Journal of Nuclear Medicine, published on September 3, 2019 as doi:10.2967/jnumed.119.228825

Recovery of decreased metabotropic glutamate receptor 5 availability in abstinent alcohol-dependent patients

Running title : Recovered mGlu5 in alcohol patients

Jenny Ceccarini PhD[1*], Gil Leurquin-Sterk MD PhD[1*], Cleo Lina Crunelle PhD[2,3], Bart de Laat PhD[1,4], Guy Bormans Pharm PhD[5], Hendrik Peuskens MD PhD[6], Koen Van Laere MD PhD DSc[1,4]

*These authors contributed equally to this work

1.Department of Nuclear Medicine and Molecular Imaging, UZ Leuven and Department of Imaging and Pathology, KU Leuven, 3000 Leuven, Belgium

2.Toxicological Center, University of Antwerp, 2610 Wilrijk, Belgium

3.Department of Psychiatry, Universitair Ziekenhuis Brussel, 1090 Brussels, Belgium

4.MoSAIC, Molecular Small Animal Imaging Center, KU Leuven, 3000 Leuven, Belgium

5.Laboratory for Radiopharmacy, KU Leuven, 3000 Leuven, Belgium

6.University Psychiatric Center, KU Leuven, Kortenberg and Kliniek Broeders Alexianen, 3300 Tienen, Belgium

Corresponding Author: Jenny Ceccarini, PhD

Department of Nuclear Medicine and Molecular Imaging, Department of Imaging and Pathology, KU Leuven,

Herestraat 49, 3000 Leuven, Belgium

jenny.ceccarini@uzleuven.be; Phone: +3216343715; Fax: +3216343759.

ORCID ID : 0000-0003-2774-9516

Number of word counts : 5500

Financial support : Koen Van Laere is senior clinical research fellow for the Research Foundation-Flanders (FWO) and has received an FWO research grant for this work (FWO/G.0548.06). Jenny Ceccarini is a FWO postdoctoral fellow. Bart de Laat received a scholarship from the Flemish Agency for Innovation (IWT).

ABSTRACT

Animal models of alcohol dependence and relapse demonstrate an important role of the glutamatergic system, in particular the cerebral metabotropic glutamate receptor 5 (mGluR5). Using ^{18}F-FPEB positron emission tomography, it was found that chronic alcohol use leads to decreased limbic mGluR5 availability, where lower mGluR5 was associated with less craving. Here, we tested whether the state of decreased mGluR5 availability in alcohol-dependent patients normalizes during abstinence (at 2- and 6-month of detoxification) and whether initial mGluR5 imaging parameters can predict individual relapse. **Methods:** ^{18}F-FPEB scans were performed in 16 recently detoxified alcohol-dependent patients (baseline condition), after 2 months (n=10) and 6 months (n=8) of detoxification, in comparison to 32 age- and gender-matched controls. mGluR5 availability was quantified by ^{18}F-FPEB total distribution volume using both voxel-by-voxel and volume-of-interest analysis. During follow-up, craving was assessed using the Desire for Alcohol Questionnaire, and alcohol consumption was assessed by a Time-Line Follow Back and monitored by hair ethyl glucuronide analysis. **Results:** During abstinence, alcohol-dependent patients showed a sustained recovered mGluR5 availability in cortical and subcortical regions compared to baseline, up to the levels observed in controls after 6 months in most areas except for the hippocampus, nucleus accumbens and thalamus. A higher striato-pallidal mGluR5 availability was observed at baseline in patients who relapsed during the 6-month follow-up period (+25.1%). Also, normalization of striatal mGluR5 to control levels was associated with reduced craving ("*Desire and intention to drink*" and "*Negative reinforcement*", range r=0.72-0.94). **Conclusions:** Reduced cerebral mGluR5 availability in alcohol-dependent patients recovers during abstinence, and is associated with reduced craving. Higher striatal mGluR5 availability in alcohol-dependent users may be associated with long-term relapse.

Key Words: mGluR5, alcohol addiction, abstinence, craving

INTRODUCTION

Recovery from alcohol dependence remains challenging with high rates of relapse, with only moderate effect of available therapies (1). Understanding the neurobiological mechanisms that mediate successful recovery and identifying suitable biomarkers that may predict relapse and/or guide therapeutic intervention have become central in alcohol addiction outcome research. PET neuroimaging in particular holds promise because of its capacity to link molecular mechanism and behavior (2). Using the highly selective and potent metabotropic glutamate receptor 5 (mGluR5) PET radioligand ^{18}F-FPEB (3–6), it was recently shown that alcohol-dependent patients show decreased cerebral mGluR5 availability in various cortical and subcortical limbic areas during the first two weeks of abstinence (7), that was associated with higher alcohol consumption in the period preceding abstinence. Furthermore, decreasing mGluR5 function by specific pharmacotherapy such as negative allosteric modulation or antagonism might improve outcome in abstinent alcohol-dependent patients. Indeed, the mGluR5 antagonists were able to prevent relapse in animal models (8–10). Additionally, although the anti-craving effect of Acamprosate was initially believed to act in part by inhibiting N-methyl-D-aspartate receptor and mGluR5 function (11), actions on these targets remain not fully clear. On the other hand, the development of positive allosteric mGluR5 modulators have revealed that allosteric activation of this receptor may also be of potential therapeutic benefit for the treatment of numerous CNS disorders, including drug addiction and deficits in extinction learning (12–15). The reduced mGluR5 availability observed in alcohol-dependent patients could also represent a pre-existing condition. Genetic studies have shown that individuals with specific mGluR5 polymorphisms are at higher risk for developing alcohol dependence (16,17). Also, our previous PET work in healthy social drinkers found that higher mGluR5 availability is associated with higher novelty-seeking (18). Moreover, lower mGluR5 binding in alcohol-dependent patients was associated with lower craving (7), and therefore with a lower relapse risk.

So far, no longitudinal PET investigations of mGluR5 in alcohol-dependent patients or other substance use dependence have been reported. In a longitudinal rat model of alcohol consumption, ^{18}F-FPEB PET showed that self-administration of alcohol resulted in a decreased mGluR5 availability in the hippocampus

and amygdala compared with baseline (19). Similarly, mGluR5 decreased during exposure to cocaine in the hippocampus (20). Regarding glutamate changes during withdrawal, to date, few studies provide evidence for a hyperglutamatergic state during early abstinence, which normalize within 14 days (21,22). Additionally, a short-lasting increase in glutamate concentration has been demonstrated after 8-12 hours upon acute forced alcohol administration (21,23,24). Recent preclinical mGluR5 imaging data have complemented these previous results showing a decreased prefrontal glutamate concentration during alcohol or cocaine exposure which normalized after few weeks of abstinence (19,20), whereas, over longer abstinence periods, other findings showed a trend toward an increase in central glutamate levels (25,26), or no changes (27).

Here, cerebral mGluR5 availability in alcohol-dependent patients was assessed at 2- and 6-month after alcohol cessation and compared to mGluR5 availability in the recent detoxification (within two weeks of abstinence). Additionally, we investigated the relation of imaging parameters to craving, as well as potential predictive value to baseline imaging parameters by investigating whether regional initial mGluR5 availability in alcohol-dependent patients would be different in patients who relapsed during the 6-month follow-up period versus those who did not.

MATERIALS AND METHODS

Participants

The local University Ethics Committee approved this study and all subjects signed a written informed consent. The group of 16 alcohol-dependent patients included in the current study was identical as in Leurquin-Sterk et al.´s study (7). In this subsequent study, all alcohol-dependent patients engaged in a standard detoxification program consisting of a 2-week in-patient period with medically supervised abstinence followed by a regular follow-up by a board-certified psychiatrist specialized in addiction. Briefly, exclusion criteria were substance use disorders other than alcohol, with the exception of nicotine dependence, any other psychiatric diseases, chronic use of benzodiazepines, and abnormal findings on physical examination, blood test, urine toxicology or structural magnetic resonance imaging (MRI) (7). The first ^{18}F-FPEB PET scan (baseline condition) was performed within the 2 first weeks of medically supervised abstinence (7). Participants agreed to come back at 2 and 6 months after the start of the alcohol detoxification program for a follow-up visit including a physical examination, blood tests, urine toxicology, standard questionnaires and a structural MRI and ^{18}F-FPEB PET scans. Craving at these evaluation moments was assessed using the shortened 13-item version of the Desire for Alcohol Questionnaire (28).

The sample of healthy controls (n=32) represents a subsample of a previous study (18) and was randomly selected based on age by a person blind to both study protocols in order to obtain two age-matched controls for each patient. All controls were non-smokers, had negative urine toxicology, normal structural MRI, free of current or past psychiatric disorders including substance use disorders, reported low alcohol consumption (≤7 units per week) and were excluded if they reported regular binge drinking (≥5 units in one occasion) (7,18).

Assessment of Alcohol Consumption and Relapse During Follow-up

For the entire follow-up period, self-reported alcohol consumption was recorded daily using the Time-Line Follow Back method (29). Relapse was defined when patients reported at least one drinking

day with an alcohol consumption equivalent to the period before the start of the initial detoxification program before the baseline scan. Effective reduction in alcohol consumption compared to baseline was also objectively verified at the 2- and 6-month follow-up visit by quantitative measurements of the alcohol metabolite hair ethyl glucuronide (hEtG) (30). As described previously (7), a 3-cm strand of scalp hair cut as proximally as possible was analyzed, to obtain a quantitative measure of alcohol consumption over the prior 3 months.

MRI and mGluR5 PET imaging

Brain MRI and PET scans were repeated at 2- and 6 months during follow-up using the same procedures as for the baseline scans (3,18) (Table 1). Individual MRI images were used for automatic volumes-of-interest (VOI) determination and co-registration with PET images, as well as for a voxel-based morphometry (VBM) analysis. ^{18}F-FPEB PET data were acquired on a HiRez Biograph 16 slice PET/CT camera (Siemens Inc, Erlangen, Germany) between 12:00 and 14:00. A dynamic PET acquisition was performed for 90 min following bolus injection of ^{18}F-FPEB (3).

PET data were analyzed using PMOD software (v3.605, PMOD Technologies, Zurich, Switzerland) and statistical parametric mapping (SPM 12). Parametric maps of ^{18}F-FPEB total distribution volume (V_T) were generated using the Logan graphical approach (31), and used as surrogate for mGluR5 availability. Additionally, a VOI analysis was performed using the N30R83 Hammers probabilistic atlas (32) in which V_T values were derived from a reversible two-tissue compartment model (3,4). Because brain atrophy in alcohol-dependent patients may be partially reversible already during the first month of abstinence (33,34), voxel- and VOI-based PET data were also calculated with partial volume correction (PVC) using Muller-Gartner (35) and Geometric Transfer Matrix (36) methods, respectively. Effective changes in gray matter volumes at 2- and 6-month abstinence compared to the baseline condition were assessed using a VBM analysis (with SPM12, using the VBM8 toolbox, (7)).

Statistical Analysis

Statistical analyses were performed using Statistica version 12 (Statsoft, Tusla, Oklahoma). Normally distributed variables are reported as mean±SD and skewed variables as median (interquartile range). Potential within-subject changes in mGluR5 availability at the 2- and 6-month follow-up visits compared to the baseline condition were assessed at the voxel level using SPM12 with paired *t*-tests. For the SPM analysis, V_T images were spatially smoothed using a Gaussian kernel of 12 mm. The SPM threshold was set at $p_{height}<0.001$ with a minimal cluster size extent (k_{ext}) of 200 voxels. Only significant clusters with $p_{cluster}<0.05$ (corrected for multiple comparisons) were retained. VOI-based ^{18}F-FPEB V_T values were used to investigate whether mGluR5 availability in alcohol-dependent patients during follow-up remains significantly lower compared to controls (n=32; (7)) using two-sided t-tests for independent samples. On the basis of previous evidence (37), regarding the group differences in mGluR5 availability between the alcohol-dependent patients at baseline and controls, the results were controlled for smoking status (7) supporting that group differences in smoking did not affect the present mGluR5 findings. The results have been controlled for smoking status using a multivariate general linear model with V_T values as dependent variables and group status, smoking status as independent predictors. Noteworthy, no significant V_T differences were observed between smokers and non-smokers in alcohol-dependent subjects in any region. For the current study, the smoking patients did not change their amount of smoking during the follow-up.

Moreover, an independent samples *t*-test was used for comparing baseline V_T between patients with and without relapse, and Pearson correlations were used to estimate the association between baseline V_T values and alcohol consumption during follow-up. Correlation analyses were performed between mGluR5 availability and craving dimensions changes in those regions showing a difference in baseline V_T between patients with and without relapse. In addition, the effect of baseline V_T values on alcohol consumption during follow-up was tested by using a Poisson model for the outcome "*number of drinks/week*" and a linear model for the outcome "*number of drinks/drinking day*".

RESULTS

Subjects and Follow-up Data

From the 16 alcohol-dependent patients that were initially recruited for the baseline condition, 10 and 8 patients underwent the 2- and 6- month follow-up evaluation, respectively (Table 1). Subjects that did not come back for the study had a documented relapse by either a board-certified psychiatrist specialized in addiction or a family member during follow-up. Moreover, 2 patients had a relapse during the 2–6 months interval (i.e., resumed alcohol consumption corresponding to the one prior detoxification), and they were consequently considered as relapsers. Eleven alcohol-dependent patients were cigarette smokers (as reported in (7), average daily number of cigarettes=18.5, Fagerstrom Test for Nicotine Dependence score=5.5±2.7). Among this group of smokers, six patients scanned at the 2- and 6-month visits did not change their smoking pattern during follow-up (16.2±4.8 cigarettes per day).

Changes in Brain Volumes and in mGluR5 Availability Over Time

VBM and VOI-based analyses did not show changes in gray matter volumes at 2- and 6-month abstinence in comparison to the baseline condition (Supplemental Fig. 1). Comparing PVC ^{18}F-FPEB V_T obtained after 2-month of abstinence with the baseline condition, the SPM analysis showed a significantly increased mGluR5 availability in large bilateral cortical and subcortical clusters, covering the hippocampus and parahippocampal gyrus, middle and superior frontal gyrus, anterior cingulate cortex, insula, putamen and left inferior temporal lobe (p_{height}<0.001, t>5.7) (Fig. 1A). When the T-maps were interrogated at the most stringent peak voxel level of $p_{height(FWE)}$=0.05, the most significant clusters showing higher mGluR5 binding were found in the hippocampus/parahippocampal gyrus and orbitofrontal gyrus (Table 2). Comparing the 6-month follow-up to the baseline condition demonstrated a similar regional increase in mGluR5 binding as observed at 2-month follow-up (p_{height}<0.001, t>6.5) (Fig. 1B, Supplemental Table 1).

mGluR5 Availability in Alcohol-Dependent Patients during Abstinence compared to Healthy Controls

At baseline, alcohol-dependent patients demonstrated lower mGluR5 binding in corticolimbic regions (14-36%) compared to controls (7). After 2 months of abstinence, mGluR5 levels in alcohol-dependent patients were still significantly lower compared to the controls (16-33%), particularly in the thalamus (-33±10%; p=0.00001, Bonferroni corrected) and hippocampus (-28±1%; p=0.002, Bonferroni corrected) (Fig. 2). In contrast, at 6-month follow-up, mGluR5 availability reached the level of controls in most brain regions (-6±5%; range p=0.12-0.79) (Fig. 2), except for the hippocampus (-26±21%; p=0.002), thalamus (-21±20%, p=0.01) and nucleus accumbens (-18±19%; p=0.04). At 2-month follow-up, none of the regions had higher V_T values than controls. At 6-month follow-up, the orbitofrontal cortex (OFC) was the only region that showed significantly higher V_T values (+19%, p=0.01) compared to controls. Similar mGluR5 changes were observed using no PVC data (Supplemental Fig. 2), except for the increased V_T found in the OFC at 6-month follow-up compared to controls.

Relationship between mGluR5 Availability, Relapse and Craving during Follow-up

Voxel-based analysis demonstrated that patients who relapsed during the 6-month follow-up (*relapsers*) had a higher baseline mGluR5 availability (+25.1%) compared to patients who did not relapse (*abstainers*), in a cluster located in the anterior putamen and globus pallidus (Fig. 3). When the number of drinks per week and the days between the last alcoholic consumption and the baseline scan were taken as covariates, the results were unaltered. No significant effects of baseline V_T values on the number of drinks per week (Supplemental Table 2) or the number of drinks per drinking day during follow-up were found (all p>0.3) or changes in hEtG values during follow-up.

There was a positive correlation between the % positive change in mGluR5 binding in striatal regions and the reduction in the craving dimensions "*Desire and intention to drink"* (caudate: r=0.94, p=0.001; putamen: r=0.75, p=0.032; nucleus accumbens: r=0.77, p=0.025) and "*Negative reinforcement"* (caudate: r=0.92, p=0.001; putamen: r=0.72, p=0.045; nucleus accumbens: r=0.74, p=0.038).

DISCUSSION

The reduced cerebral mGluR5 availability in alcohol-dependent patients after a severe drinking period (7), represents either a pre-existing biological trait, predisposing to alcohol dependence, or, alternatively, a state of mGluR5 downregulation. Regardless whether decreased mGluR5 availability in alcohol-dependent patients is a consequence of alcohol dependence, mGluR5 reduction may influence recovery from the disease. Indeed, excessive glutamatergic neurotransmission associated with alcohol withdrawal and prolonged abstinence is thought to be a central mechanism contributing to craving and relapse (38). Thus, a decrease in post-synaptic mGluR5 could help compensate for this hyperglutamatergic state, thereby improving the odds of patients to remain sober in the long term.

The current study is the first human study where mGluR5 is longitudinally assessed in vivo. Already after 2 and certainly after 6 months of alcohol detoxification, the recovery of mGluR5 availability in patients achieved levels comparable to healthy controls for all brain regions except for the hippocampus, nucleus accumbens and thalamus. In patients with chronic alcohol dependence, a persistent sensitization of these subcortical limbic-striatal regions that are involved in reward and memory processes could compromise the prefrontal regulatory function, which in turn affects the recovery from alcohol dependence (39). Overall, these findings support the hypothesis that mGluR5 downregulation represents a lasting but reversible consequence of chronic alcohol consumption, rather than a pre-existing trait. However, based on the long-lasting reduction of mGluR5 signaling after 6 months of abstinence in limbic-striatal regions such as hippocampus and nucleus accumbens, specific regional selective mGluR5 decrease may be considered as a risk factor for alcohol dependence. Noteworthy, in recent cross-sectional PET study in smokers and ex-smokers, similar reversible effects were suggested (40). These findings are in accordance with preclinical studies suggesting that mGluR5 downregulation represents a common neuro-adaptation in the transition from controlled use to dependence for several drugs of abuse (41,42). A longitudinal microPET experiment on a cocaine self-administration rat model also showed decreased mGluR5 availability after cocaine exposure, more pronounced in the hippocampus (20). Similarly, ^{18}F-FPEB PET

showed that self-administration of alcohol resulted in a decreased mGluR5 availability in the hippocampus and amygdala compared with baseline (19). Similarly, extinction from the drug was associated with a full recovery of mGluR5 availability after 14 days. Interestingly, animals with lower cocaine-induced reduction in mGluR5 availability, specifically in the hippocampus, showed more severe relapse-like behaviors following withdrawal. This observation was consistent with the hypothesis that mGluR5 downregulation represents a beneficial adaptation to chronic drug use, potentially by regulating craving behavior during abstinence. Nevertheless, translating findings derived by animal models of alcoholism to human should be approached and interpreted with caution.

Based on the current findings, some hypotheses can be formulated about the functional and clinical implications of reduced mGluR5 availability in alcohol dependence and its partial recovery during abstinence. Within 2 weeks of reaching abstinence, lower mGluR5 availability was associated with lower craving for alcohol (7). Patients who relapsed during the 6-month follow-up period had higher baseline mGluR5 availability compared to abstainers in a cluster comprising the anterior putamen and internal globus pallidus. These two subcortical nuclei are part of the dorsal striatum, which is centrally involved in habit formation and where lasting drug-induced neuroadaptations driving relapse behaviors are suggested to occur (16,41,43).

Furthermore, a better striatal mGluR5 recovery toward normalization was associated with higher reduction in the *desire and intention to drink* and *negative reinforcement* aspects of craving. The direction of this association was unexpected, as lower mGluR5 availability at the start of detoxification was associated with lower craving levels. A possible explanation could be that the normalization of the alcohol-induced changes in the glutamatergic state that occurs during abstinence (21–24) represents the causal factor for both the recovery of mGluR5 and the reduction in craving. In a recent longitudinal alcohol rat model, prefrontal glutamate concentration decreased during alcohol exposure, but normalized after 1 week of abstinence (19). Noteworthy, full recovery of mGluR5 availability in the hippocampus, nucleus accumbens and thalamus was not achieved yet at 6-months after alcohol detoxification. Given the important role of these limbic-striatal regions in regulating addictive behaviors as emotion regulation,

decision making, impulse control and alcohol craving (39,44), longitudinal studies with longer follow-up may be warranted. For instance, lasting impairment of mGluR5-dependent plasticity in these regions might contribute to the relative inability of dependent individuals to extinguish alcohol-associated memories and to form adaptive behaviors to prevent alcohol consumption (45,46). As such, future studies could also include other behavioral outcomes such as impaired memory and cognitive control processes which are inherent to drug dependence (47,48) and are suggested to depend on the mGluR5 (45,49). Collectively, the present data supports the view that modulating mGluR5 signaling by pharmaceutical interventions might be effective in preventing relapse (12–14), as has been suggested earlier in preclinical studies (8–10,45,46).

Some limitations of this study merit comment. First, due to the small sample size, the correlations between mGluR5 changes and craving during follow-up should be considered preliminary and await independent replications in larger samples. Another potential confound is the fact that true mGluR5-related relapse risk may depends on the effect of two interacting factors: *1)* the natural receptor state, where higher mGluR5 availability may be associated with higher novelty-seeking (18) and thereby conferring a higher relapse risk, and *2)*, the receptor state after exposure to chronic, large amounts of alcohol which likely downregulates mGluR5, where lower mGluR5 availability is associated with lower craving and relapse risk. Moreover, although an appealing underlying mechanism for the reversible decrease in mGluR5 availability in patients is the changes in the glutamatergic activity varying over the time of abstinence, this hypothesis was not tested here, and could be addressed by combining longitudinal mGluR5 PET with MR spectroscopy (MRS) of glutamate and its metabolites, in relation to withdrawal severity during early and prolonged abstinence. Indeed, although using MRS no straightforward conclusions can be made for an excess or deficit of available glutamate at the mGluR5 receptor site, combining PET and MRS and receptor blockade might be useful for assessing the effect of modulating mGluR5 signaling on alcohol addiction.

CONCLUSION

In summary, we found that decreased cerebral mGluR5 availability in alcohol-dependent patients partially recovers during abstinence, with observable changes already after two months. Higher striatal mGluR5 availability was observed in relapsers compared to abstainers, and higher mGluR5 recovery was associated with higher reduction in craving scores during abstinence. As such, mGluR5 availability could be a marker of disease progression.

DISCLOSURE

Authors report no biomedical financial interests or potential conflicts of interest.

ACKNOWLEDGEMENTS

We acknowledge Kwinten Porters, Jef Van Loock, the radiopharmacy and radiology team UZ Leuven. Koen Van Laere is senior clinical research fellow for the Research Foundation-Flanders (FWO) and has received an FWO research grant for this work (FWO/G.0548.06). Jenny Ceccarini is a FWO postdoctoral fellow. Bart de Laat received a scholarship from the Flemish Agency for Innovation (IWT).

KEY POINTS

QUESTION: Does the state of decreased mGluR5 availability in alcohol-dependent patients normalize during abstinence ?

PERTINENT FINDINGS: In a case-control study comparing cerebral mGluR5 availability in alcohol-dependent patients during abstinence to the recent detoxification (baseline) phase, we found that after 6 months of detoxification, alcohol-dependent patients showed a sustained recovered mGluR5 availability in cortical and subcortical regions compared to baseline, up to the levels observed in controls, in most areas except for the hippocampus, nucleus accumbens and thalamus. Also, higher striatal mGluR5 availability was observed in relapsers compared to abstainers, and higher mGluR5 recovery was associated with higher reduction in craving scores during abstinence.

IMPLICATIONS FOR PATIENT CARE: In vivo assessment of cerebral mGluR5 availability could be a potential biomarker in the development of treatments for alcoholism.

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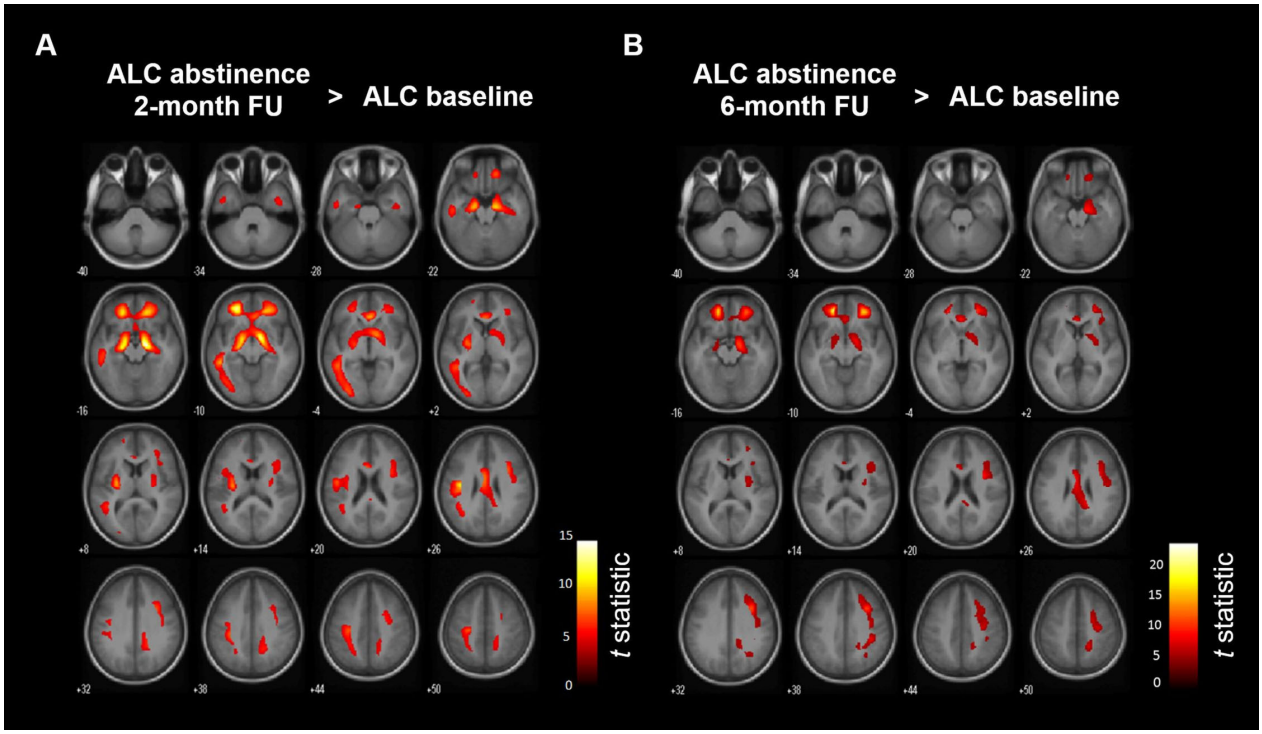


FIGURE 1. SPM of increased mGluR5 availability in alcohol-dependent patients (ALC) after A) 2 months (*n*=10) and B) 6 months of abstinence (*n*=8) compared to baseline (*n*=16).

ALC, alcohol-dependent patients; FU, follow-up.

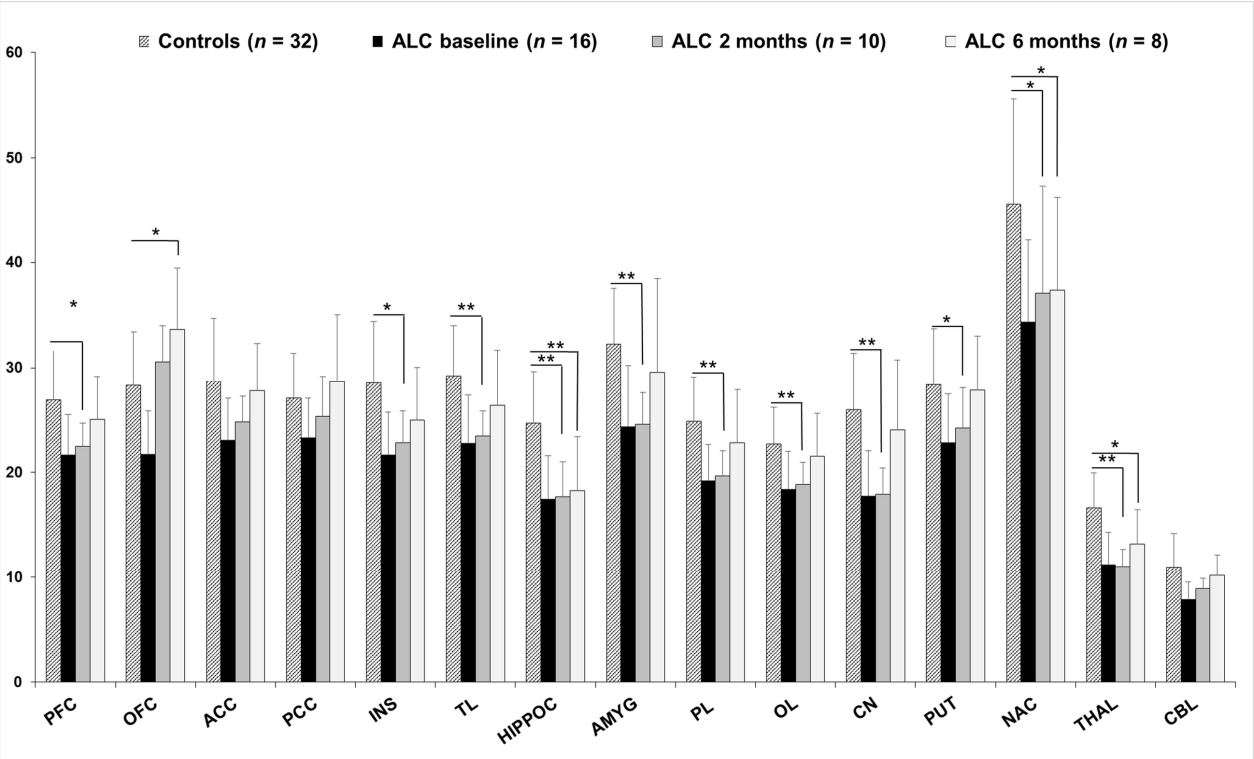


FIGURE 2. Average ^{18}F-FPEB V_T in healthy controls and in alcohol-dependent patients (ALC) at baseline, and at 2- and 6-month follow-up during abstinence. Error bars represent one SD. Statistical significances were reported only for ALC 2 and 6 months compared to Controls. ALC baseline versus Controls was reported in (7).

*p<0.05, Two-sided independent t-test; **p<0.05, Bonferroni post-hoc tests.

PFC, prefrontal cortex; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; INS, insula; TL, temporal lobe; HIPPOC, hippocampus AMYG, amygdala; PL, parietal lobe; OL, occipital lobe; CN, caudate nucleus; PUT, putamen; NAC, nucleus accumbens; THAL, thalamus; CBL, cerebellum.

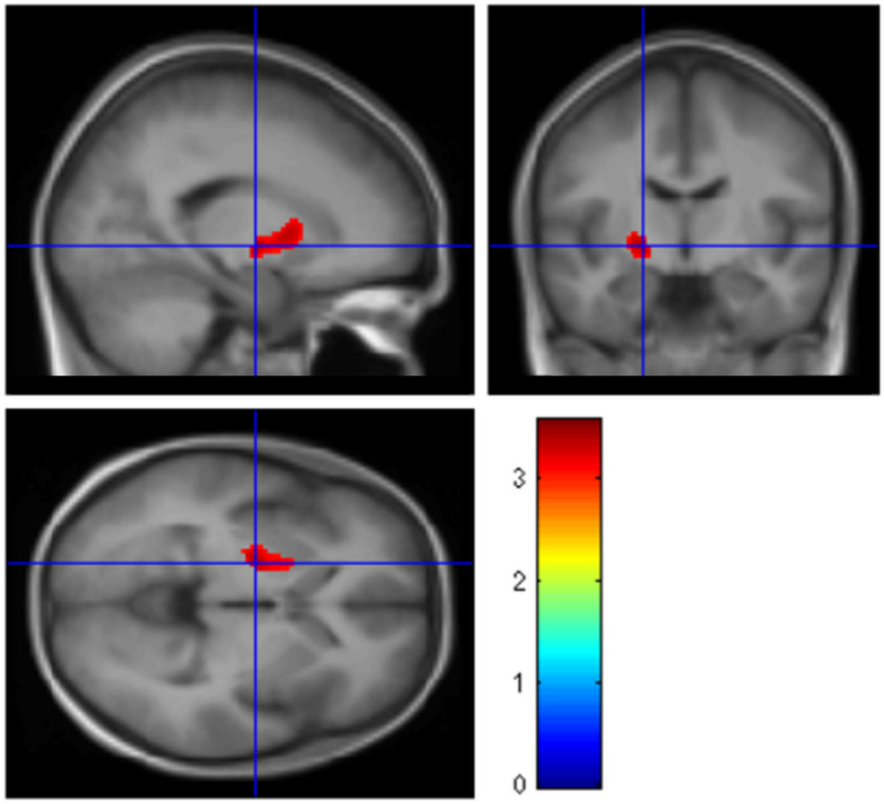


FIGURE 3. SPM showing the higher mGluR5 availability in patients who relapsed during the 6-month follow-up period (*n*=8) compared to the patients who abstained from alcohol (*n*=8).

TABLE 1. Demographic Data

	Controls	**ALC baseline**	**ALC abstinence**	**ALC abstinence**
Patients, no	32	16	10	8
Age (years)	45±13	45±8	46±8	44±8
Gender (female/male)	14/18	3/13	3/7	2/6
ALC craving (DAQ scores)				
Desire and intention	-	16±7	9±4	10±4
Negative reinforcement	-	14±7	6±3	9±7
Control	-	5±3	4±3	7±5
ALC consumption parameters			***Baseline-2 mo interval***	***2-6 mo interval***
Reported ALC use (years)	26.6±8.1	26.5±12.9	-	-
Abstinence (days)	5.8±6.6[§]	7±4*	56±12[§]	101±20[§]
Drinks/week (TLFB)	2.4±2.1	133 (124-184)	1 (0-4.1)	6 (1-8)
Drinks/drinking day (TLFB)	-	19 (18-26)	2 (0-10)	5 (3-10)
hEtG (pg/mg)	8.1 (5.3-17.5)[††]	221 (143-486)[†]	51 (9-118)	61 (30-76)
^{18}F-FPEB imaging				
ID (MBq)	177±7	175±11	174±7	174±5
SA (MBq/nmol)	90±52	97±51	81±38	140±67
IM (μg)	0.63±0.60	0.56±0.43	0.58±0.24	0.34±0.18

Continuous data are mean±SD or median (interquartile range).

ALC, alcohol-dependent patients; mo, months; FU, follow-up; TLFB, Time-Line Follow Back questionnaire; hEtG, hair ethyl glucuronide; ID, injected dose; SA, specific activity; IM, injected mass.

*Medically supervised. [§]Self-reported. [†]*n*=14 (two alcohol-dependent patients refused hair sampling). [††]*n*=29 (three controls refused hair sampling).

TABLE 2. Statistical Parametric Mapping of increased mGluR5 availability in alcohol-dependent patients after 2 months of abstinence compared to the baseline condition.

Cluster Level		**Voxel Level**		**Peak Voxel MNI coordinate**			**Anatomical area**
K_{ext}	***P***	***T***	***P***	**x**	**y**	**z**	
11805	<0.001*	14.7	0.002*	16	-12	-16	R ParaHpc gyrus
		13.8	0.004*	14	-4	-12	R Hippocampus
		13.4	0.004*	-24	42	10	L Middle Frontal gyrus
		11.5	0.010*	-18	-16	-16	L ParaHpc gyrus
		11.0	0.013*	-14	-6	-12	L Hippocampus
		10.6	0.015*	-38	-14	26	L Insula
		10.2	0.020*	20	38	-14	R Superior Orbitofrontal gyrus
		9.2	0.035*	-44	-12	24	L Insula
		9.0	0.039*	4	30	-4	R Ant Cing Cortex
		8.7	0.045*	-28	-12	8	L Putamen
		7.9	$1.2 \cdot 10^{-5}$	-2	0	26	L Middle Cingulum
		7.7	$1.6 \cdot 10^{-5}$	-32	-28	40	L Postcentral gyrus
		7.7	$1.6 \cdot 10^{-5}$	34	10	28	R Inferior frontal cortex
2614	0.006*	9.00	0.038*	-48	-40	-10	L Inferior temporal lobe
		6.53	$5.4 \cdot 10^{-5}$	-50	-24	-20	L Inferior temporal lobe
		6.16	$8.3 \cdot 10^{-5}$	-38	-48	24	L Angular gyrus

K_{ext}, cluster size extent; MNI, Montreal Neurological Institute; L, left; R, right

*Corrected for familywise error.

SUPPLEMENTAL DATA

Supplemental Table 1. Statistical Parametric Mapping of increased mGluR5 availability in alcohol-dependent subject after 6 months of abstinence compared to the baseline condition ($p_{height} < 0.001$).

Cluster Level		**Voxel Level**		**Peak Voxel MNI coordinate**			**Location**	
K_E	***P value***	***T***	***P value***	**x**	**y**	**z**	**Anatomical area**	**Hemisphere**
6058	$6.1 \cdot 10^{-5}$*	22.98	0.004	-18	44	-12	Sup Orbitofront gyr	Left
		15.60	0.018	30	42	-10	Mid Orbitofront gyr	Right
		13.61	0.031	34	12	34	Mid frontal gyr	Right
		8.48	$3.1 \cdot 10^{-5}$	-2	0	26	Middle Cing	Left
		8.47	$3.2 \cdot 10^{-5}$	36	4	20	Insula	Right
		8.36	$3.4 \cdot 10^{-5}$	0	30	-4	Ant Cing Cortex	Right
		7.86	$5.1 \cdot 10^{-5}$	40	-36	38	Supramarginal gyr	Right
		7.64	$6.1 \cdot 10^{-5}$	14	-42	26	Post Cing Cortex	Right
1344	0.034*	10.59	$7.3 \cdot 10^{-6}$	16	-8	-16	ParaHpc gyr	Right
		6.14	$2.4 \cdot 10^{-4}$	28	-2	4	Putamen	Right

BA, Brodmann area; K_E, cluster size extent (number of 2 x 2 x 2 mm3 voxels); mGlu5, metabotropic glutamate receptor subtype 5; MNI, Montreal Neurological Institute; gyr, gyrus.

* Corrected for familywise error.

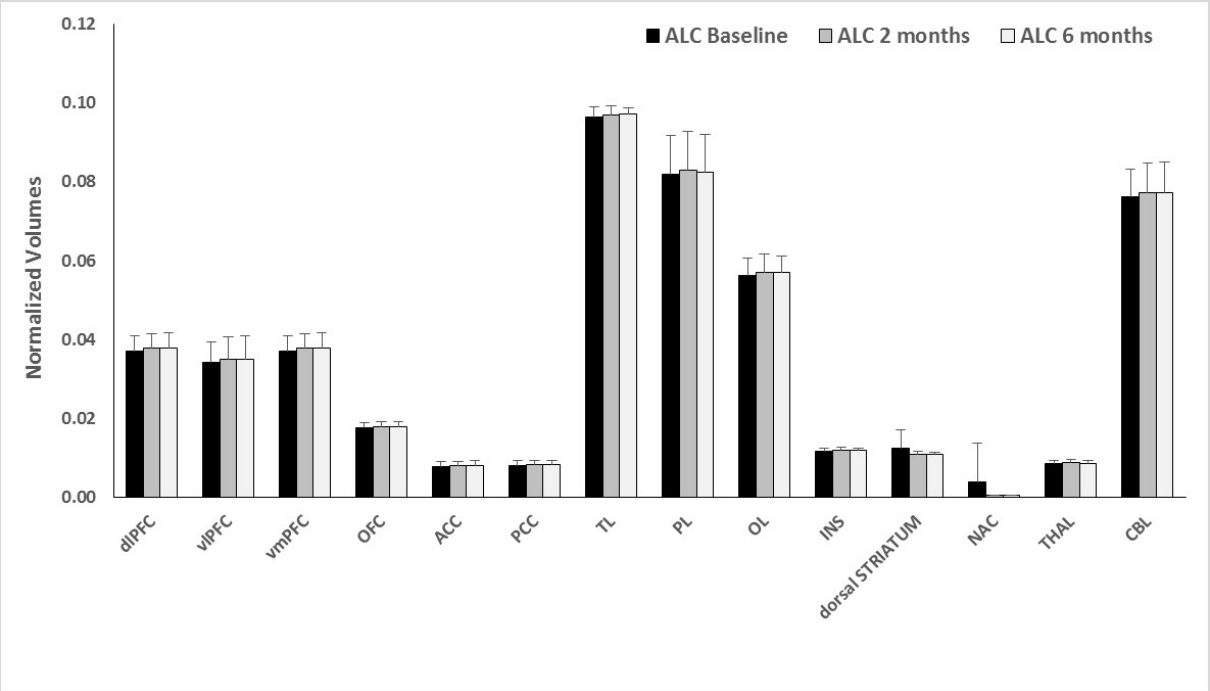
Supplemental Table 2. Effect of baseline mGluR5 availability ([^{18}F]FPEB V_T) on the number of drinks consumed per week during follow-up.

VOI	IRR (95% CI)	*p*
PFC	0.94 (0.57; 1.57)	0.82
Dorsolateral PFC	0.99 (0.58; 1.72)	0.99
Ventrolateral PFC	0.91 (0.56; 1.49)	0.70
Ventromedial PFC	0.92 (0.62; 1.39)	0.71
Orbitofrontal cortex	0.96 (0.59; 1.55)	0.86
Anterior cingulate	0.95 (0.63; 1.43)	0.80
Posterior cingulate	0.92 (0.59; 1.46)	0.74
Insula	0.95 (0.61; 1.47)	0.80
Temporal lobe	0.90 (0.57; 1.43)	0.66
Hippocampus	0.93 (0.57; 1.51)	0.77
Amygdala	1.03 (0.72; 1.47)	0.87
Parietal lobe	0.89 (0.51; 1.53)	0.66
Occipital lobe	0.90 (0.52; 1.58)	0.72
Caudate nucleus	0.80 (0.42; 1.53)	0.49
Putamen	1.07 (0.67; 1.69)	0.77
Nucleus accumbens	1.03 (0.69; 1.54)	0.88
Thalamus	1.24 (0.59; 2.58)	0.56
Cerebellum	1.02 (0.40; 2.58)	0.96

For each patient, the average weekly consumption (number of drinks) was calculated per month. Results are presented as incidence rate ratios (with 95% CIs), expressing the percentage change in consumption associated with a one-unit increase of the predictor (V_T). IRR > (<) 1 indicates higher (lower) number of drinks with increasing V_T (e.g., IRR = 0.9 (1.1) means 10% decrease (increase) in number of drinks with 1 unit increase in V_T.

Abbreviations: IRR, incidence rate ratio; CI, confidence interval.

Supplemental Figure 1. Grey matter volumes normalized by the total intra-cranial volume in alcohol-dependent patients at baseline (ALC baseline, *n* = 16), at 2 and 6 months of abstinence follow-up (ALC 2 months, *n* = 10, and ALC 6 months, *n* = 8).

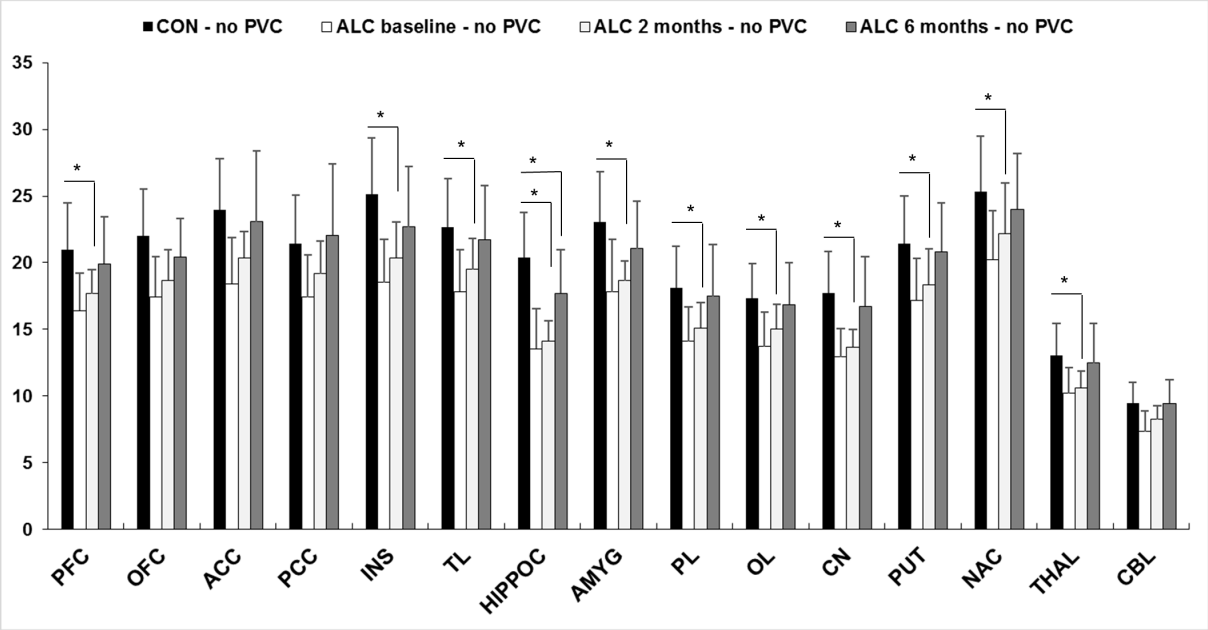


Abbreviations: dlPFC, dorsolateral prefrontal cortex; vlPFC, ventrolateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; TL, temporal lobe; PL, parietal lobe; OL, occipital lobe; INS, insula; NAC, nucleus accumbens; THAL, thalamus; CBL, cerebellum.

Supplemental Figure 2. Average [^{18}F]FPEB V_T (no partial volume correction, no PVC) in healthy controls (CON, *n* = 32) and in alcohol-dependent subjects (ALC) at baseline (recent detoxification; *n* = 16) and at the 2-month (*n* = 10) and 6-month (*n* = 8) follow-up during abstinence. Error bars represent one SD.

Statistical significances were reported only for ALC 2 and 6 months compared to CON. ALC baseline versus Controls was reported in (7).

*p<0.05, Two-sided independent t-test;



Abbreviations: PFC, prefrontal cortex; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; INS, insula; TL, temporal lobe; HIPPOC, hippocampus AMYG, amygdala; PL, parietal lobe; OL, occipital lobe; CN, caudate nucleus; PUT, putamen; NAC, nucleus accumbens; THAL, thalamus; CBL, cerebellum.