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DOI: https://doi.org/10.1038/mp.2013.51

Smoking but not cocaine use is associated with lower cerebral metabotropic glutamate receptor 5 density in humans

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Abbreviated Running Title: mGluR5 density in smokers and cocaine users

Manuscript Category: Original article

Submitted: March 7th, 2013

Manuscript statistics:
Number of words in the abstract: 244
Number of words in the text: 3491
Number of figures: 3
Number of tables: 2
Supplemental information: yes

Financial support: This study was supported by grants from the Swiss National Science Foundation (SNSF; grant No. PP00P1-123516/1) and the Novartis Foundation for Medical Biological Research (11B51).

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Abstract

Long-lasting neuroadaptations in the glutamatergic corticostriatal circuitry have been suggested to be responsible for the persisting nature of drug addiction. In particular, animal models have linked the metabotropic glutamate receptor 5 (mGluR5) to drug-seeking behavior and extinction learning. Accordingly, blocking mGluR5s attenuated self-administration of cocaine and other addictive drugs in rats. How these animal findings extend to humans remains unclear. Therefore, we investigated if human cocaine users exhibit altered mGluR5 availability compared to drug-naïve control subjects. Seventeen male controls (11 smokers) and 18 male cocaine users (13 smokers) underwent positron emission tomography with ^{11}C-ABP688 to quantify mGluR5 availability in 12 volumes of interest in addiction-related brain areas. Drug use was assessed by self-report and quantitative hair toxicology. Cocaine users and controls did not significantly differ in regional mGluR5 availability. In contrast, smokers (n=24) showed significantly lower mGluR5 density throughout the brain (mean 20%) compared to non-smokers (n=11). In terms of effect sizes, lower mGluR5 availability was most pronounced in the caudate nucleus (d=1.50, 21%), insula (d=1.47, 20%), and putamen (d=1.46, 18%). Duration of smoking abstinence was positively associated with mGluR5 density in all brain regions of interest, indicating that lower mGluR5 availability was particularly pronounced in individuals who had smoked very recently.

Specifically tobacco smoking was associated with lower mGluR5 availability in both cocaine users and controls, while cocaine use was not linked to detectable mGluR5 alterations. These findings have important implications regarding the development of novel pharmacotherapies aimed at facilitating smoking cessation.

Keywords: addiction, glutamate, PET, cocaine, nicotine, treatment

Introduction

Cocaine addiction continues to be a major public health concern resulting in high societal and economic costs.[1,2] Notably, cocaine is the second most used illicit drug with 13 to 20 million users worldwide. Annual prevalence rates are relatively high with 1.6% in North America, 1.3% in Central Europe, and 1.5-1.9% in Oceania[3] and the harmful effects associated with cocaine use also become evident through the high treatment demand related to psychostimulants. Accordingly, 17% of all patients who entered drug treatment programs in the USA,[4] and 23% of the drug-seeking patients in Europe did so for the treatment of stimulant addiction.[5] Despite the high treatment demand, effective pharmacotherapies for cocaine addiction are currently lacking.[6]

While acutely reinforcing effects of addictive drugs are mainly dependent on the mesocorticolimbic dopamine system,[7] accumulating preclinical research has provided evidence that longer-lasting neuroadaptations in glutamatergic corticolimbic circuitries may be responsible for the persisting nature of cocaine addiction.[8-10] In particular, glutamatergic projections from the prefrontal cortex (PFC) to the nucleus accumbens (NAcc) appear to be relevant for drug reinstatement and drug-seeking behavior in animals and may also play an important role in craving and relapse-related behaviors in psychostimulant-addicted humans.[9,11] Chronic self-administration in rats resulted in lower basal extracellular, non-synaptic glutamate levels in the NAcc, which in turn has been associated with down-regulation of group I and II metabotropic glutamate receptor (mGluR) expression and function in rats,[12-17] resulting in altered synaptic plasticity. Impaired plasticity has been suggested to contribute to the relative inability to form adaptive behaviors that help inhibiting relapse in drug users.[9]

The mGluR of type 5 (mGluR5), belonging to group I mGluRs, has gained growing attention in addiction research due to its high expression in corticolimbic regions implicated in drug addiction[18] and involvement in drug-seeking behavior and extinction learning in animals withdrawn after cocaine self-administration (for reviews[19,20]). In fact, mGluR5 null-mutant mice neither self-administered cocaine nor exhibited increased locomotor activity after cocaine treatment.[21] Moreover, self-administration and reinstatement of cocaine, nicotine, alcohol, methamphetamine, and heroin was attenuated by mGluR5 antagonists.[22-32] Therefore, much effort is being undertaken to develop

pharmacological compounds with the ability to block mGluR5s, which is assumed to be one efficacious way to treat psychostimulant-addiction.

How preclinical findings regarding changes in mGluR5 expression extend to human cocaine users (CU) has not been investigated to date. The positron emission tomography (PET) radioligand ^{11}C-ABP688 (3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-enone-O-carbon-11-methyl-oxime)[33,34] has made it possible to quantify mGluR5 availability in humans. A recent PET study applying this ligand has demonstrated that smokers exhibit globally lower cerebral mGluR5 density.[35] However, it is not clear if mGluR5 alterations are specific for nicotine addiction or a common phenomenon in drug addiction. Therefore, the aim of the present study was to quantify mGluR5 availability in addiction-related brain regions employing ^{11}C-ABP688 PET in CU and drug-naïve controls. Additionally, we investigated the potential impact of smoking on mGluR5 availability in CU. We expected that both cocaine and tobacco use decrease mGluR5 availability in corticolimbic brain regions.

Materials and Methods

Participants

The study sample consisted of 18 male drug-naïve healthy control subjects (HC) and 18 male CU who also took part in the longitudinal Zurich Cocaine Cognition Study ($ZuCo^2St$).[36-38] Participants were recruited via advertisements in local newspapers, different drug prevention and treatment centers, psychiatric hospitals, internet platforms, and word-of-mouth communication (for details see[37]). Inclusion criteria for CU were 1) diagnoses of cocaine abuse or dependence according to DSM-IV,[39] 2) age between 20 to 50 years, 3) proficiency in German language, 4) no polytoxic drug use pattern and no use of prescription drugs affecting the CNS, 5) no current or previous Axis I DSM-IV psychiatric disorder (other than cocaine abuse/dependence, alcohol abuse in CU, and a former depression), 6) no neurological disorder or head injury, 7) no family history of a severe DSM-IV psychiatric disorder such as schizophrenia, bipolar disorder or obsessive-compulsive disorder, and 8) no metal parts in the body. The same inclusion criteria applied to HC except for 1). Additionally, HC were excluded if they regularly engaged in illegal drug use (>15 occasions) with exception of cannabis use. Participants were instructed to abstain from illegal drugs for ≥3 days and ≥24 hours from alcohol. Urine samples were collected to control for recent drug use. To objectively characterize drug use over the past six months, hair samples (6cm) were collected and analyzed with liquid chromatography-mass spectrometry (see **Supplement**). Participants received financial compensation. This study was approved by the Ethics Committee of the Canton Zurich and the Swiss Federal Office of Public Health. All participants provided written informed-consent before inclusion.

Clinical Interviews and Questionnaires

The *Structured Clinical Interview for DSM-IV Disorders* was carried out by a trained psychologist.[39] Drug use was assessed by means of the *Interview for Psychotropic Drug Consumption*, which has been described in detail elsewhere.[40] The *Beck Depression Inventory* (BDI) was used to assess symptoms of depression.[41] The *Symptom Checklist-90-R* (SCL-90-R) served as a screening measure of general psychiatric symptoms.[42] The brief version of the *Cocaine Craving Questionnaire*

(CCQ) was used to assess current cocaine craving.[43,44] The *Fagerström Test for Nicotine Dependence* (FTND) was used to determine severity of nicotine dependence.[45]

Image Acquisition and Data Analyses

Magnetic Resonance Image Acquisition. T1-weighted magnetic resonance (MR) imaging was acquired for each participant on a Philips Achieva 3T whole-body scanner equipped with a transmit/receive head coil (Philips Healthcare, Best, The Netherlands) to rule out structural abnormalities and for the preprocessing of PET images.

PET Acquisition. The synthesis of ^{11}C-ABP688 has been described in detail elsewhere.[33,34] PET image acquisition was conducted in 3-D mode in the whole-body scanner DSTX PET/computed tomography scanner (B. Braun Medical, Sempach, Switzerland) at the Division of Nuclear Medicine of the University Hospital Zurich. Before the PET acquisition, a catheter was placed in the left antecubital vein for tracer injection and a low-dose CT was conducted for attenuation correction. In order to avoid inconvenient arterial blood sampling, we relied on a previously evaluated equilibrium paradigm[46,47] based on the premise that a steady-state between tracer concentration in tissue and blood can be obtained.[48,49] ^{11}C-ABP688 was administered according to a bolus-infusion protocol,[46,49] where half of the tracer was administered as a bolus over 2min and the other half was infused over 58min (Perfusor FM, Braun Medical). Groups did not differ regarding injected activity [HC: 604±29 MBq, CU: 600±24 MBq, $t(33)=-0.42$, $p=.68$ (two-tailed)]. At tracer injection, a series of 20 scans was recorded over a total duration of 60min (10x60sec, 10x300sec). Applying filtered back-projection, transaxial images were reconstructed to a 128x128 matrix with 47 transaxial slices of 2.3x2.3x3.2mm voxel size.

PET Image Processing and Quantification. Image processing and quantification steps were performed with the PMOD software, version 3.307 (PMOD Technologies, Zurich, Switzerland). Frames 17 to 19 were averaged and spatially normalized to the Montreal Neurological Institute (MNI) template brain. Twelve volumes of interest (VOIs) and a cerebellar reference VOI were generated

based on the standard VOIs of the MNI template brain[50] comprising the following brain regions: ACC, dorsolateral PFC (DLPFC), MPFC, OFC, ventrolateral PFC (VLPFC), caudatus nucleus, putamen, insula, amygdala, parahippocampal gyrus, hippocampus, and thalamus.

To verify that steady-state of receptor binding was reached after 45min (frames 17 to 19), time-activity curves were generated for high (ACC, putamen), medium (thalamus), and low receptor density regions (cerebellum) in both hemispheres for all participants. Steady state of ^{11}C-ABP688 uptake 45min after tracer injection was reached in all participants, save one control subject, who was therefore excluded from the subsequent statistical analyses. Normalized values of distribution ($V_{norm}=C_{T[VOI]}/C_{T[Cer]}$) of ^{11}C-ABP688 uptake constituted the quantitative PET outcome measure and were derived by dividing the average radioactivity concentration between 45 and 55min (frames 17 to 19) of each VOI ($C_{T[VOI]}$) with the commensurate cerebellar radioactivity concentration ($C_{T[Cer]}$). V_{norm} is equivalent to $BP_{ND}+1=((V_T-V_{ND})/V_{ND})+1)$.[51] mGluR5 has been successfully quantified with this approach in previous studies[46,52,53] and a preclinical study showed that the cerebellum is relatively devoid of mGluR5 and thus suitable as a reference region.[54]

Statistical Analysis

Statistical analyses were performed with the PASW 19.0 software (SPSS Inc.). Independent t-tests and frequency analyses (Pearson's Chi^2 test) were conducted to compare groups among demographic and clinical questionnaire data. PET data were analyzed with a mixed-effect analysis of variance (ANOVA) including the between-subject factors *group* (HC, CU) and *smoking status* (non-smokers, smokers), the within-subject factors *brain region* (12 VOIs) and *hemisphere* (left, right), and their interactions. Two-tailed independent t-tests were conducted to identify differences of ^{11}C-ABP688 uptake in the selected VOIs between groups. To account for multiple testing, the Bonferroni-correction was applied, resulting in a significance level of $p<.0042$ ($p=.05/12$). Potential associations of drug use parameters and mGluR5 availability were examined with correlation analyses (Pearson's product-moment). Effect sizes (Cohen's *d*) were calculated with G*Power 3.1.[55] As the assumption of parametric distribution was not met by certain variables, the drug use parameters weekly alcohol, cigarette, cannabis and cocaine consumption (in grams/cigarettes), last alcohol, cigarette, cannabis and

cocaine use (h), cumulative cannabis and cocaine dose (g), and concentrations of cocaine and its metabolites in the hair samples (pg/mg) were log-transformed (log10) and the constant 1 was added because the data contained 0 values.

Results

Demographic Information and Drug Use Patterns

HC and CU were well-matched for age, smoking status, symptoms of depression, years of education, and did not differ with regard to SCL-90 and FTND scores **(Table 1)**. Nine (50%) of the CU met the DSM-IV criteria for current cocaine dependence, while the remaining 9 CU met cocaine abuse criteria. Except for one CU who reported smoking crack cocaine, all other CU indicated to use cocaine nasally. None of the participants met the criteria for alcohol dependence. Drug use patterns are shown in **Table 2**. CU and HC did not differ in cigarette use. However, CU reported higher weekly alcohol consumption [$t(33)=-2.84$, $p<.05$] and higher cumulative doses of cannabis [$t(33)=-2.17$, $p<.05$]. CU had a mean weekly cocaine consumption of 1.5g, while they reported very little co-use of amphetamine and MDMA. Hair toxicology analyses capturing the past six months confirmed that cocaine was the primary drug of choice in all CU. Methamphetamine and opiates were not detected, while mean amphetamine values were very low. It is noteworthy that 44% of the CU tested positive for cocaine in the urine toxicology. Therefore, additional analyses were carried out to investigate post-acute effects of cocaine use.

^{11}C-ABP688 Uptake in Controls, Cocaine Users, and Smokers

In accordance with previous reports,[34,53,56] regional ^{11}C-ABP688 uptake was highest in mGluR5-rich regions such as the ACC, caudate, insula, and putamen, while ^{11}C-ABP688 binding was lower in the thalamus (**Figure 1A/B, 2A/B**).

^{11}C-ABP688 uptake did not differ between HC and CU (**Figure 1A, 2A**), yet differed substantially between non-smokers (n=11) and smokers (n=24)(**Figure 1B, 2B**). The mixed-effect ANOVA revealed significant main effects of *smoking status* [$F(1,31)=29.46$, $p<.0001$] and *brain region* [$F(11,341)=127.22$, $p<.0001$] but not of *group* [$F(1,31)=0.09$, $p=.77$] and *hemisphere* [$F(1,31)=0.86$, $p=0.36$]. Therefore, V_{norm} values of both hemispheres were averaged for further calculations. Smokers showed lower (14-21%) mGluR5 availability in all VOIs compared to non-smokers (**Figure 1B**, **Table S1**) and the effect was present in CU and controls (**Figure S1**). Notably, when only non-smoking CU (n=5) were compared with non-smoking HC (n=6), there was a weak

trend for lower mGluR5 availability in the caudate nucleus of CU [$t(9)=1.87$, $p=.095$, $d=1.01$]. Moreover, heavy smokers showed slightly lower mGluR5 availability than light smokers (**Figure S2**).

^{11}C-ABP688 uptake in all VOIs correlated significantly with age in CU (n=18, $r=.51$-$.58$, $p<.05$) and smokers [(n=24, 13 CU and 11 HC), $r=.41$-$.52$, $p<.05$; except for the hippocampus and amygdala $p=.12$] but not in HC in general (n=17, $p>.88$). Moreover, ^{11}C-ABP688 uptake was significantly negatively associated with age in non-smokers in the putamen [(n=11, CU=5, HC=6), $r=-.65$, $p<.05$)].

Drug Use Parameters and ^{11}C-ABP688 Uptake

In order to reduce the probability of α-error accumulation, three combined brain regions were derived by averaging the V_{norm} values of the OFC, ACC, MPFC, DLPFC, VLPFC (*Frontal Cortex)*, the V_{norm} values of the caudate and the putamen (*Striatum)*, and the V_{norm} values of the amygdala, parahippocampal gyrus, and the hippocampus (*Medial Temporal Lobe [MTL]*). The insula and the thalamus were not combined with other brain regions. To prevent inflating potential associations, for correlation analyses regarding drug use only users of the specific drug were included.

There were no significant associations of alcohol, cannabis, and cocaine use (times/g per week, last use, duration in years, cumulative dose in g) with mGluR5 availability, except for age of cocaine onset ($r_{\text{Striatum VOI}}=.47$, $p=.05$; $r_{\text{MTL VOI}}=.55$, $p<.05$; $r_{\text{Thalamus VOI}}=.48$, $p<.04$). However, when corrected for age, these correlations were not statistically significant anymore ($p>.30$). Weekly cigarette consumption and smoking duration did not correlate with mGluR5 availability, yet last cigarette use (h) correlated strongly with mGluR5 availability ($r_{\text{Frontal VOI}}=.60$, $p<.01$; $r_{\text{Striatum VOI}}=.61$, $p<.01$; $r_{\text{MTL VOI}}=.57$, $p<.01$; $r_{\text{Insula VOI}}=.63$, $p<.01$; $r_{\text{Thalamus VOI}}=.57$, $p<.01$; **Figure 3**). Moreover, age of onset of smoking correlated positively with mGluR5 availability in the frontal VOI ($r=.41$, $p<.05$) and thalamus ($r=.45$, $p<.05$), but this result also did not survive correction for age ($p>.31$).

Eight out of 18 CU tested positive for cocaine in the urine toxicology. Therefore, mGluR5 availability was compared among controls (n=17), CU who tested negative (n=10), and CU who tested positive for cocaine (n=8). There was no significant group difference for all VOIs indicating that recent cocaine use did not alter mGluR5 density in CU. The same analysis was repeated with cannabis urine toxicology status, but recent cannabis use did also not affect our results. Cocaine craving scores did not correlate with mGluR5 availability in CU.

Discussion

This is the first study to investigate mGluR5 availability in human CU vs. psychostimulant-naïve control subjects. The CU sample was unique in terms of very little co-use of other illicit drugs verified by hair toxicology, and sparse psychiatric co-morbidities. The key findings are that cocaine use was not significantly associated with altered mGluR5 density. However, in a small subsample of non-smokers, CU exhibited a trend for lower mGluR5 availability in the caudate nucleus in comparison to HC. In line with a recent study,[35] smokers exhibited globally lower mGluR5 density of on average 20% compared to non-smokers. The time interval since smoking the last cigarette correlated with mGluR5 density in all regions of interest, reflecting that the lower mGluR5 availability was particularly pronounced in individuals who had smoked very recently. Interestingly, while older age was associated with higher mGluR5 availability in CU and smokers, an opposing trend was observed in non-smokers, whereas no significant correlations between age and mGluR5 availability were found in controls.

With the present study, we replicated the recent finding that smoking is associated with markedly lower ^{11}C-ABP688 uptake[35] either representing altered affinity of the binding site or lower mGluR5 density. Because G-protein coupled receptors have the ability to undergo endocytosis in response to changes in extracellular levels of receptor agonists,[57] the notion that the 20% lower mGluR5 availability in smokers constitutes a mechanism to attenuate excessive signaling by down-regulation or internalization seems probable.[58-60] Indeed, the fact that longer smoking abstinence was associated with increased mGluR5 availability may reflect the dynamic nature of mGluR5 trafficking and supports the assumption of nicotine-induced changes. Alternatively, reduced mGluR5 availability may constitute a pre-existing condition that increases the risk for nicotine dependence. The average mGluR5 reduction observed in our study was remarkably in accordance with recent independent data.[35] Although mGluR5 availability was slightly lower in the caudate nucleus compared to other brain regions in smokers, we did not find a strong region-specific decrease as was previously revealed for the medial OFC in smokers.[35] Moreover, cigarettes smoked per week and severity of nicotine dependence in our study did also not correlate with mGluR5 availability. Furthermore, we failed to find a significant association between smoking duration and mGluR5 availability. However, when

Akkus et al.[35] took age into account the association was not significant either. A notable difference emerged with regard to smoking abstinence, where we found that briefer smoking abstinence was associated with lower mGluR5 availability. This divergent finding is likely explained by the fact that smokers exhibited a larger variance of abstinence periods in our study increasing the likelihood to detect significant correlations.

mGluR5s have directly been linked to drug-seeking behavior and extinction learning in animals withdrawn after cocaine self-administration (for reviews[19,20,61]). Preclinical studies have shown that chronic self-administration of cocaine disrupts synaptic communication between the PFC and the striatum and results in decreased basal levels of non-synaptic, extracellular glutamate in the NAcc.[11,62-65] Reduced extracellular glutamate tone in the NAcc core has in turn been associated with down-regulation of mGluR5 expression and function in rats,[12-17] resulting in disrupted synaptic plasticity, which is assumed to contribute to the persisting nature of addiction.[9] It has been proposed, that the observed down-regulation of mGluR5s and Homer1b/c, post-synaptic scaffolding proteins influencing mGluR5 trafficking and signal transduction, after chronic cocaine administration may constitute a compensatory mechanism, as mGluR5 antagonists attenuate drug-seeking behaviors.[9] Interestingly, extinction training was also associated with a marked decrease in mGluR5 expression and elevated levels of Homer proteins, possibly providing further support for compensatory adaptation, which may inhibit relapse.[66] Moreover, it has been suggested that genetic variations in mGluR5s, when resulting in decreased mGluR5-mediated neurotransmission, may render such individuals less sensitive to the reinforcing effects of cocaine and nicotine, as well as aversive states during withdrawal as the chromosomal region 11q14, on which the mGluR5 gene (GRM5) is located has been associated with habitual smoking behaviour.[67,68]

Even though findings from preclinical and human studies are not directly comparable, it is noteworthy that preclinical cocaine studies found specifically reduced mGluR5 expression in the NAcc core and failed to find alterations in mGluR5 expression in the PFC and the amygdala, which is consistent with our study.[13,16,17,69] As we only found a trend for lower mGluR5 availability in the caudate in a small subsample of non-smoking CU compared to non-smoking HC, it is possible that the small statistical power and the strong global effect associated with tobacco smoking in the overall

study sample may have masked cocaine-related effects on mGluR5 availability. Moreover, not all CU met criteria for dependence and 44% tested positive for cocaine in the urine toxicology.

The question arises why cocaine use did not seem to alter mGluR5 availability while tobacco smoking did so in a very pronounced and global manner. In contrast to cocaine, nicotine has the ability to directly increase glutamate release by binding to nicotinic acetylcholine receptors (nAChR) that are located on presynaptic terminals of glutamatergic neurons in the PFC, ventral tegmental area (VTA), NAcc, amygdala, and the hippocampus.[70] This might explain the strong smoking effect on mGluR5 availability observed in the present study. In contrast, cocaine binds to monoamine transporters (dopamine, serotonin, noradrenaline), which are mainly localized in the basal ganglia but only show low density in most regions of the neocortex.[71] Acute cocaine intake may therefore exert its function on glutamatergic transmission in a more indirect and less global manner compared to nicotine. Finally, in agreement with our findings, preclinical studies demonstrated specific cocaine-induced alterations of mGluR5 expression primarily in the NAcc core rather than globally.[12-17]

Some limitations of the present report merit comment: i) Due to limited spatial resolution of PET imaging, we cannot fully exclude the possibility that CU may have exhibited subtle differences especially in the NAcc core region in comparison to controls. ii) The present study cannot conclusively answer if the reduced mGluR5 availability in smokers represents a pre-existent condition or is indeed acquired. iii) A PET study using the radioligand ^{18}F-FPEB reported that the human cerebellum is not entirely devoid of mGluR5s.[72] However, a more recent study with ^{18}F-FPEB demonstrated that using the cerebellum as a reference region may be feasible to quantify mGluR5 density.[73] Additional support for negligible mGluR5 binding in the cerebellum comes from prior in vitro and in vivo studies with the radioligand ^{11}C-ABP688.[54,74] iv) The strong influence of recent tobacco use may have masked less pronounced cocaine-related effects on mGluR5 expression.

In conclusion, tobacco smoking was associated with a globally lower mGluR5 density of 20%, while only a weak trend was found for decreased mGluR5 availability specifically in the caudate nucleus in a small non-smoking CU subsample. In particular, briefer smoking abstinence duration was associated with lower mGluR5 density pointing to the possibility of nicotine-induced down-regulation or internalization of mGluR5s. These findings provide evidence that mGluR5s are involved in nicotine

addiction and may have important implications with regard to the development of potential pharmacotherapies aimed at facilitating smoking cessation. If compounds targeting mGluR5s are effective in preventing relapses in human CU akin to preclinical findings remains to be determined. Since the systematic investigation of neuroadaptations in humans is much more complicated due to constraints in experimental manipulation that is only possible in preclinical research, it would be informative to apply multimodal imaging in human CU. For instance glutamate levels in the NAcc and PFC measured by means of magnetic resonance spectroscopy and how these levels are related to mGluR5 availability, PFC metabolism and perfusion in different stages of addiction (e.g., acute drug intake/relapse, craving, withdrawal) could be insightful to unmask neuroadaptations in drug-addicted humans.

Acknowledgments

We would like to acknowledge Josephine Trinckauf, Verena Weichselbaumer, and Marlena Hofbauer from the Division of Nuclear Medicine of the University Hospital Zurich for their assistance with data acquisition. Moreover, we thank Franziska Minder, Stephanie Turin, and Valery Rohrbach for excellent study support.

Financial Disclosures

The study was supported by grants from the Swiss National Science Foundation (SNSF; grant No. PP00P1-123516/1) and the Novartis Foundation for Medical Biological Research (11B51). None of the authors report any conflicts of interest with respect to this study.

Supplementary information is available at *Molecular Psychiatry*'s website.

References

1. Degenhardt L, Hall W. Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *Lancet* 2012; **379**(9810)**:** 55-70.
2. Olesen J, Gustavsson A, Svensson M, Wittchen HU, Jonsson B. The economic cost of brain disorders in Europe. *Eur J Neurol* 2012; **19**(1)**:** 155-162.
3. UNODC. World Drug Report. Geneva2012.
4. SAMHSA. Treatment Episode Data Set (TEDS) 1998-2008. Rockville2010.
5. EMCDDA. Annual report 2011: The state of the drug problem in Europe. Luxembourg2011.
6. Olive MF, Cleva RM, Kalivas PW, Malcolm RJ. Glutamatergic medications for the treatment of drug and behavioral addictions. *Pharmacol Biochem Behav* 2012; **100**(4)**:** 801-810.
7. Volkow ND, Wang GJ, Fowler JS, Tomasi D. Addiction circuitry in the human brain. *Annu Rev Pharmacol Toxicol* 2012; **52:** 321-336.
8. Everitt BJ, Robbins TW. Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* 2005; **8**(11)**:** 1481-1489.
9. Kalivas PW. The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci* 2009; **10**(8)**:** 561-572.
10. Nestler EJ. Common molecular and cellular substrates of addiction and memory. *Neurobiol Learn Mem* 2002; **78**(3)**:** 637-647.
11. McFarland K, Lapish CC, Kalivas PW. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci* 2003; **23**(8)**:** 3531-3537.
12. Ary AW, Szumlinski KK. Regional differences in the effects of withdrawal from repeated cocaine upon Homer and glutamate receptor expression: a two-species comparison. *Brain Res* 2007; **1184:** 295-305.
13. Ben-Shahar O, Obara I, Ary AW, Ma N, Mangiardi MA, Medina RL *et al.* Extended daily access to cocaine results in distinct alterations in Homer 1b/c and NMDA receptor subunit expression within the medial prefrontal cortex. *Synapse* 2009; **63**(7)**:** 598-609.
14. Fourgeaud L, Mato S, Bouchet D, Hemar A, Worley PF, Manzoni OJ. A single in vivo exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. *J Neurosci* 2004; **24**(31)**:** 6939-6945.
15. Ghasemzadeh MB, Vasudevan P, Mueller C, Seubert C, Mantsch JR. Neuroadaptations in the cellular and postsynaptic group 1 metabotropic glutamate receptor mGluR5 and Homer proteins following extinction of cocaine self-administration. *Neurosci Lett* 2009; **452**(2)**:** 167-171.
16. Hao Y, Martin-Fardon R, Weiss F. Behavioral and functional evidence of metabotropic glutamate receptor 2/3 and metabotropic glutamate receptor 5 dysregulation in cocaine-escalated rats: factor in the transition to dependence. *Biol Psychiatry* 2010; **68**(3)**:** 240-248.
17. Swanson CJ, Baker DA, Carson D, Worley PF, Kalivas PW. Repeated cocaine administration attenuates group I metabotropic glutamate receptor-mediated glutamate release and behavioral activation: a potential role for Homer. *J Neurosci* 2001; **21**(22)**:** 9043-9052.
18. Abe T, Sugihara H, Nawa H, Shigemoto R, Mizuno N, Nakanishi S. Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca2+ signal transduction. *J Biol Chem* 1992; **267**(19)**:** 13361-13368.
19. Bellone C, Mameli M. mGluR-Dependent Synaptic Plasticity in Drug-Seeking. *Front Pharmacol* 2012; **3:** 159.
20. Duncan JR, Lawrence AJ. The role of metabotropic glutamate receptors in addiction: evidence from preclinical models. *Pharmacol Biochem Behav* 2012; **100**(4)**:** 811-824.
21. Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S *et al.* Reinforcing and locomotor stimulant effects of cocaine are absent in mGluR5 null mutant mice. *Nat Neurosci* 2001; **4**(9)**:** 873-874.
22. Backstrom P, Hyytia P. Ionotropic and metabotropic glutamate receptor antagonism attenuates cue-induced cocaine seeking. *Neuropsychopharmacology* 2006; **31**(4)**:** 778-786.
23. Besheer J, Faccidomo S, Grondin JJ, Hodge CW. Regulation of motivation to self-administer ethanol by mGluR5 in alcohol-preferring (P) rats. *Alcohol Clin Exp Res* 2008; **32**(2)**:** 209-221.
24. Gass JT, Osborne MP, Watson NL, Brown JL, Olive MF. mGluR5 antagonism attenuates methamphetamine reinforcement and prevents reinstatement of methamphetamine-seeking behavior in rats. *Neuropsychopharmacology* 2009; **34**(4)**:** 820-833.
25. Kenny PJ, Boutrel B, Gasparini F, Koob GF, Markou A. Metabotropic glutamate 5 receptor blockade may attenuate cocaine self-administration by decreasing brain reward function in rats. *Psychopharmacology (Berl)* 2005; **179**(1)**:** 247-254.
26. Kumaresan V, Yuan M, Yee J, Famous KR, Anderson SM, Schmidt HD *et al.* Metabotropic glutamate receptor 5 (mGluR5) antagonists attenuate cocaine priming- and cue-induced reinstatement of cocaine seeking. *Behav Brain Res* 2009; **202**(2)**:** 238-244.
27. Lee B, Platt DM, Rowlett JK, Adewale AS, Spealman RD. Attenuation of behavioral effects of cocaine by the Metabotropic Glutamate Receptor 5 Antagonist 2-Methyl-6-(phenylethynyl)-pyridine in squirrel monkeys: comparison with dizocilpine. *J Pharmacol Exp Ther* 2005; **312**(3)**:** 1232-1240.
28. Martin-Fardon R, Baptista MA, Dayas CV, Weiss F. Dissociation of the effects of MTEP [3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]piperidine] on conditioned reinstatement and reinforcement: comparison between cocaine and a conventional reinforcer. *J Pharmacol Exp Ther* 2009; **329**(3)**:** 1084-1090.
29. Paterson NE, Markou A. The metabotropic glutamate receptor 5 antagonist MPEP decreased break points for nicotine, cocaine and food in rats. *Psychopharmacology (Berl)* 2005; **179**(1)**:** 255-261.
30. Paterson NE, Semenova S, Gasparini F, Markou A. The mGluR5 antagonist MPEP decreased nicotine self-administration in rats and mice. *Psychopharmacology (Berl)* 2003; **167**(3)**:** 257-264.

31. Platt DM, Rowlett JK, Spealman RD. Attenuation of cocaine self-administration in squirrel monkeys following repeated administration of the mGluR5 antagonist MPEP: comparison with dizocilpine. *Psychopharmacology* 2008; **200**(2)**:** 167-176.
32. van der Kam EL, de Vry J, Tzschentke TM. Effect of 2-methyl-6-(phenylethynyl) pyridine on intravenous self-administration of ketamine and heroin in the rat. *Behav Pharmacol* 2007; **18**(8)**:** 717-724.
33. Ametamey SM, Kessler LJ, Honer M, Wyss MT, Buck A, Hintermann S *et al.* Radiosynthesis and preclinical evaluation of 11C-ABP688 as a probe for imaging the metabotropic glutamate receptor subtype 5. *J Nucl Med* 2006; **47**(4)**:** 698-705.
34. Ametamey SM, Treyer V, Streffer J, Wyss MT, Schmidt M, Blagoev M *et al.* Human PET studies of metabotropic glutamate receptor subtype 5 with 11C-ABP688. *J Nucl Med* 2007; **48**(2)**:** 247-252.
35. Akkus F, Ametamey SM, Treyer V, Burger C, Johayem A, Umbricht D *et al.* Marked global reduction in mGluR5 receptor binding in smokers and ex-smokers determined by [11C]ABP688 positron emission tomography. *Proc Natl Acad Sci U S A* 2013; **110**(2)**:** 737-742.
36. Hulka LM, Wagner M, Preller KH, Jenni D, Quednow BB. Blue-yellow colour vision impairment and cognitive deficits in occasional and dependent stimulant users. *Int J Neuropsychopharmacol* 2013; **16**(3)**:** 535-547.
37. Preller KH, Ingold N, Hulka LM, Vonmoos M, Jenni D, Baumgartner MR *et al.* Increased Sensorimotor Gating in Recreational and Dependent Cocaine Users Is Modulated by Craving and Attention-Deficit/Hyperactivity Disorder Symptoms. *Biol Psychiatry* 2013; **73**(3)**:** 225-234.
38. Vonmoos M, Hulka LM, Preller KH, Jenni D, Baumgartner MR, Stohler R *et al.* Recreational and dependent cocaine users both display cognitive dysfunctions. 2013**: Submitted**.
39. Wittchen HU, Wunderlich U, Gruschwitz S, Zaudig M. *SKID-I. Strukturiertes Klinisches Interview für DSM- IV Achse I: Psychische Störungen. Interviewheft und Beurteilungsheft*. Hogrefe: Göttingen, 1997.
40. Quednow BB, Kuhn KU, Hoenig K, Maier W, Wagner M. Prepulse inhibition and habituation of acoustic startle response in male MDMA ('ecstasy') users, cannabis users, and healthy controls. *Neuropsychopharmacology* 2004; **29**(5)**:** 982-990.
41. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry* 1961; **4:** 561-571.
42. Franke G. *SCL-90-R: Die Symptom-Check-Liste von Derogatis - German Version*. Beltz Test Gesellschaft: Göttingen, 1995.
43. Sussner BD, Smelson DA, Rodrigues S, Kline A, Losonczy M, Ziedonis D. The validity and reliability of a brief measure of cocaine craving. *Drug Alcohol Depend* 2006; **83**(3)**:** 233-237.
44. Tiffany ST, Singleton E, Haertzen CA, Henningfield JE. The development of a cocaine craving questionnaire. *Drug Alcohol Depend* 1993; **34**(1)**:** 19-28.
45. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict* 1991; **86**(9)**:** 1119-1127.
46. Burger C, Deschwanden A, Ametamey S, Johayem A, Mancosu B, Wyss M *et al.* Evaluation of a bolus/infusion protocol for 11C-ABP688, a PET tracer for mGluR5. *Nucl Med Biol* 2010; **37**(7)**:** 845-851.
47. Treyer V, Streffer J, Wyss MT, Bettio A, Ametamey SM, Fischer U *et al.* Evaluation of the metabotropic glutamate receptor subtype 5 using PET and 11C-ABP688: assessment of methods. *J Nucl Med* 2007; **48**(7)**:** 1207-1215.
48. Blasberg RG, Carson RE, Kawai R, Patlak CG, Sawada Y, Channing MA *et al.* Strategies for the study of the opiate receptor in brain: application to the opiate antagonist cyclofoxy. *J Cereb Blood Flow Metab* 1989; **9:** 732.
49. Carson RE, Channing MA, Blasberg RG, Dunn BB, Cohen RM, Rice KC *et al.* Comparison of bolus and infusion methods for receptor quantitation: application to [18F]cyclofoxy and positron emission tomography. *J Cereb Blood Flow Metab* 1993; **13**(1)**:** 24-42.
50. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N *et al.* Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 2002; **15**(1)**:** 273-289.
51. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN *et al.* Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab* 2007; **27**(9)**:** 1533-1539.
52. Deschwanden A, Karolewicz B, Feyissa AM, Treyer V, Ametamey SM, Johayem A *et al.* Reduced metabotropic glutamate receptor 5 density in major depression determined by [(11)C]ABP688 PET and postmortem study. *Am J Psychiatry* 2011; **168**(7)**:** 727-734.
53. Hefti K, Holst SC, Sovago J, Bachmann V, Buck A, Ametamey SM *et al.* Increased Metabotropic Glutamate Receptor Subtype 5 Availability in Human Brain After One Night Without Sleep. *Biol Psychiatry* 2012.
54. Elmenhorst D, Minuzzi L, Aliaga A, Rowley J, Massarweh G, Diksic M *et al.* In vivo and in vitro validation of reference tissue models for the mGluR(5) ligand [(11)C]ABP688. *J Cereb Blood Flow Metab* 2010; **30**(8)**:** 1538-1549.
55. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Meth* 2007; **39**(2)**:** 175-191.
56. DeLorenzo C, Kumar JS, Mann JJ, Parsey RV. In vivo variation in metabotropic glutamate receptor subtype 5 binding using positron emission tomography and [11C]ABP688. *J Cereb Blood Flow Metab* 2011; **31**(11)**:** 2169-2180.
57. Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. *Annu Rev Neurosci* 2004; **27:** 107-144.
58. Dhami GK, Ferguson SS. Regulation of metabotropic glutamate receptor signaling, desensitization and endocytosis. *Pharmacol Ther* 2006; **111**(1)**:** 260-271.
59. Fourgeaud L, Bessis AS, Rossignol F, Pin JP, Olivo-Marin JC, Hemar A. The metabotropic glutamate receptor mGluR5 is endocytosed by a clathrin-independent pathway. *J Biol Chem* 2003; **278**(14)**:** 12222-12230.
60. Trivedi RR, Bhattacharyya S. Constitutive internalization and recycling of metabotropic glutamate receptor 5 (mGluR5). *Biochem Biophys Res Commun* 2012; **427**(1)**:** 185-190.

61. Kenny PJ, Markou A. The ups and downs of addiction: role of metabotropic glutamate receptors. *Trends Pharmacol Sci* 2004; **25**(5)**:** 265-272.
62. Baker DA, McFarland K, Lake RW, Shen H, Tang XC, Toda S *et al.* Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nat Neurosci* 2003; **6**(7)**:** 743-749.
63. Kalivas PW, Brady K. Getting to the core of addiction: hatching the addiction egg. *Nat Med* 2012; **18**(4)**:** 502-503.
64. Madayag A, Lobner D, Kau KS, Mantsch JR, Abdulhameed O, Hearing M *et al.* Repeated N-acetylcysteine administration alters plasticity-dependent effects of cocaine. *J Neurosci* 2007; **27**(51)**:** 13968-13976.
65. Miguens M, Del Olmo N, Higuera-Matas A, Torres I, Garcia-Lecumberri C, Ambrosio E. Glutamate and aspartate levels in the nucleus accumbens during cocaine self-administration and extinction: a time course microdialysis study. *Psychopharmacology (Berl)* 2008; **196**(2)**:** 303-313.
66. Kalivas PW, Volkow ND. New medications for drug addiction hiding in glutamatergic neuroplasticity. *Mol Psychiatry* 2011; **16**(10)**:** 974-986.
67. Bierut LJ, Rice JP, Goate A, Hinrichs AL, Saccone NL, Foroud T *et al.* A genomic scan for habitual smoking in families of alcoholics: common and specific genetic factors in substance dependence. *Am J Med Genet A* 2004; **124A**(1)**:** 19-27.
68. Stoker AK, Olivier B, Markou A. Involvement of metabotropic glutamate receptor 5 in brain reward deficits associated with cocaine and nicotine withdrawal and somatic signs of nicotine withdrawal. *Psychopharmacology (Berl)* 2012; **221**(2)**:** 317-327.
69. Schmidt K, Krishnan B, Xia Y, Sun A, Orozco-Cabal L, Pollandt S *et al.* Cocaine withdrawal reduces group I mGluR-mediated long-term potentiation via decreased GABAergic transmission in the amygdala. *Eur J Neurosci* 2011; **34**(2)**:** 177-189.
70. Mansvelder HD, McGehee DS. Cellular and synaptic mechanisms of nicotine addiction. *J Neurobiol* 2002; **53**(4)**:** 606-617.
71. Telang FW, Volkow ND, Levy A, Logan J, Fowler JS, Felder C *et al.* Distribution of tracer levels of cocaine in the human brain as assessed with averaged [11C]cocaine images. *Synapse* 1999; **31**(4)**:** 290-296.
72. Patel S, Hamill TG, Connolly B, Jagoda E, Li W, Gibson RE. Species differences in mGluR5 binding sites in mammalian central nervous system determined using in vitro binding with [18F]F-PEB. *Nucl Med Biol* 2007; **34**(8)**:** 1009-1017.
73. Barret O, Tamagnan G, Batis J, Jennings D, Zubal G, Russell D *et al.* Quantitation of glutamate mGluR5 receptor with 18F-FPEB PET in humans. *J Nucl Med* 2010; **51**(Supplement 2)**:** 215.
74. Hamill TG, Krause S, Ryan C, Bonnefous C, Govek S, Seiders TJ *et al.* Synthesis, characterization, and first successful monkey imaging studies of metabotropic glutamate receptor subtype 5 (mGluR5) PET radiotracers. *Synapse* 2005; **56**(4)**:** 205-216.

Figure Legends

Figure 1. Regional differences in mGluR5 density (means and standard errors). **(A)** Normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 uptake did not differ between controls (n=17) and cocaine users (n=18) in 12 predefined regions of interest. **(B)** Smokers (n=24) showed significantly lower mGluR5 density in all regions of interest compared to non-smokers (n=11) irrespective of cocaine use. The percent difference of mGluR5 density in non-smokers and smokers ranged from 14 to 21 percent. *Indicates significant differences (p<.0001). Ant. cingulate cortex, anterior cingulate cortex; ventrolat. prefrontal cortex, ventrolateral prefrontal cortex; dorsolat. prefrontal cortex, dorsolateral prefrontal cortex.

Figure 2. Axial, sagittal, and coronal views of ^{11}C-ABP688 binding. **(A)** ^{11}C-ABP688 binding did not differ between controls and cocaine users. **(B)** Smokers exhibited a marked global reduction of ^{11}C-ABP688 binding compared to non-smokers. V_{norm} = normalized volume of distribution. Crosshair position: (45, 39, 37; Montreal Neurological Institute brain atlas coordinates).

Figure 3. Correlations between last cigarette use and mGluR5 availability in smokers (n=24). Longer duration of smoking abstinence (hours) was associated with higher mGluR5 availability. V_{norm}, normalized volume of distribution of ^{11}C-ABP688; Frontal VOI, average of V_{norm} values of the OFC, ACC, MPFC, DLPFC, VLPFC; Striatum VOI, average of the V_{norm} values of the caudate and putamen; Medial Temporal Lobe VOI, V_{norm} values of the amygdala, parahippocampal gyrus, and the hippocampus; r_p, Pearson's product-moment correlation coefficient.

Table 1. Demographic data (means and standard deviations, number of subjects and percent)

	Controls (n=17)	**Cocaine users (n=18)**	**Value[a]**	***p*[a]**	***df***
Age	36.24 (±8.34)	36.17 (±7.64)	0.03	0.98	33
Body Mass Index (kg/m^2)	23.72 (±2.77)	25.09 (±3.51)	-1.28	0.21	33
Years of education	10.53 (±1.94)	10.50 (±1.89)	0.05	0.96	33
Beck Depression Index (BDI)	5.59 (±6.54)	5.89 (±3.91)	-0.17	0.87	33
SCL-90-R Global Severity Index (GSI)	0.31 (±0.42)	0.38 (±0.24)	-0.61	0.55	33
SCL-90-R Positive Symptom Distress Index (PSDI)	1.19 (±0.28)	1.28 (±0.25)	-1.00	0.33	33
SCL-90-R Positive Symptom Total (PST)	20.18 (±22.35)	25.72 (±14.09)	-0.88	0.38	33
Cocaine Craving Questionnaire (CCQ) (sum)	-	20.06 (±6.03)	-	-	-
Age at cocaine use onset	-	26.03 (±6.32)	-	-	-
Smoking Status (yes/no)	11, 6 (65, 35%)	13, 5 (72, 28%)	0.23	0.63	1
Fagerström (sum)	2.55 (±2.73)	4.85 (±3.21)	-1.87	0.08	22

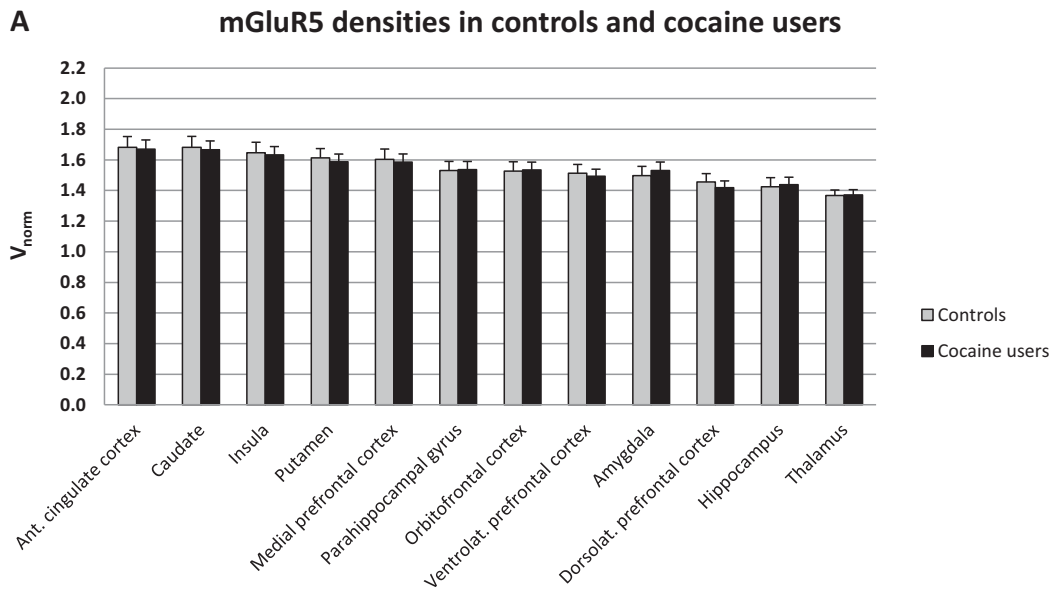
[a]Independent T-test, [b]Chi^2-test for frequency data.

Table 2. Drug use patterns (means and standard deviations)

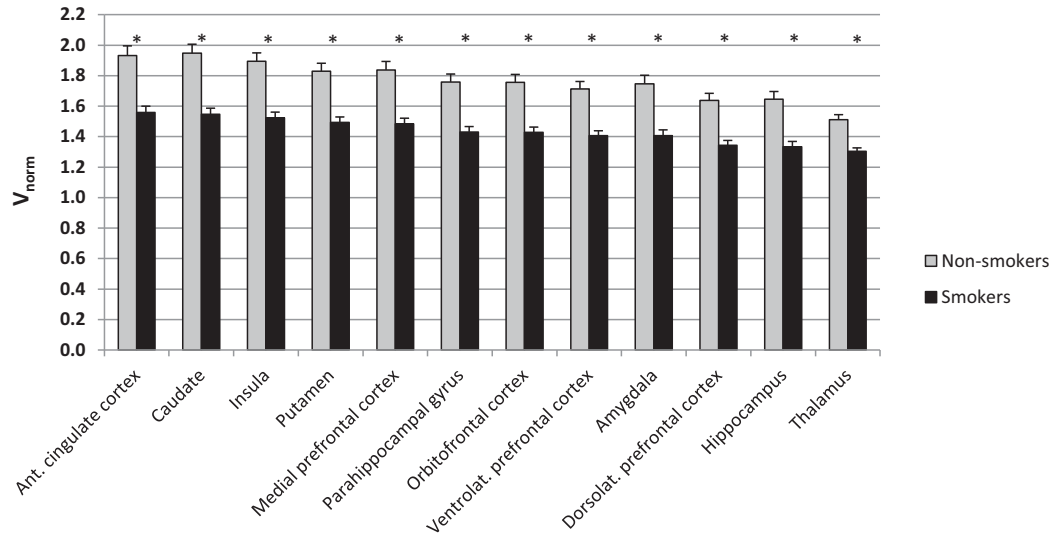
	Controls (n=17)	Cocaine users (n=18)
Alcohol		
Grams per week	88.10 (±62.90)	262.00 (±251.48)
Years of use	17.41 (±8.83)	17.58 (±6.46)
Tobacco		
Cigarettes per week	54.15 (±63.08)	85.19 (±73.74)
Years of use	11.32 (±11.09)	12.06 (±9.18)
Last consumption (hours)	14.47 (±22.36) n=11	123.86 (±320.35) n=13
Cannabis		
Grams per week	0.16 (±0.43)	1.07 (±1.92)
Years of use	4.79 (±7.59)	7.83 (±8.67)
Cumulative dose (grams)	177.23 (±290.81)	1563.85 (±2689.90)
Last consumption (days)	50.76 (±74.39) n=5	3.81 (±2.14) n=8
Urine toxicology (pos./neg.)	2, 15 (12, 88%)	6, 12 (33, 67%)
Cocaine		
Times per week	0.00 (±0.00)	1.28 (±1.39)
Grams per week	0.00 (±0.00)	1.46 (±1.36)
Years of use	0.00 (±0.00)	10.14 (±5.55)
Maximum dose (grams/day)	-	3.94 (±2.89)
Cumulative dose (grams)	0.00 (±0.00)	1056.56 (±977.50)
Last consumption (days)	-	7.60 (±6.84)
Cocaine in hair (pg/mg)	0.00 (±0.00)	18915 (±19665)
Benzoylecgonine in hair (pg/mg)	0.00 (±0.00)	3460 (±3575)
Ethylcocaine in hair (pg/mg)	0.00 (±0.00)	1490 (±2710)
Norcocaine in hair (pg/mg)	0.00 (±0.00)	395 (±500)
Urine toxicology (pos./neg.)	0, 100 (0, 100%)	8, 10 (44, 56%)
Amphetamine		
Grams per week	0.00 (±0.00)	0.0043 (±0.01)
Years of use	0.00 (±0.00)	0.64 (±1.30)
Cumulative dose (grams)	0.00 (±0.00)	10.77 (±25.86)
Last consumption (days)	-	76.00 (±40.22) n=3
Amphetamine in hair (pg/mg)	0.00 (±0.00)	12 (±30) n=3
Methamphetamine in hair (pg/mg)	0.00 (±0.00)	0.00 (±0.00)
MDMA		
Pills per week	0.00 (±0.00)	0.03 (±0.06)
Years of use	0.00 (±0.00)	3.58 (±4.94)
Cumulative dose (pills)	0.00 (±0.00)	24.88 (±63.74)
Last consumption (days)	-	70.63 (±47.67) n=7
MDMA in hair (pg/mg)	0.00 (±0.00)	1644.44 (±4859.92) n=6
MDEA in hair (pg/mg)	0.00 (±0.00)	10.00 (±42.43) n=1
MDA in hair (pg/mg)	0.00 (±0.00)	68.33 (±259.28) n=2

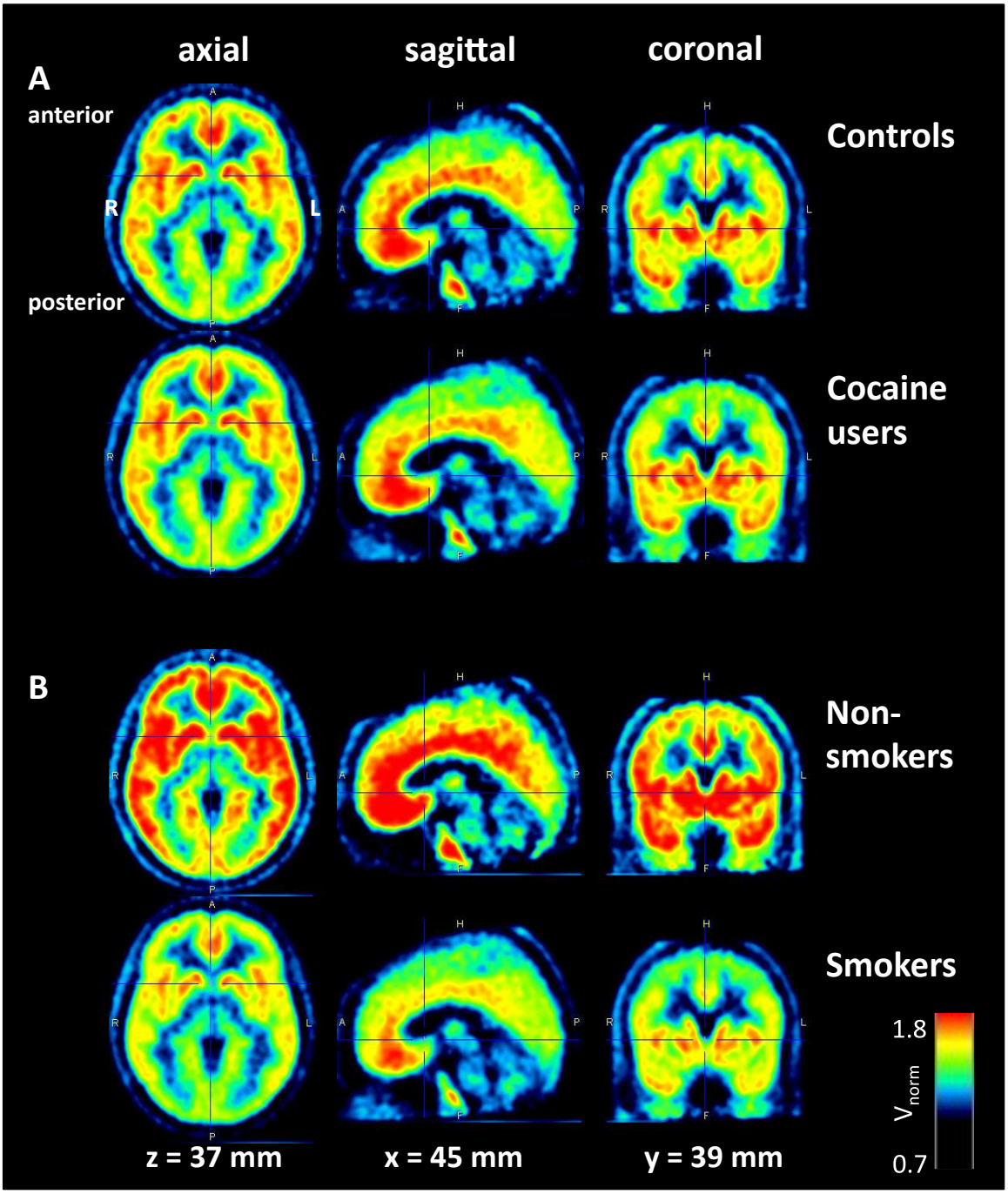
[a]Consumption per day/week captures the last six months, duration of use, and cumulative dose are averaged within the total group. Last consumption is averaged only for subjects who used the drug in the last six months. In this case, sample size is shown. The hair analysis was performed on two hair samples (each 3 cm in length) per participant capturing drug use over the last six months. Concentrations were averaged over the two samples. If the hair sample was not long enough, only one sample was analyzed (3 cm, 3 months). MDMA = 3,4-methylenedioxy-N-methylamphetamine; methylenedioxymethamphetamine, MDEA = methylenedioxyethylamphetamine, MDA = 3,4-methylenedioxyamphetamine.

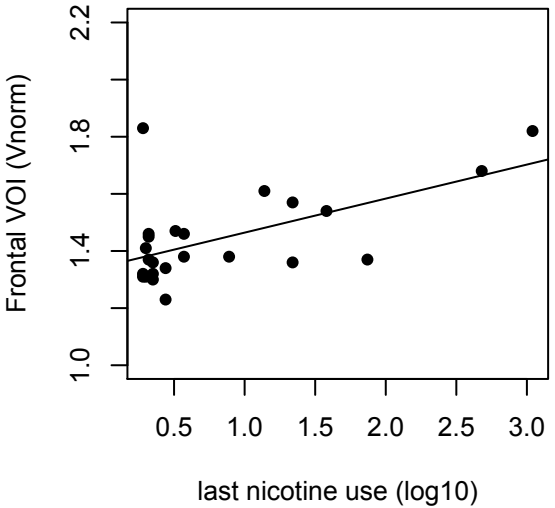
A

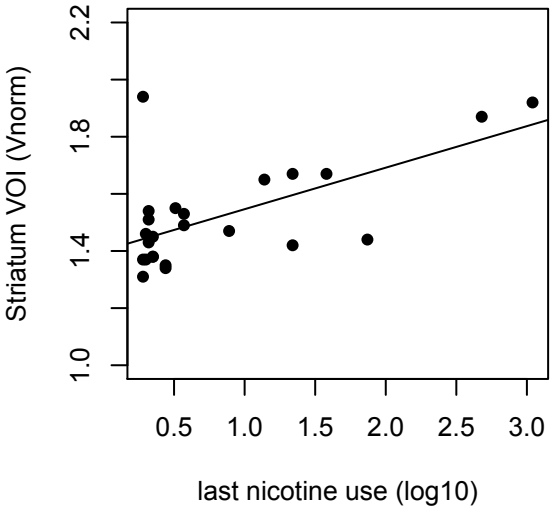

mGluR5 densities in controls and cocaine users
V_{norm}
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ant. cingulate cortex
Caudate
Insula
Putamen
Medial prefrontal cortex
Parahippocampal gyrus
Orbitofrontal cortex
Ventrolat. prefrontal cortex
Amygdala
Dorsolat. prefrontal cortex
Hippocampus
Thalamus
Controls
Cocaine users

B


mGluR5 densities in non-smokers and smokers
V_{norm}
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
Ant. cingulate cortex
Caudate
Insula
Putamen
Medial prefrontal cortex
Parahippocampal gyrus
Orbitofrontal cortex
Ventrolat. prefrontal cortex
Amygdala
Dorsolat. prefrontal cortex
Hippocampus
Thalamus
Non-smokers
Smokers


axial
sagittal
coronal
A
anterior
R
L
posterior
Controls
Cocaine users
B
Non-smokers
Smokers
1.8
V norm
0.7
z = 37 mm
x = 45 mm
y = 39 mm


Frontal VOI (Vnorm)
1.0
1.4
1.8
2.2
0.5
1.0
1.5
2.0
2.5
3.0
last nicotine use (log10)


Striatum VOI (Vnorm)
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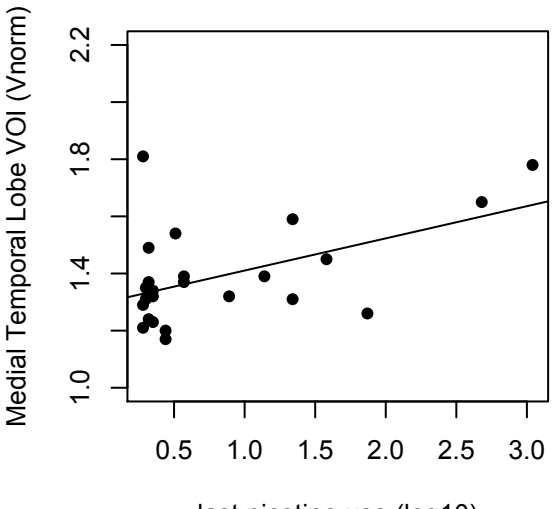
1.8
2.2
0.5
1.0
1.5
2.0
2.5
3.0
last nicotine use (log10)


Medial Temporal Lobe VOI (Vnorm)
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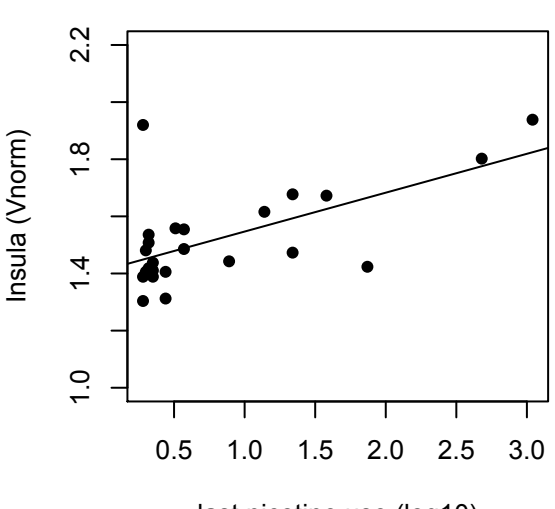
1.8
2.2
0.5
1.0
1.5
2.0
2.5
3.0
last nicotine use (log10)


Insula (Vnorm)
1.0
1.4
1.8
2.2
0.5
1.0
1.5
2.0
2.5
3.0
last nicotine use (log10)

Supplementary Information

Smoking but not cocaine use is associated with lower cerebral metabotropic glutamate receptor 5 density in humans

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Methods

Methodology of the Urine Analysis

Urine toxicology analysis comprised the compounds/substances tetrahydrocannabinol, cocaine, amphetamines, benzodiazepines, opioids, and methadone and was assessed by a semi-quantitative Enzyme Multiplied Immunoassay method (Dimension RXL Max, Siemens, Erlangen, Germany).

Methodology of the Hair Analysis

If participants' hair was long enough, one sample of six cm hair (from the scalp) was taken and subsequently divided into two subsamples of three cm length. The following compounds were assessed: cocaine, benzoylecgonine, ethylcocaine, norcocaine, amphetamine, methamphetamine, MDMA, MDEA, MDA, morphine, codeine, methadone EDDP (primary methadone metabolite), tramadol, and methylphenidate.

For our routine protocol for drugs of abuse analysis a three step washing procedure with water (2 minutes shaking, 15ml), acetone (2min., 10ml) and finally hexane (2min., 10ml) of hair was performed. Then the hair samples were dried at ambient temperatures, cut into small snippets and extracted in two steps, first with methanol (5ml, 16hours, ultrasonication) and a second step with 3 ml MeOH acidified with 50 µL hydrochloric acid 33 % (3 hours, ultrasonication). The extracts were dried and the residue reconstituted with 50 µL MeOH and 500 µL 0.2 mM ammonium formate (analytical grade) in water. As internal standards deuterated standards of the following compounds were used, added as mixture of the following compounds: cocaine-d3, benzoylecgonine-d3, ethylcocaine-d3, morphine-d3, MAM-d3, codeine-d3, dihydrocodeine-d3, amphetamine-d6, methamphetamine-d9, MDMA-d5. MDEA-d6, MDA-d5, methadone-d9, EDDP-d3, methylphenidate-d9, tramadol-d3, oxycodone-d3, and ephedrine-d3. All deuterated standards were from ReseaChem (Burgdorf, Switzerland), the solvents for washing and extraction were of analysis grade and obtained from Merck (Darmstadt, Germany); LC-solvents were of HPLC grade and were obtained from Sigma Aldrich (Buchs, Switzerland).

The LC-MS/MS apparatus was an ABSciex QTrap 3200 (Analyst software Version 1.5, Turbo V ion source operated in the ESI mode, gas 1, nitrogen (50 psi); gas 2, nitrogen (60 psi); ion spray voltage, 3500V; ion source temperature, 450°C; curtain gas, nitrogen (20 psi) collision gas, medium), with a Shimadzu Prominence LC-system (Shimadzu CBM 20 A controller, two Shimadzu LC 20 AD pumps including a degasser, a Shimadzu SIL 20 AC autosampler and a Shimadzu CTO 20 AC column oven, Shimadzu, Duisburg, Germany). Gradient elution was performed on a separation column (Synergi 4µ POLAR-RP 80A, 150x2.0 with a POLAR-RP 4x2.0 Security Guard Cartridge, (Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of 1mM ammonium formate buffer adjusted to pH 3,5 with formic acid (eluent A) and acetonitrile containing 1mM ammonium formate and 1 mM formic acid (eluent B). The Analysis was performed in MRM mode with two transitions per analyte and one transition for each deuterated internal standard, respectively.

Table S1. Independent t-tests of age, tobacco use and brain regions for non-smokers and smokers (means and standard deviations)

	Nonsmokers (n = 11)	**Smokers (n = 24)**	***t*-value**	**df**	**% difference**	**Cohen's *d***
Age	38.09 (± 6.07)	35.33 (± 8.54)	-0.96	33	-	-
Cigarettes per week	0.00 (± 0.00)	101.83 (± 61.72)	8.08*	33	-	-
Smoking duration (years)	0.00 (± 0.00)	16.02 (± 7.94)	10.01*	33	-	-
Age at smoking onset	-	19.31 (± 7.40)	-	-	-	-
Last cigarette (hours)	-	73.73 (± 238.45)	-	-	-	-
Anterior cingulate cortex	1.93 (± 0.24)	1.56 (± 0.19)	-4.98	33	-19 ± 9.96%	1.39*
Caudate	1.95 (± 0.18)	1.55 (± 0.20)	-5.75	33	-21 ± 10.14%	1.50*
Insula	1.89 (± 0.21)	1.52 (± 0.17)	-5.53	33	-20 ± 9.04%	1.47*
Putamen	1.83 (± 0.18)	1.49 (± 0.17)	-5.42	33	-18 ± 9.17%	1.46*
Medial prefrontal cortex	1.84 (± 0.22)	1.48 (± 0.16)	-5.30	33	-19 ± 8.91%	1.44*
Parahippocampal gyrus	1.76 (± 0.16)	1.43 (± 0.18)	-5.24	33	-19 ± 9.96%	1.43*
Orbitofrontal cortex	1.76 (± 0.19)	1.43 (± 0.17)	-5.18	33	-19 ± 9.55%	1.42*
Ventrolater prefrontal cortex	1.71 (± 0.19)	1.41 (± 0.15)	-5.28	33	-18 ± 8.60%	1.44*
Amygdala	1.75 (± 0.17)	1.41 (± 0.19)	-5.08	33	-19 ± 10.82%	1.41*
Dorsolateral prefrontal cortex	1.64 (± 0.20)	1.34 (± 0.13)	-5.31	33	-18 ± 7.84%	1.44*
Hippocampus	1.64 (± 0.18)	1.33 (± 0.17)	-5.00	33	-19 ± 10.12%	1.39*
Thalamus	1.51 (± 0.11)	1.30 (± 0.10)	-5.30	33	-14 ± 6.91%	1.44*

*p<.0001

Figure Legend

Figure S1. Regional differences in mGluR5 density (means and standard errors) in controls and cocaine users depend on smoking status. **(A)** Normalized volumes of distribution (V_{norm}) of ^{11}C-ABP688 uptake differed significantly between non-smokers and smokers in all regions of interest irrespective of whether they used cocaine (a mixed-effect ANOVA revealed significant main effects of *group* [$F(3,31)=11.07$, $p<.0001$] and *brain region* [$F(11,341)=125.46$, $p<.0001$]). More specifically, smoking controls ($p<.0001$, Bonferroni-corrected post hoc comparisons) and smoking cocaine users ($p<.01$) had significantly lower mGluR5 density than non-smoking controls, and smoking controls ($p<.01$) and by trend smoking cocaine users ($p=.069$) had lower mGluR5 density than non-smoking cocaine users. **Indicates significant differences ($p<.0001$). **(B)** Percent difference in V_{norm} between non-smoking controls (n=6), smoking controls (n=11), non-smoking cocaine users (n=5), and smoking cocaine users (n=13). **Indicates significant differences ($p<.0001$), *stands for ($p<.01$). Ant. cingulate cortex, anterior cingulate cortex; ventrolat. prefrontal cortex, ventrolateral prefrontal cortex; dorsolat. prefrontal cortex, dorsolateral prefrontal cortex.

Figure S2. Regional differences in mGluR5 density (means and standard errors) in never smokers (n=11), light smokers (n=12; FTND score <4) and heavy smokers (n=12; FTND score ≥4). Never smokers had significantly higher mGluR5 availability than light ($p<.01$) and heavy smokers ($p<.0001$), while light and heavy smokers did not significantly differ from each other ($p=.47$) (main effects of *group* [$F(2,32)=16.50$, $p<.0001$; linear contrast $p<.0001$] and *brain region* [$F(11,352)=125.19$, $p<.0001$] in mixed-effect ANOVA). FTND, Fagerström Test for Nicotine Dependence; V_{norm}, normalized volumes of distribution of ^{11}C-ABP688 uptake. **Indicates significant differences ($p<.0001$), *stands for ($p<.01$). Ant. cingulate cortex, anterior cingulate cortex; ventrolat. prefrontal cortex, ventrolateral prefrontal cortex; dorsolat. prefrontal cortex, dorsolateral prefrontal cortex.

Figure S1

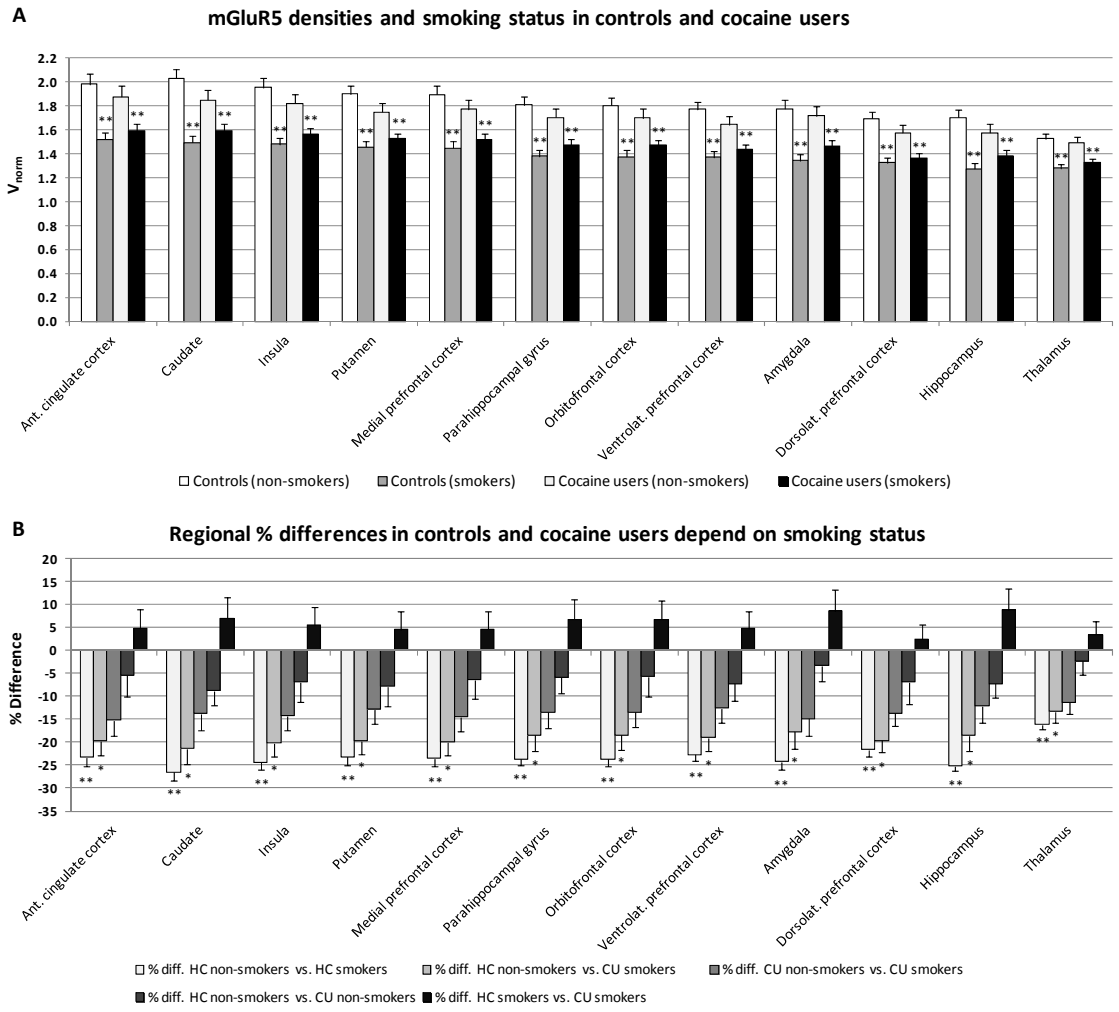
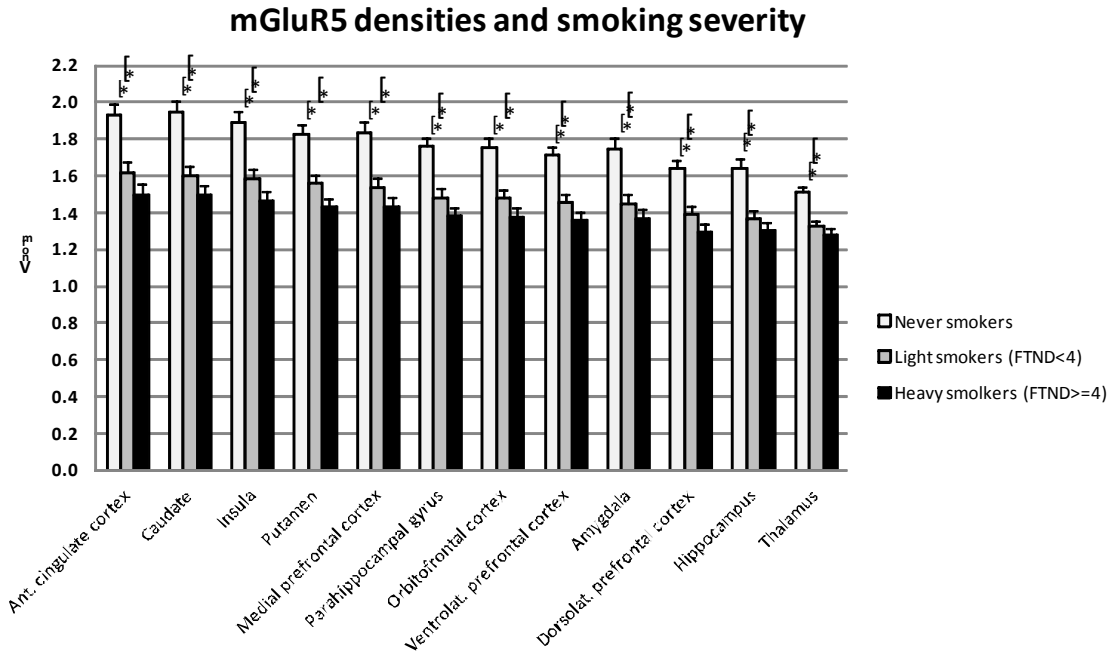

A
mGluR5 densities and smoking status in controls and cocaine users
V_{norm}
Ant. cingulate cortex
Caudate
Insula
Putamen
Medial prefrontal cortex
Parahippocampal gyrus
Orbitofrontal cortex
Ventrolat. prefrontal cortex
Amygdala
Dorsolat. prefrontal cortex
Hippocampus
Thalamus
Controls (non-smokers)
Controls (smokers)
Cocaine users (non-smokers)
Cocaine users (smokers)
B
Regional % differences in controls and cocaine users depend on smoking status
% Difference
% diff. HC non-smokers vs. HC smokers
% diff. HC non-smokers vs. CU smokers
% diff. CU non-smokers vs. CU smokers
% diff. HC non-smokers vs. CU non-smokers
% diff. HC smokers vs. CU smokers

Figure S2


mGluR5 densities and smoking severity
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Never smokers
Light smokers (FTND<4)
Heavy smolkers (FTND>=4)
Ant. cingulate cortex
Caudate
Insula
Putamen
Medial prefrontal cortex
Parahippocampal gyrus
Orbitofrontal cortex
Ventrolat. prefrontal cortex
Amygdala
Dorsolat. prefrontal cortex
Hippocampus
Thalamus