findings by confirming lower GABA + levels in young BDs in the occipital cortex. The group effect was tied to the levels of high-intensity drinking.

The earliest ^{1}H-MRS studies reported lower GABA in the occipital cortex in small samples of abstinent participants with AUD (Behar et al.,

Table 3
Sex-based participant characteristics for BD and LD groups.

Participants	BDm (n = 25)	BDw (n = 27)	LDm (n = 26)	LDw (n = 23)	BDm vs BDw	LDm vs LDw	BDm vs LDm	BDw vs LDw
Age	22.4 ± 3.3	21.2 ± 2.1	23.4 ± 3.4	22.0 ± 2.6				
Ethnicity[a]	60%	52%	50%	48%				
Fam. Hist.[a]	8%	22%	8%	21%				
WASI-II % rank	69.8 ± 19.4	71.6 ± 15.8	70.6 ± 21.2	74.6 ± 16.8				
Past six months								
Drink days/week	2.3 ± 1.3	2.2 ± 1.0	1.0 ± 0.9	0.8 ± 0.7			***	***
Drinks/occasion	6.1 ± 3.4	3.7 ± 1.4	1.7 ± 1.6	1.6 ± 1.3	**		***	***
Drinks per week	12.2 ± 6.7	7.6 ± 4.0	2.1 ± 2.2	1.9 ± 2.0	**		***	***
Binge episodes	21.8 ± 19.2	16.1 ± 11.1	1.2 ± 1.2	1.2 ± 1.2			***	***
Blackouts	4.6 ± 7.1	2.6 ± 3.5	0.3 ± 0.7	0.1 ± 0.3			***	***
Max drinks in 24 h	15.3 ± 5.9	10.5 ± 4.0	5.6 ± 3.0	4.4 ± 2.3	***		***	***
Alcohol onset	16.4 ± 2.6	16.2 ± 1.5	18.0 ± 1.6	16.9 ± 1.8		*	**	
AUDIT	11.3 ± 4.1	9.3 ± 4.2	4.3 ± 3.0	4.8 ± 2.7			***	***
PACS	5.4 ± 4.4	5.0 ± 3.9	2.3 ± 2.5	2.6 ± 3.2			**	**
DSM-5 checklist	2.6 ± 1.6	3.0 ± 2.2	1.5 ± 2.1	1.9 ± 1.6			***	
B-YAACQ	6.9 ± 3.9	7.0 ± 5.6	2.3 ± 3.6	3.1 ± 5.0			***	***
DMQ-R								
Social	7.3 ± 1.3	7.7 ± 1.3	6.2 ± 2.0	6.4 ± 1.9			*	**
Coping	4.3 ± 1.5	4.8 ± 1.9	3.8 ± 1.2	3.9 ± 1.2				
Conformity	4.3 ± 1.6	4.3 ± 1.1	3.8 ± 1.2	4.3 ± 1.4				
Enhancement	6.8 ± 1.3	6.6 ± 1.3	5.5 ± 1.8	5.0 ± 1.6			**	***
GAD-7	4.1 ± 4.0	6.3 ± 5.2	4.0 ± 5.1	6.0 ± 5.4				
PHQ-9	4.3 ± 4.5	4.7 ± 4.5	4.1 ± 4.5	4.5 ± 4.9				
ABIS								
Motor	8.0 ± 1.8	8.0 ± 2.3	7.4 ± 1.9	7.1 ± 2.2				
Attention	9.3 ± 1.5	9.0 ± 2.0	9.2 ± 2.3	8.7 ± 2.2				
Non-planning	8.0 ± 2.9	6.9 ± 1.7	7.2 ± 2.5	6.8 ± 2.8				
BSSS								
Experience	8.1 ± 1.4	7.5 ± 1.6	7.3 ± 1.9	7.1 ± 1.5				
Boredom	7.6 ± 1.4	7.3 ± 1.4	6.5 ± 1.9	6.6 ± 1.5			*	
Thrill	7.1 ± 2.0	5.9 ± 2.1	6.7 ± 1.9	5.8 ± 2.3	*			
Disinhibition	7.5 ± 1.3	6.3 ± 1.4	6.0 ± 2.0	5.4 ± 2.0	**		**	*
PSS	19.4 ± 3.6	21.4 ± 3.6	19.2 ± 4.4	20.4 ± 5.9	*			
PSQI	4.1 ± 2.2	5.2 ± 3.2	4.0 ± 2.2	5.0 ± 2.9				

Group × Sex means ± standard deviations for all continuous variables. BDm: binge drinking men; BDw: binge drinking women; LDm: light drinking men; LDw: light drinking women. [a]Tested with chi-square; all other comparisons performed using non-parametric Mann-Whitney *U* test. Significant differences are marked with * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. WASI-II: Wechsler Abbreviated Scale of Intelligence; AUDIT: Alcohol Use Disorder Identification Test; PACS: Penn Alcohol Craving Scale; DSM-5: Diagnostic Criteria for Alcohol Use Disorder (self-reported checklist that tallies the number of endorsed diagnostic criteria); B-YAACQ: Brief Young Adult Alcohol Consequences Questionnaire; DMQ-R: Drinking Motivations Questionnaire Revised; GAD-7: Generalized Anxiety Disorder; PHQ-9: Patient Health Questionnaire; ABIS: Abbreviated Impulsiveness Scale; BSSS: Brief Sensation Seeking Scale; PSS: Perceived Stress Scale; PSQI: Pittsburgh Sleep Quality Index.

1999; Mason et al., 2005). In a follow-up report, GABA concentration was modulated by smoking status as only those recently abstinent AUD participants who were also smokers had lower GABA levels in the occipital cortex one week into abstinence (Mason et al., 2006), but that difference normalized after 1 month. Other, well-powered studies used a voxel placement in the ACC and reported lower GABA levels in participants with AUD scanned 2.5 days after the last drink on average (Prisciandaro et al., 2019). The same research group observed lower GABA levels in individuals with co-occurring dual diagnosis of AUD and bipolar disorder within approximately two weeks of abstinence, but not in those with a singular AUD status (Prisciandaro et al., 2017). Meyerhoff and colleagues examined a range of metabolites in the ACC, dorsolateral prefrontal, and posterior cortices, and reported no differences in GABA levels between AUD and control groups at one week (Mon et al., 2012), four (Abe et al., 2013), or five weeks of abstinence (Mon et al., 2012). However, participants with AUD and polysubstance abuse comorbidity showed a strong trend towards lower GABA (Abe et al., 2013). Despite its paucity, the available ^{1}H-MRS evidence is broadly indicative of lower GABA levels in young binge drinkers and in older individuals with AUD, consistent with reduced inhibitory function in alcohol misuse. However, this effect is rather subtle and is not readily observed even in relatively well-powered studies.

Resulting from a confluence of methodological and physiological factors, the lack of robust evidence underscores the fact that GABA signal is very weak, and that its detection is hindered by overlapping resonances of other metabolites, and large voxel sizes. Furthermore, its variability derives from the temporal dynamics of the allostatic excitatory dominance which is sensitive to the length of abstinence (Moeller et al., 2016). In a recent study, Prisciandaro and colleagues (Prisciandaro et al., 2020) examined the time course of GABA changes in the ACC in a group (N = 23) of treatment-naïve, non-treatment seeking individuals with AUD. They were scanned repeatedly after delays of one, three, and seven days since the last drink in a longitudinal design. The AUD participants showed an increase in GABA between days 1 and 3, suggesting GABA normalization within that time span. Even though the AUD group was categorized as non-severe, all participants in that study met the DSM-IV criteria for alcohol dependence. They all reported consuming at least 20 drinks per week, and experiencing 7.8 weekly binge episodes in the two weeks preceding the study, indicating very heavy alcohol use. At 27 ± 6 years of age, the AUD participants were ~ 5 years older than the BD participants in the present study. Our BD participants reported consuming 10.3 drinks and engaging in 0.8 binge episodes per week, which is considerably less than the AUD participants in the study by Prisciandaro and colleagues. Nonetheless, our results (Fig. 5) replicate their findings rather faithfully in a cross-sectional design. In both studies, reduced GABA levels were measured one day after the last drink, which increased at the next scan that took place two days after the last drink (in the present study), or with a three-day delay (Prisciandaro et al., 2020). Comparing GABA levels with 1-day temporal resolution is indicative of the time course of GABA neuroplasticity.

Importantly, it confirms that the E/I balance is tipped towards hyperexcitability as a function of alcohol misuse, complementing direct measures of neural excitability (Correas et al., 2020). Furthermore, small sample size notwithstanding, the present study has found lower GABA + in BDs compared to LDs when measured with one-day delay since the last drink (Fig. 5 a). The drinking patterns characterizing BD and LD groups were consistent across all three delays, confirming that the GABA changes reflected inherent neuroplasticity, and not sensitivity to incidental aberrations in consumption (Fig. 5b). Indeed, it has been established that GABA homeostatic plasticity is characterized by relatively short time scales. A direct relationship between GABA levels and visually-evoked neural activity in the occipital cortex has been demonstrated by Lunghi and colleagues who cleverly manipulated neural plasticity of the visual cortex (Lunghi et al., 2015a; Lunghi et al., 2015b). Combining ultra-high-field ^{1}H-MRS and event-related potentials, they showed that a short-term (150 min) monocular deprivation reduced GABA levels in the occipital cortex, while increasing the early visual responsivity. Taken together, these lines of evidence strongly suggest that the future ^{1}H-MRS studies of alcohol misuse need to consider the temporal sensitivity of GABA changes.

The present results also have implications for subclinical neuroadaptation to alcohol at the levels of consumption that are within a normative range. Our study indicates that compensatory neuroadaptation can be detected in young emerging adults who engage in binge drinking occasionally. To put this in perspective, our BD group comprised individuals who reported engaging in 3.2 binge episodes per month which is below the national average of 4.4 monthly binge drinking episodes for 2015 (Kanny et al., 2018). Similarly, our BD group reported consuming 10.3 drinks per week on average, which does not quite reach the national average of 12.5 drinks per week reported for people 14 years or above in the USA in 2018, accounting for the proportion of the population that drinks (Slater and Alpert, 2021). Furthermore, our group of light, social drinkers included only those individuals who drink at least once per month, though at low levels, averaging at 2.1 drinks per week. People who do not imbibe alcohol were not included. These findings suggest that the neural tissue is sensitive to alcohol consumption at the levels that are within the socially acceptable and expected range and may not be perceived as harmful. This is consistent with recent evidence from large-scale studies indicating that there are no “safe drinking” levels and that harmful health outcomes are minimized by avoiding alcohol (Griswold et al., 2018).

Most ^{1}H-MRS studies focusing on neurochemical markers of alcohol misuse are not sufficiently powered to additionally examine possible differences between men and women as a function of drinking history. The present study was designed to address the question of sexually dimorphic impact of heavy drinking on GABA+, given its sensitivity to the hormonal status (Belelli and Lambert, 2005; Puia et al., 1990). While the BD vs LD differences were primarily driven by LD women, there was no interaction between Sex and a history of binge drinking (Fig. 3). Higher GABA + levels were observed in LDw compared to LDm. Because cycling neurosteroids interact with GABA signaling and with alcohol in complex ways (Barth et al., 2015; Del Rio et al., 2018; Gunn et al., 2011), further research is needed. Please see Supplementary Material for more details and discussion of the Sex-related findings.

It should be noted that participants completed a DSM-5 checklist and endorsed the criteria for AUD diagnosis, but were not evaluated for psychiatric diagnoses using standard interview methods such as the Structured Clinical Interview for DSM-5 (First et al., 2015). This represents a limitation of the study, since the participants’ AUD status was not ascertained in a standard manner, limiting direct comparisons with studies on AUD.

In sum, GABA + levels are lower in young emerging adults who engage in binge drinking than in their light-drinking counterparts, suggesting that subclinical neuroadaptation is present even at the levels of consumption that are within a normative range. The GABA + reduction is associated with bouts of high-intensity drinking, which is consistent with compensatory increase in neural excitability in BDs (Correas et al., 2020), as predicted by a mechanistic model of allostasis (Koob and Le Moal, 2008). The study provides insight into the time course of GABA + changes, indicating that GABA + is particularly reduced one day after drinking. While the BD vs LD differences were observed reliably in women, sex differences did not depend on a recent history of binge drinking.

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CRediT authorship contribution statement

Ksenija Marinkovic: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Austin B. Alderson Myers:** Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Donatello Arienzo:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – review & editing. **Martin I. Sereno:** Methodology, Software. **Graeme F. Mason:** Methodology, Software, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2022.103091.

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