To be submitted as an Archival Report to Biological Psychiatry (revised version)

Association of Long Term Abstinence with Normal Metabotropic Glutamate Receptor-5 Binding

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Short Title: Nicotine Abstinence and mGluR5 binding in humans

Key Words: nicotine, addiction, glutamate, relapse, abstinence, mGluR5

Number of words in abstract: 203

Number of words in article body: 2948

Number of figures: 2

Number of tables: 2

Supplemental Information: 0

Abstract

Background: Nicotine addiction is a major public health problem and is associated with primary glutamatergic dysfunction. We recently showed marked global reductions in metabotropic glutamate receptor type 5 (mGluR5) binding in smokers and recent ex-smokers (average abstinence duration of 25 weeks). The goal of this study was to examine the role of mGluR5 down-regulation in nicotine addiction by investigating a group of long-term ex-smokers (abstinence greater 1.5 years), and to explore associations between mGluR5 binding and relapse in recent ex-smokers.

Methods: Images of mGluR5 receptor binding were acquired in 14 long-term ex-smokers, using PET with [(11)C]ABP688, which binds to an allosteric site with high specificity.

Results: Long-term ex-smokers and individuals who had never smoked showed no difference in mGluR5 binding in any of the brain region examined. Long-term ex-smokers showed significantly higher mGluR5 binding compared with recent ex-smokers, most prominently in the frontal cortex (42%) and thalamus (57%).

Conclusions: Our findings suggest that down-regulation of mGluR5 is a pathogenetic mechanism underlying nicotine dependence and the high relapse rate in individuals previously exposed to nicotine. Therefore, mGluR5 receptor binding appears to be an effective biomarker in smoking and a promising target for the discovery of novel medication for nicotine dependence and other substance-related disorders.

Introduction

The recidivism rate for nicotine consumption is extraordinarily high. Each year, 40% of smokers try to quit but only 3–5% achieve prolonged abstinence for 6–12 months after a given quit attempt (1). Although the neurobiological mechanisms underlying relapse are largely unknown, there is increasing evidence that molecular and neurochemical adaptations in the glutamatergic system play an important role in the recidivism for cocaine and nicotine abuse (2).

There is strong evidence that the metabotropic glutamate receptor type 5 (mGluR5) has a specific role in addiction. In 2001, Chiamulera et al. demonstrated that mGluR5 gene knock-out mice do not respond to acute administration of various doses of cocaine and fail to acquire intravenous self-administration of cocaine (3). Multiple studies have demonstrated that negative allosteric modulators of the mGluR5 receptor, such as 2-methyl-6-(phenylethynyl)pyridine (MPEP) and 3-((2-methyl-4-thiazolyl)ethynyl)pyridine (MTEP), reduce the self-administration of addictive drugs such as cocaine and nicotine (4-10). In addition, there is direct evidence that mGluR5 receptor antagonism attenuates reinstatement to nicotine (6, 7).

We used positron emission tomography (PET) to measure mGluR5 availability with the radiolabeled mGluR5 antagonist 3-(6-methyl-pyridin-2-ylethynyl)-cyclohex-2-enone-O-11C-methyl-oxime ([^{11}C]ABP688) (11), which binds with high selectivity to an allosteric site. We have previously shown a marked reduction in mGluR5 at that time measured as DVR (V_T/V_{ND}) based on a ratio of cortical uptake and cerebellum uptake at equilibrium in a bolus infusion setting (12). We found a strong reduction in gray matter DVR between smokers and non-smokers (20.6% and between ex-smokers and non-smokers (11.5%). Our finding in smokers has recently been replicated by another research group (13).

To test the association between relapse of nicotine addiction and mGluR5 binding, we conducted two follow-up studies. First, we measured mGluR5 binding in subjects with a long abstinence duration of 78 to 1144 weeks. Given that these subjects belong to about 3% of ex-smokers with the ability to abstain for a longer period (1), we expected their mGluR5 binding to

be positively associated with abstinence duration. Second, we followed the ex-smokers assessed in our previous study over time and tested for an association between relapse and mGluR5 binding in this subsample. Based on our previous study, we hypothesized that abnormally low mGluR5 binding predicts a high risk of relapse. In this paper, we have changed our nomenclature to the one by Innis et al. (14). Upon request we do not calculate DVR but BP_{ND} which is calculated as V_T/V_{ND} - 1 and therefore we had to recalculate the outcome variables which resulted in different % changes compared to our previous publication, while the raw uptake data remained the same.

Material and Methods

Subjects

Participants were recruited using local newspaper advertisements and screened at Zurich University hospital. Inclusion criterion for the group of long-term ex-smokers was duration of nicotine abstinence of more than 1.5 years, having smoked at least 11 cigarettes per day.

Exclusion criteria included neurological or medical disorders, pregnancy, breast-feeding, history of psychosis, manic episodes, current depressive episodes, substance dependence, and autism (based on clinical interview as described below). Subjects were enrolled into the study after a full explanation of the study design and procedures and after written consent was obtained, which was approved by the local ethics committee (Kantonale Ethikkommission Zürich).

All subjects were assessed using an unstructured clinical interview by a psychiatrist and the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision. Clinical measures included the Beck Anxiety Inventory (BAI) and the Beck Depression Inventory (BDI). Magnetic resonance images were assessed for each subject to exclude any structural brain pathology.

At the time of the PET scan, an additional clinical evaluation, comprising a physical examination, BAI, and BDI, was performed. These scores from the BAI and BDI were used for further statistical evaluations.

Subjects in the recent ex-smoker group were questioned regarding potential relapse over the telephone 5–12 months (mean 8.4 ± 2.5) after the scanning session. They were asked if, and when they had started smoking again and their daily cigarette consumption.

Positron Emission Tomography

The description of the PET with [^{11}C]ABP688 can be found in detail in a previous study (12). In this article, we changed the outcome variable from previously defined DVR to BP_{ND} at the equilibrium (14). In the current study we included a new group of long ex-smokers, with whom we used the same bolus/infusion protocol as with the previous study with[^{11}C]ABP688 in smokers (12). We have shown previously that the bolus infusion method gives reliable results using the cerebellum as reference region (15, 16).

Statistical Analysis

We used PMOD software (Version 3.0; PMOD Technologies, Zurich, Switzerland (www.pmod.com) for all analyses. Uptake images were transformed to a common space, the Montreal Neurological Institute template. The same 23 regions of interest (ROI) definitions in our previous study were used (except brain stem). These included 2 regions within the cingulate gyrus (anterior and posterior), 4 cortical regions (frontal, parietal, temporal, and occipital), 3 regions in the limbic system (medial orbitofrontal cortex, amygdala, and medial temporal lobe), 3 in the prosencephalon (caudate, putamen, and thalamus) (12). The same grey matter mask created for the previous study was used in this analysis. To test for differences in mGluR5 BP_{ND} between the groups, we used two sample 2-tailed *t*-tests. Spearman correlations were used to assess the relationship between clinical variables and mGluR5 BP_{ND}.

Results

The clinical characteristics of 14 smokers, 14 age- and gender-matched non-smokers, 14 recent ex-smokers, and14 long-term ex-smokers are shown in Table 1. Only one to two subjects per group had a history of one episode of depression but did not show any sign of depression at time of scanning. Age did not differ across the groups ($F_{(3,55)} = 2.46$, $p > 0.05$), and there was no significant difference in age between male and female subjects overall or within the smoker, non-smoker, recent ex-smoker, or long-term ex-smoker groups ($F_{(1,55)} = 0.03$, $p > 0.05$). The BDI scores across all groups did not differ ($F_{(3,55)}=0.55$, $p>0.6$). BAI scores on the day of scanning were significantly different across all groups ($F_{(3,55)} = 3.2$ $p < 0.05$). Scheffe post hoc tests revealed that this significant effect was not due to the comparisons with long-term ex-smokers ($p > 0.4$ in all cases). There was no significant difference in the number of cigarettes smoked per day ($t_{26} = 0.05$; $p > 0.9$), number of years smoking ($t_{26} = 0.7$; $p > 0.4$), or age of onset ($t_{26} = 0.02$; $p > 0.9$) between recent and long-term ex-smokers. There was no significant difference in number of cigarettes smoked per day ($t_{26} = 0.9$; $p >0.3$), number of years smoking ($t_{26} = 0.66$; $p >0.5$), or age of onset ($t_{26} = 0.96$; $p > 0.3$) between long-term ex-smokers and smokers.

We did not see a significant effect of age of smoking onset on mGluR5 BP_{ND} in the long-term ex-smoker group, after correction for multiple comparisons. Testing for an effect of age of smoking onset as a covariate in a repeated measures analysis of all smoking groups we did not see a significant between subjects effect of this factor ($F_{(1,37)}=0.026$, $p>0.8$) nor did we see a potential effect of the variable age in this comparison between all groups ($F_{(1,50)}=3.27$ $p>0.05$).

We did perform also a repeated measures analysis (regions) combining all groups and did not found a significant between subjects effect of gender on the mGluR5 binding ($F_{(1,53)}=0.225$ $p>0.6$). In the long-term ex-smoker group, there was no difference in mGluR5 BP_{ND} between male and female subjects ($p > 0.05$ for all ROIs), with the exception of the left mediotemporal region, which showed an uncorrected significant difference in mGluR5 BP_{ND} ($p < 0.05$). Performing a repeated measures analysis (using regions as repeat factor) combining all groups no significance between subjects effect of gender on the mGluR5 binding ($F_{(1,53)}=0.225$ $p>0.6$) was

found. The mean [^{11}C]ABP688 activity did not differ significantly among non-smokers, smokers, and ex-smokers (729 ± 71, 765 ± 80, 740 ± 34, and 697 ± 61MBq, respectively). Table 1 and Figure 1summarize the results of the mGluR5 BP_{ND} comparisons across the 4 clinical groups in the 23 ROIs examined. Overall, there was a significant regions × group effect ($F_{(66,99)}$ = 2.214; $p < 0.0001$). The post hoc comparisons between long-term ex-smokers and all other groups revealed that all regions showed a significant difference, after Bonferroni correction, in mGluR5 BP_{ND} between long-term ex-smokers and smokers, except for right thalamus ($p < 0.01$). Percent differences in [^{11}C]ABP688 uptake ranged from 39% to 68% between all groups. When we compared long-term ex-smokers and recent ex-smokers, we found a significant difference, before Bonferroni correction, in mGluR5 BP_{ND} in all regions, except the parietal cortex. After Bonferroni correction, only differences in the bilateral frontal, thalamic, and occipital regions remained significant. The highest percentage differences were found in the frontal cortex (left: 42%), occipital cortex (left: 37%), and thalamus (left: 57%). Comparison between long-term ex-smokers and healthy controls did not reveal any significant effects, after correction, in any region, with up to 17% (Figure 1).

There was no significant correlation between age and mGluR5 BP_{ND} in any region in long-term ex-smokers after Bonferroni correction. However, there was a significant correlation in the left medial orbitofrontal region, before correction ($r = 0.56$, $p < 0.05$). Performing a repeated measures analysis (using regions as repeat factor) combining all groups with age as covariate no significance between subjects effect of age on the mGluR5 binding ($F_{(1,50)}$=3.271 $p>0.07$) was found. Group effects remained significant ($F_{(3,50)}$=20.185 $p<0.001$). No significant correlation, after correction, was observed between mGluR5 BP_{ND} and daily consumption of cigarettes, years of consumption, abstinence duration, BDI or BAI scores in any regions in long-term ex-smokers.

Figure 2 shows the results of our analysis of the follow-up information from the recent ex-smoker group. Four recent ex-smokers stayed completely abstinent and another three started smoking only 1–2 cigarettes per day (below the WHO criteria for nicotine addiction). Seven

recent ex-smokers started smoking again with more than 11 cigarettes per day (or 8 nicotine chewing gums). Abstinence duration in the relapsing subgroup was 3–69 weeks (mean = 22 ± 23.1 weeks) after the scan visit. Abstinence duration did not correlate with mGluR5 BP_{ND} in any region.

Discussion

In a recently published study, we provided the first human evidence of a potential role for mGluR5 in nicotine addiction and possibly other substance use disorders. We found marked global reductions in mGluR5 binding in smokers and recent ex-smokers with average nicotine-abstinence duration of 25 weeks compared to controls who had never smoked. This reduction occurred in all brain regions, except the brain stem. Current nicotine consumption and estimates of current nicotine dependence were not associated with mGluR5 binding. There was no gender difference in mGluR5 binding in smokers and recent ex-smokers, whereas female non-smokers had significantly lower mGluR5 binding than male non-smokers. In smokers, both age and age at smoking onset were positively correlated with mGluR5 binding.

We could only speculate about the mechanisms underlying the widespread decrease in [^{11}C]ABP688 binding to mGluR5 in smokers and recent ex-smokers. The finding may have represented a biological trait associated with an increased risk for nicotine dependence, possibly due to genetic factors. Alternatively, reduced mGlu5 receptor binding may have been the result of mGluR5 down-regulation, possibly because of the nicotine-induced increase in glutamate activity (17, 18). Reductions in mGluR5 binding seen in the recent ex-smokers may reflect an incomplete recovery of the mGluR5 receptors, given that the recent ex-smokers were abstinent for only 25 weeks on average. This supports the hypothesis of a lasting effect of regular nicotine consumption, which is possibly associated with the risk of relapse.

In the first follow-up study, we examined a new group of ex-smokers with average abstinence duration of 9 years. These subjects belonged to 1–3% of ex-smokers with the ability to abstain for longer periods, giving us the opportunity to investigate mGluR5 binding with respect to long-term abstinence. Our findings clearly show that mGluR5 binding across all

regions did not differ between the long-term abstinence group and the group of individuals who had never smoked. The long-term ex-smoker group showed significantly higher mGluR5 binding than the short-term ex-smoker group. This result supports the hypothesis that reduced mGluR5 binding either is a biological risk factor for nicotine dependence or reflects the amount of nicotine-induced glutamate hyperactivity in smokers. It is also consistent with the hypothesis that reduced mGluR5 binding in short-term ex-smokers represents an incomplete recovery of mGluR5 receptors, lasting for at least the first months of nicotine abstinence. In contrast, the results of the first follow-up analysis here argue against the alternative hypothesis that mGluR5 down-regulation is an effective compensatory change necessary for long-term abstinence. There is no gender difference in mGluR5 binding in long-term ex-smokers when compared with smokers and recent ex-smokers, whereas women in the control group showed lower mGluR5 binding than men. This suggests that mGluR5 was down regulated in long-term ex-smokers but normalized after extended periods of abstinence.

Independent of this strong whole brain differences between smoker, recent and long-term ex-smoker we looked also into regional differences a posteriori. The contrast between recent and long-term ex-smoker should reveal differences between early phases of abstinence and successful conquering smoking addiction. We see highest percentage reduction in recent versus long-term ex-smokers in the thalamus and frontal cortex. The thalamus is known for high densities of nicotinic receptor concentration with strong connection with the frontal cortex (16). The “normalization” of these regions seems to be delayed or over proportionally reduced in the short-term ex-smoker (from which many did relapse). In order to further analyze network related effects more subjects per group would be needed.

Looking at the differences between long-term ex-smoker and currently smoking subjects we see an overall effect of higher binding in the long-term ex-smoker group. Nevertheless, the regional analysis revealed the highest difference in percentage is found in mOFC. This is in line with various publications on addiction and reward processes. In a recent meta-analysis of studies of nicotine dependence and its treatment the authors have extracted that medial and lateral

orbitofrontal cortex aside of cingulate, striatum as well as amygdala and thalamus are heavily involved in maintenance of smoking and nicotine withdrawal (19).

The goal of the second follow-up analysis was to explore associations between mGluR5 binding and relapse in the 14 recent ex-smokers who were included in our previous report (12). There were seven ex-smokers who did not smoke at the time of follow-up or smoked only 1 or 2 cigarettes daily, their mGluR5 binding was not significantly higher than that for individuals who relapsed. However, a trend was seen (average 0.55 BP_{ND} nonrelapsing vs. 0.47 BP_{ND} relapsing, $p>0.2$) for lower mGluR5 binding predicting relapse. This trend needs further investigation in a larger sample. This finding is inconclusive since the small sample size does not allow for a definitive interpretation for or against a relationship between mGluR5 binding and risk of relapse.

Preclinical studies have found that the functional up-regulation of mGluR2/3 and down-regulation of mGluR5 are likely to be factors in the transition from drug use to drug dependence (20). Specifically, mGluR1/5 and its intracellular binding protein Homer1 were down regulated in the nucleus accumbens after withdrawal from chronic cocaine administration (21-24), and the drug-induced adaptation of mGluR5 has been implicated in brain reward deficits and somatic signs associated with nicotine withdrawal (25). Although these findings consistently suggest that mGluR5 plays an important role in the development of substance dependence, the functional role of mGluR5 down-regulation remains unclear. It has been suggested that mGluR5 down-regulation represents a compensatory neuroadaptation (26), diminishing drug-induced reward acquisition (27), and reducing the effects of contextual cues in the conditioned behavioral responses to nicotine (28). The findings of this study argue against the compensatory neuroadaptation hypothesis and favor hypotheses that regard mGluR5 down-regulation as an element in the pathogenesis of drug dependence and/or a factor underlying the high rate of relapse in nicotine and stimulant consumption.

Several methodological limitations merit comment. Firstly, the design of this study with no repeated assessments of mGluR5 binding cannot conclusively distinguish whether

abnormalities in mGluR5 receptor binding reflect a biological vulnerability for nicotine dependence or are a consequence of nicotine intake. Secondly, undetected psychiatric or neurological conditions may have confounded the results of this study. However, the exclusion of subjects with a history of psychiatric disorders did not alter the results. Finally, we used a bolus-infusion technique and normalized PET images to the cerebellar radioactivity concentration to avoid the need for potentially painful arterial cannulation. This reference tissue method was based on the assumption that mGluR5 levels are extremely low in the cerebellum relative to the predefined ROIs in other brain areas (29). This assumption is supported by various types of evidence, including previous PET studies (30), *in vitro* and *in vivo* studies on ABP688 binding in the cerebellum (29, 31), a postmortem study on cerebellar mGluR5 protein expression (32), and studies on cerebellar mRNA expression (33-35).

In conclusion, we showed that there is normal mGluR5 binding (as compared to healthy non-smokers) in ex-smokers who have stayed abstinent for more than a year. These individuals represent the 1–3% of smokers who have the ability to stay abstinent over long periods. This result suggests that normal mGluR5 binding reflects the relative insensitivity of the glutamate system to prolonged nicotine consumption or the full recovery of mGluR5 during nicotine abstinence. These findings suggest that down-regulation of mGluR5 in smokers is a pathogenetic mechanism underlying nicotine dependence and the high relapse rate in individuals who have been previously exposed to nicotine. Therefore, mGluR5 receptor binding appears to be an effective biomarker in smoking and a good target for the discovery of novel medication for treatment of nicotine dependence and other substance-related disorders.

Acknowledgments

The authors would like to thank the PET team of Zurich University for help with data acquisition. The PET study was supported by Novartis Pharma AG, OPO Foundation, Zurich, Switzerland; Olga Mayenfisch Foundation, Zurich, Switzerland; Vontobel Foundation, Zurich, Switzerland; Hartmann Muller Foundation, Zurich, Switzerland. The PET data were analyzed at

Zurich University Hospital and at Psychiatric University Hospital Bern by Alfred Buck, Valerie Treyer, Funda Akkus, and Gregor Hasler.

Author Contributions

The research was designed by G.H. and performed by F.A., V.T., and A.J.

S.M.A., A.B., B.G-M., and J.S. contributed new reagents for analytic tools. The data were analyzed by V.T., F.A., and G.H., and F.A. and G.H. wrote the paper.

Financial Disclosures

Funda Akkus, Valerie Treyer, Simon M. Ametamey, Anass Johayem, Alfred Buck, and Gregor Hasler do not have any conflict of interest regarding the content of this article. Baltazar Gomez-Mancilla and Judit Sovago work for Novartis Pharma AG (Basel, Switzerland), which is developing and testing drugs targeting the mGlu5 receptor.

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Figures and Table Legends

Figure 1. mGluR5 BP_{ND} across all groups

The bars represent mean (±SD) of mGluR5 BP_{ND}) values in selected brain regions: anterior cingulate cortex (ACC), mediotemporal cortex (MTL), and medial orbitofrontal cortex (mOFC) (mean for left and right sides). Significant differences in all these regions were observed between the long-term ex-smoker, recent ex-smoker, and smoker groups. There was no significant difference in mGlu5R values between the long-term ex-smoker and healthy control group. Statistical details are described in the results section.

Figure 2. Follow-up of recent ex-smokers assessing relapse in relation to grey matter uptake

This figure shows the follow-up of recent ex-smokers in relation to relapse rate. Seven recent ex-smokers are still abstinent; 4 did not smoke at all and 3 smoked 1 or 2 cigarettes per day. Seven participants relapsed to smoking more than 11 cigarettes per day. Comparing regional uptake between the groups did not reveal any significant (with and without correction) effects. However, there was a tendency for smaller baseline values in ($p>0.2$) the relapsed subjects. Bars represent mean ± SD. Cig./day = number of cigarettes per day.

Table 1. Clinical characteristics of study groups

Clinical characteristic	Non-smokers (n = 14)	Smokers (n = 14)	Recent ex-smokers (n = 14)	Long-term ex-smokers (n = 14)
Gender (f/m)	8/6	8/6	6/8	6/8
Age (years)	36.8 (9.6)	36.1 (10.2)	37.7 (10.1)	37.8 (10.1)
Age of smoking onset (years)	--	20.4 (5.9)	18.4 (5.3)	18.0 (5.0)
Number of cigarettes smoked per day	--	17.0 (4.5)	17.9 (6.0)	19.6 (8.2)
Number of years smoking	--	16.8 (7.6)	19.6 (8.8)	19.6 (7.7)
Duration of nicotine abstinence (weeks)	--	--	25.0 (19.4)	473.6 (336.1)
Fagerstrom test for nicotine dependence (FTND) score	--	4.5 (2.0)	--	--
Beck Anxiety Inventory (BAI) score	1.5 (1.2)	4.3 (4.3)	4.6 (3.3)	2.8 (2.5)
Beck Depression Inventory (BDI) score	1.8 (2.1)	2.7 (2.6)	2.6 (2.0)	2.4 (2.0)
Past psychiatric history (n)				
Alcohol abuse	1	1	0	0

Major depression	1	2	1	2
Anorexia nervosa	0	1	0	0
Highest educational qualification (n)				
High school, completed	0	2	4	2
	13	12	10	9
College, completed				
	1	0	0	3
Academic completed,				

The data is displayed as mean (SD), unless indicated otherwise.

n refers to the number of participants within this category. The new information from the long ex-smokers is displayed together with the information of the other groups, which were already described in our previous study (12).

Table 2

Regions of interest	Long ex-smoker	Non smoker	Recent ex-smoker	Smoker
	p is cacluated vs. Long-term ex-smoker			
Gray matter				
P value		0.826	0.003	0.000
$p_{adjusted}$		18.999	0.072	0.000
BPND	0.688	0.701	0.516	0.383
ACC				
P value		0.743	0.015	0.000
$p_{adjusted}$		17.100	0.334	0.000
BPND	0.964	0.992	0.762	0.574
Amgdala left				
P value		0.693	0.012	0.000
$p_{adjusted}$		15.934	0.268	0.003
BPND	0.851	0.887	0.619	0.462
Amygdala right				
P value		0.417	0.020	0.000
$p_{adjusted}$		9.597	0.466	0.005
BPND	0.802	0.869	0.606	0.462
Caudatus left				
P value		0.247	0.003	0.000
$p_{adjusted}$		5.687	0.075	0.000
BPND	0.779	0.867	0.556	0.409
Caudatus right				
P value		0.550	0.005	0.000
$p_{adjusted}$		12.657	0.104	0.000
BPND	0.783	0.832	0.566	0.417
Frontal left				
P value		0.875	0.000	0.000
$p_{adjusted}$		20.118	0.007	0.000
BPND	0.745	0.757	0.484	0.370
Frontal right				
P value		0.507	0.002	0.000
$p_{adjusted}$		11.651	0.042	0.000
BPND	0.727	0.774	0.508	0.380
Medial temporal left				
P value		0.844	0.009	0.000
$p_{adjusted}$		19.416	0.216	0.000
BPND	0.77	0.755	0.572	0.401
Medial temporal right				
P value		0.825	0.589	0.001
$p_{adjusted}$		18.983	0.589	0.001
BPND	0.579	0.724	0.550	0.380
mOFC left				
P value		0.783	0.015	0.000
$p_{adjusted}$		18.000	0.356	0.002
BPND	0.605	0.633	0.412	0.196
mOFC right				
Occipital left				
P value		0.669	0.002	0.000
$p_{adjusted}$		15.394	0.014	0.000
BPND	0.568	0.586	0.386	0.290
Occiptal right				
P value		0.548	0.000	0.000
$p_{adjusted}$		12.602	0.007	0.000
BPND	0.613	0.580	0.403	0.314
Parietal left				
P value		0.564	0.106	0.000
$p_{adjusted}$		12.974	2.431	0.003
BPND	0.525	0.560	0.423	0.276
Parietal right				
P value		0.268	0.091	0.000
$p_{adjusted}$		6.155	2.098	0.003
BPND	0.551	0.616	0.452	0.297
PCC				
P value		0.572	0.020	0.000
$p_{adjusted}$		13.157	0.463	0.000
BPND	0.837	0.873	0.691	0.504
Putamen left				
P value		0.905	0.005	0.000
$p_{adjusted}$		20.826	0.125	0.000
BPND	0.776	0.769	0.581	0.450
Putamen right				
P value		0.818	0.007	0.000
$p_{adjusted}$		18.820	0.168	0.001
BPND	0.758	0.772	0.557	0.435
Temporal left				
P value		0.546	0.003	0.000
$p_{adjusted}$		12.563	0.064	0.000
BPND	0.807	0.765	0.593	0.418
Temporal right				
P value		0.609	0.003	0.000
$p_{adjusted}$		14.015	0.062	0.000
BPND	0.786	0.754	0.596	0.415
Thalamus left				
P value		0.164	0.001	0.001
$p_{adjusted}$		3.777	0.021	0.033
BPND	0.481	0.572	0.021	0.033
Thalamus right				
P value		0.187	0.001	0.003
$p_{adjusted}$		4.291	0.023	0.080
BPND	0.513	0.606	0.276	0.313

P value		0.599	0.006	0.000
p_{adjusted}		13.786	0.147	0.001
BPND	0.579	0.629	0.386	0.202