Striatal cue-reactivity and neurotransmitter function in gambling disorder

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ABSTRACT

Background. Abnormal striatal cue reactivity is one of the neurobiological hallmarks of substance use disorders (SUDs). Cue reactivity is associated with relapse, prompting efforts to target its underlying mechanisms for therapeutic interventions. However, the neural correlates of cue reactivity in behavioral addictions, such as gambling disorder (GD), remain less understood. In this pilot study, we investigated striatal cue reactivity and its neurotransmitter associations in individuals with GD using multimodal imaging.

Methods. Thirteen subjects with GD and 16 healthy controls underwent fMRI using a block-design consisting of three different types of visual stimuli: gambling-related, erotic, and neutral videos. The subjects also underwent brain PET imaging with three specific radiotracers: 18F-FDOPA for striatal dopamine synthesis capacity, 11C-carfentanil for mu-opioid receptor availability and opioid and 11C-MADAM for serotonin transporter binding.

Results. Subjects with GD showed a significantly greater BOLD response in the dorsal striatum compared to healthy controls when viewing gambling-related (versus neutral) videos ($p_{FWE}<0.05$). Enhanced cue-reactivity was specific to gambling, as there were no significant differences in striatal BOLD response between the groups when watching the natural reward cues (erotic vs. neutral videos). The dorsal and ventral striatum BOLD responses to gambling videos were coupled in healthy controls ($r=0.7$, $p=0.003$) whereas no such coupling was observed in GD ($r=-0.1$, $p=0.75$, difference between the correlation coefficients $p=0.008$). In GD, dorsal striatal BOLD response to gambling cues correlated with 11C-carfentanil binding potential ($r = 0.8$, $p < 0.001$), implicating the involvement of the mu-opioid receptor system, but showed no significant association with dopamine synthesis capacity (18F-FDOPA) or serotonin transporter binding (11C-MADAM).

Conclusions. GD is characterized by increased gambling cue-induced activity in the dorsal striatum, which is linked to mu-opioid receptor availability. The findings highlight the potential role of the opioid system in mediating cue-reactivity in behavioral addictions.

Keywords: Gambling disorder, striatum, cue reactivity, opioid system

INTRODUCTION

Gambling disorder (GD) is characterized by persistent and recurrent gambling despite of harmful consequences (American Psychiatric Association, 2013). The worldwide prevalence of GD has been estimated to be 1.6-1.9% (Shaffer et al., 1999; Shaffer & Hall, 2001; Welte et al., 2002, 2015) (Welte et al., 2015). Clinically, GD shares several features with substance use disorders (SUDs), including increased impulsivity, compulsive behavior, and the pursuit of rewarding stimuli despite negative consequences (Clark, 2010; Fauth-Bühler et al., 2017; Goudriaan et al., 2006; Reuter et al., 2005).

Neurobiologically, addictive disorders have been closely associated with dysfunctions in the striatum. Under normal conditions, natural or extrinsic rewards, such as food, sex and money, are associated with hemodynamic responses in the ventral striatum (Diekhof et al., 2012; Sescousse, Caldú, et al., 2013). In SUDs, this reward circuitry becomes desensitized leading to reduced ventral striatum responses through continued excessive substance use (Koob & Volkow, 2010). This desensitization is associated with a transition to substance-related cue-induced reactivity, traditionally considered to localize to the more dorsal parts of the striatum, along with a blunted reactivity to natural reward cues (Engelmann et al., 2012; Everitt & Robbins, 2005; Sjoerds et al., 2014; Vollstädt-Klein et al., 2010; Zhou et al., 2019). Similar findings have been reported with cue reactivity in GD (García-Castro et al., 2023; Starcke et al., 2018), but the results are not uniform (Balodis et al., 2012; Choi et al., 2012; Crockford et al., 2005; Potenza et al., 2003).

Dopamine is one of the key neurotransmitters involved in reward- and cue-induced striatal responses. PET imaging studies in humans have repeatedly demonstrated striatal dopamine release in response to drugs, alcohol and monetary rewards (Hyman et al., 2006; Zald et al., 2004). In SUDs, these dopaminergic responses are blunted, with early studies suggesting an enhanced cue-induced dorsal striatal dopamine release, mediated by baseline dopaminergic tone in the striatum (Volkow et al., 2006; Wong et al., 2006). The findings in GD however differ from those of SUDs by mainly showing increased dopaminergic responses to gambling and no downregulation of postsynaptic dopamine receptors (Boileau et al., 2014; Linnet et al., 2010; O'Sullivan et al., 2011; Steeves et al., 2009; Wu et al., 2015). However, striatal dopamine function is also regulated by multiple other neurotransmitter, including endogeneous opioids and serotonin, but their role in cue-induced striatal responses is largely unstudied (Gago et al., 2007; Majuri et al., 2018; Tuominen et al., 2015; Unterwald & Cuntapay, 2000). Currently, there is no agreement on the mechanisms underlying cue reactivity in GD, and pharmacological treatments have yet to be established. Identifying the molecular mechanisms associated with cue-reactivity in GD could pave way for discovery new therapeutic options.

This study had two primary objectives: (1) to examine striatal hemodynamic responses to gambling-related cues in individuals with GD compared to healthy controls, and (2) to explore the contribution of striatal dopamine, opioid, and serotonin systems to these cue-induced responses in GD.

METHODS

Subjects

In this study, a total of 32 participants were involved, consisting of 15 individuals diagnosed with GD and 17 healthy controls (HCs) who had no history of gambling problems. Participants from both groups were age- and sex- matched. The inclusion criteria for GD was diagnoses confirmed through clinical interviews utilizing the DSM-IV criteria for pathological gambling but all GD subjects also fulfilled the diagnostic criteria for the most recent diagnostic criteria (DSM-5) for gambling disorder. For HC individuals, the inclusion criteria involved an absence of any gambling problems based on the clinical interview. Participants (from both groups) with the presence of neurological disorders, psychiatric disorders, evidence of current alcohol or substance use disorder, significant medical conditions, currently taking medications affecting the central nervous system, current pregnancy, strong susceptibility to allergic reactions or nausea, body weight exceeding the scanner limit (180 kg), and any contraindications to magnetic resonance imaging, were excluded from the study.

The study protocol received approval from the Ethics Committee of the Hospital District of Southwest Finland. All participants provided written informed consent, and the study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Clinical and behavioral measures

Clinical and behavioral data were obtained through validated questionnaires during a clinical interview at the initial study visit. This information included subject demographics, including age, gender, body mass index (BMI), and smoking status. Additionally, it included gambling-related metrics, such as weekly gambling hours, weekly gambling expenditure, and problematic gambling duration. The administered questionnaires were the South Oaks Gambling Screen (SOGS) (Lesieur & Blume, 1987), Beck Depression Inventory (BDI) (Beck et al., 1996), and the Barratt Impulsiveness Scale (BIS-11) (Barratt, 1985).

Image acquisition

Each participant completed an extensive brain imaging protocol, which encompassed structural MRI, task-functional MRI (task-fMRI), and three distinct brain PET scans designed to assess serotonin (11C-MADAM), dopamine (18F-FDOPA), and opioid (11C-carfentanil) neurotransmission.

MRI

We acquired 3D T1-weighted scans to serve as a structural reference for data analysis. These scans were obtained using a 3T PET-MRI scanner (Philips Ingenuity, Philips Healthcare, Cleveland, OH, USA) equipped with a 34-channel receiving head coil. The scanning protocol employed a sagittal 3D T1-weighted TFE sense pulse sequence with isotropic voxels using the following parameters: TR 8.1 ms, TE 3.7 ms, flip angle 7°, matrix size 256 × 256, and a total of 176 slices.

fMRI video task

fMRI scans were performed using PET- MRI scanner Philips Ingenuity (Philips Healthcare, Cleveland, OH, USA). Anatomical T1-weighted images were collected before fMRI tasks using the same scanner. Blood-oxygenation dependent (BOLD) echo-planar imaging (EPI) was applied. Whole-brain BOLD-weighted EPI sequence sensitive to the BOLD contrast was obtained during the stimuli presentations. The scanning protocol utilized a TR of 2000 msec, a TE of 20 msec, and a flip angle of 75°. It included 35 slices with a thickness of 4 mm each, operating in parallel multislice mode.

The experiments were run with the classic block design with approximately 10 blocks per condition. Participants were shown videos from three categories: neutral, natural reward (erotic), and gambling-related videos. Neutral videos depicted everyday activities, such as people walking in public spaces. Erotic videos primarily featured "soft-core" content, including scenes of nudity and intercourse. Gambling-related videos portrayed individuals engaged in casino games, such as poker and roulette, highlighting actions like handling chips and placing bets. Each category consisted of ten unique video clips from existing movies, which were presented twice in randomized order. Thus, every subject saw altogether 60 video clips with breaks of 6-8 seconds where participants were watching a black screen. Each video clip lasted approximately 9-14 seconds. The total stimulus presentation time was approximately 17 minutes. The schematic study design can be seen in **Fig. 1**. Due to scanner malfunction, two individuals with GD were not scanned with fMRI at all. In addition, one HC subject was excluded from the analysis due to lack of occipital BOLD response during the video task, suggesting that this subject was not viewing the videos as instructed. The fMRI paradigm was slightly shorter for three participants because of scanner storage space temporarily running out. To ensure that the shorter paradigm doesn’t bias the results, the main results were confirmed excluding these subjects from the analyses.

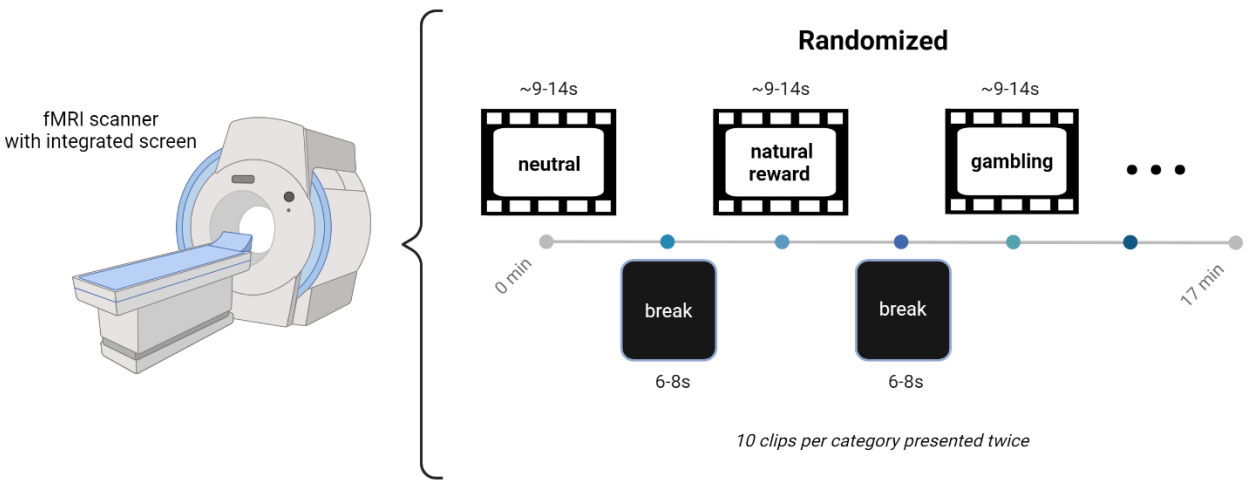

Figure 1. Task-fMRI study design
Block design to study the brain activation patterns while being presented videos of different categories, including gambling, natural reward, and neutral.

PET imaging

PET imaging protocols have been previously detailed in (Majuri, Joutsa, Johansson, Voon, Alakurtti, et al., 2017; Majuri, Joutsa, Johansson, Voon, Parkkola, et al., 2017). Imaging was conducted using a high-resolution research tomography (HRRT) PET scanner from Siemens Medical Solutions, with an intrinsic spatial resolution of 2.5 mm. Scanning times were 51 minutes for 11C-carfentanil, and 90 minutes each for 18F-FDOPA and 11C-MADAM. The 3D mode with scatter correction was applied. All three tracer PET scans were performed within a single day at fixed intervals. In specific cases due to logistical issues, three subjects underwent PET scans on two separate days. To minimize head movements during scanning, an individually shaped thermoplastic mask was typically used, except for three GD patients who utilized a Velcro strap due to discomfort with the mask. Head motion was tracked using a stereotaxic infrared camera (Polaris vicar, Northern Digital, Waterloo, Canada).

In the 11C-MADAM analysis, one participant with GD was excluded due to the use of SSRI medication during imaging, and two other participants (one with GD and one HC) were excluded due to excessive head movement while scanning. Additionally, technical problems during scanning resulted in the unavailability of one HC for the 11C-carfentanil analysis, and one HC along with two GD participants were unavailable for the 18F-FDOPA analysis as explained previously (Majuri, Joutsa, Johansson, Voon, Alakurtti, et al., 2017).

Task-fMRI data preprocessing and analyses

Anatomical data preprocessing

Anatomical preprocessing was performed with fMRIPrep 23.1.0 (Esteban et al. 2019). The T1-weighted (T1) images were corrected for intensity non-uniformity (INU) with

N4BiasFieldCorrection (Tustison et al. 2010), distributed with ANTs 2.3.3 (Avants et al. 2008, RRID:SCR_004757), and used as T1w-reference throughout the workflow. The T1w-reference was then skull-stripped with a Nipype implementation of the antsBrainExtraction.sh workflow (from ANTs). Brain tissue segmentation of cerebrospinal fluid (CSF), white-matter (WM) and gray-matter (GM) was performed on the brain-extracted T1w using fast (FSL 6.0.5.1:57b017774, Zhang, Brady, and Smith 2001). Brain surfaces were reconstructed using recon-all (FreeSurfer 7.3.2, RRID:SCR_001847, Dale, Fischl, and Sereno 1999). Volume-based spatial normalization to the Montreal Neurological Institute (MNI) space was performed through nonlinear registration with antsRegistration (ANTs 2.3.3).

Functional data preprocessing and analyses

Functional preprocessing was performed with fMRIPrep 23.1.0 (Esteban et al. 2019). Briefly, the functional preprocessing pipeline involved the following steps: Head-motion parameters were estimated and corrected using mcflirt (FSL, Jenkinson et al. 2002), aligning the BOLD time-series back to its original space. Co-registration between BOLD and T1w references was done using bbregister (FreeSurfer). Various confounding time-series were derived from the preprocessed BOLD data, including framewise displacement (FD), DVARS, and three global signals (CSF, WM, whole-brain masks). Physiological regressors were also extracted for noise correction using CompCor (tCompCor, aCompCor). Frames that exceeded a threshold of 0.5 mm FD or 1.5 standardized DVARS were classified as motion outliers. The preprocessed BOLD runs were resampled into MNI space. The internal operations of fMRIPrep relied on Nilearn 0.10.1 within the functional processing workflow. For more comprehensive details, refer to fMRIPrep's documentation on workflows.

Individual and group level analyses were performed with SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) in MATLAB (2021b; MathWorks, Natick, MA, USA). First, for the individual level analysis, we corrected for motion-related signal changes within each subject by including regressors for the 6 rigid body realignment parameters (3 translations and 3 rotations) as well as motion outlier volumes. Then, for the voxel-wise group-level analyses we restricted to the striatum (Mawlawi et al., 2001). Peak-level Family-Wise Error (FWE) correction was applied with a corrected threshold of $p < 0.05$ was considered significant to control for multiple comparisons in the fMRI data (Eklund, Nichols, & Knutsson, 2016). Age and sex were included as nuisance covariates to the group-level analyses. Extracted mean connectivity values from significant clusters and striatal ROIs were used for visualization and correlation analyses with clinical and PET imaging data. Pearson or Spearman correlations were used for these analyses.

PET imaging data preprocessing and analyses

PET imaging data preprocessing procedures have been previously documented (Majuri, Joutsa, Johansson, Voon, Alakurtti, et al., 2017; Majuri, Joutsa, Johansson, Voon, Parkkola, et al., 2017). Briefly, image realignment and coregistration were performed using SPM8 software in MATLAB R2012a. Individual PET images were realigned to correct for any head movement during scanning, and the scan reconstruction details were as described (Johansson et al., 2016). Regional data were extracted from regions of interest (ROIs) generated using FreeSurfer's recon-all (version 5.3.0). These ROIs were employed to extract average time-activity courses for modeling. 18F-FDOPA Ki images were computed using a Patlak plot, while 11C-MADAM and 11C-carfentanil BPND images were calculated using a simplified reference tissue model. The cerebellar cortex served as the reference region for 11C-MADAM, while the occipital cortex was designated for 18F-FDOPA and 11C-carfentanil. Parametric images were normalized to the Montreal Neurological Institute standard space (MNI152) using T1 information with DARTEL and subsequently smoothed with a 6mm Gaussian kernel for enhanced signal-to-noise ratio in statistical analyses restricted to the striatum (Mawlawi et al., 2001). Two subjects, whose measurements from the right NAcc 11C-MADAM BPND showed standard deviations greater than 2, were identified as outliers and were excluded from the analyses involving this variable.

To investigate neurotransmitters underlying the identified abnormal functional activation, BPND/Ki values were extracted from the significant connectivity cluster. In addition, for further analyses, BPND/Ki values were also obtained from the dorsal and ventral striatum.

Statistical analyses

Statistical analyses for ROI and clinical data were performed using IBM SPSS Statistics, version 27 (Armonk, NY, USA). Group differences in demographic and clinical data were assessed using independent samples t-tests, Mann-Whitney tests, and chi-square tests. To explore relationships between clinical/behavioral and/or imaging data, Pearson and Spearman correlation coefficients were used. To analyze if correlations differ significantly from each other Fisher's *r* to *z* transformation was used.

RESULTS

Demographic and clinical measures

Table 1 presents all demographic and clinical information. No significant group differences were found in age, sex, AUDIT and BIS-11 attention subdomain. The GD group showed significantly higher scores in all gambling-related variables, BIS-11 motor and nonplanning subdomains, BDI, and smoking.

TABLE 1. Demographic and clinical characteristics

Variables (mean ± SD)	*GD (n = 13)*	*HC (n = 16)*	*p value*
Age (years)	43.9 ± 12.2	43.5 ± 11.4	0.94
Sex (male/female)	6/7	8/8	0.84
Gambling hours per week	8.6 ± 6.6	0.6 ± 1.3	<0.001
Gambling euros per week	175.4 ± 146.5	3.7 ± 7.5	<0.001
Problem gambling years	11.9 ± 7	0.0 ± 0.0	<0.001
PG DSM-IV points	7.5 ± 1.5	0.1 ± 0.3	<0.001
SOGS	13.3 ± 2.4	0.1 ± 0.3	<0.001
BIS11_attention	19.5 ± 2.9	17.8 ± 1.9	0.054
BIS11_motor	26.3 ± 2.1	22.3 ± 2.5	<0.001
BIS11_nonplanning	28.4 ± 1.9	23.1 ± 4.6	<0.001
BDI	14.7 ± 8	2.9 ± 3.2	<0.001
Smoking	11/2	6/10	0.01
AUDIT	6.4 ± 4	5.4 ± 3.4	0.45

SD: Standard deviation; GD: Gambling disorder; HC: Healthy controls; AUDIT: Alcohol Use Disorders Identification Test; SOGS: South Oaks Gambling Screen; BIS: Barratt Impulsiveness Scale; BDI: Beck Depression Inventory; PG: Pathological gambling

Striatal hemodynamic responses to visual stimuli

Individuals with GD showed significantly greater BOLD response in the dorsal striatum than healthy controls when watching gambling-related versus neutral videos (**Fig. 2**). Overall, BOLD response in individuals with GD tended to be higher in the dorsal striatum and lower in the ventral striatum (**Fig. 2A).** Removing the subjects with shorter tasks did not change the results either. No significant group differences were found in the erotic videos contrasted to gambling or neutral videos.

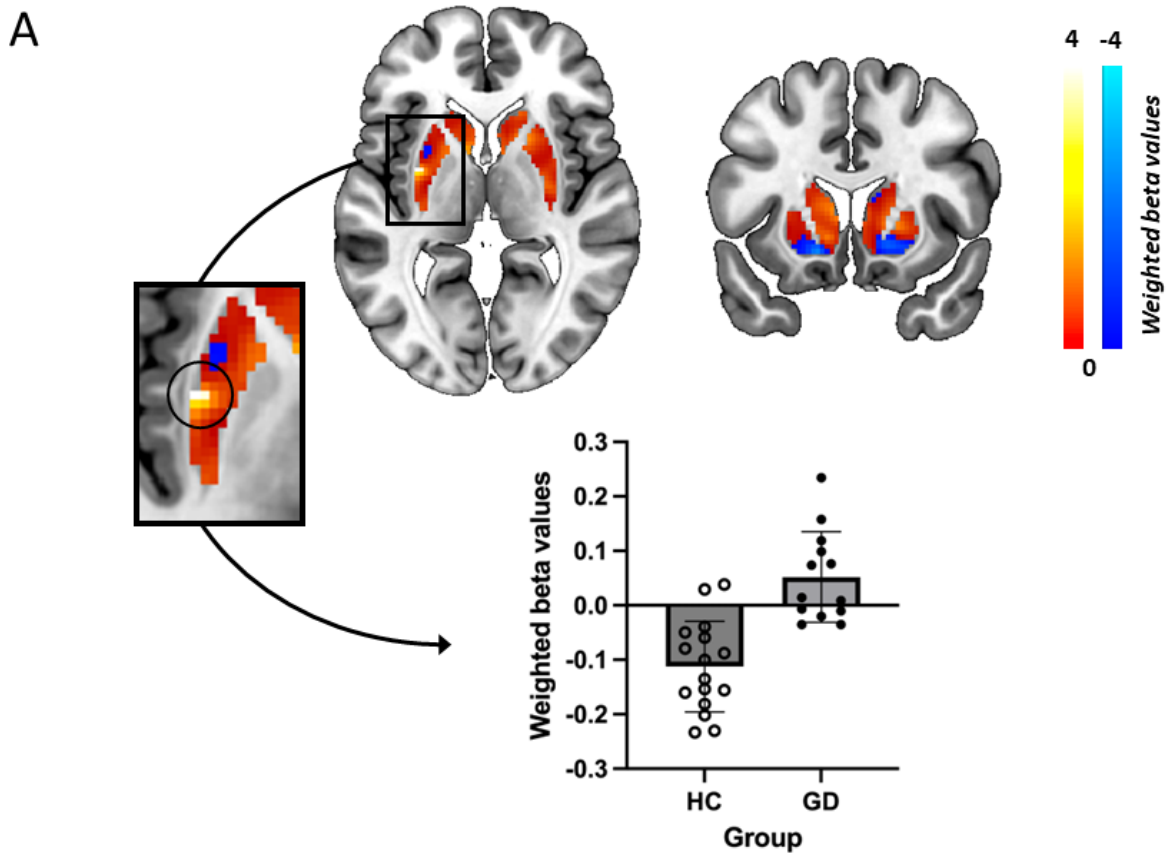

Figure 2. Striatal BOLD response to gambling videos in individuals with GD and HC
A) Unthresholded GD>HC BOLD T-map. Significant cluster showed within the zoomed box (peak coordinates at -32 -2 2, cluster size 2 voxels, P_{FWE}=0.004) B) Plotted raw values within the significant cluster (HC: -0.11(0.08), GD: 0.05(0.08)).

The BOLD response within the dorsal striatum cluster did not significantly correlate with GD symptom severity or other gambling-related variables (p>0.18). In addition, there were no significant correlations between this BOLD response and BDI score (r = 0.12, p = 0.72), AUDIT score (r = -0.36, p = 0.23) or smoking (r = 0.4, p = 0.17).

There was a significant positive correlation between ventral and dorsal striatum BOLD response to gambling contrasted to neutral videos in healthy volunteers (p=0.003) but not in individuals with GD (p=0.75). The difference between both correlation coefficients was significant (p=0.008) (**Fig. 3**).

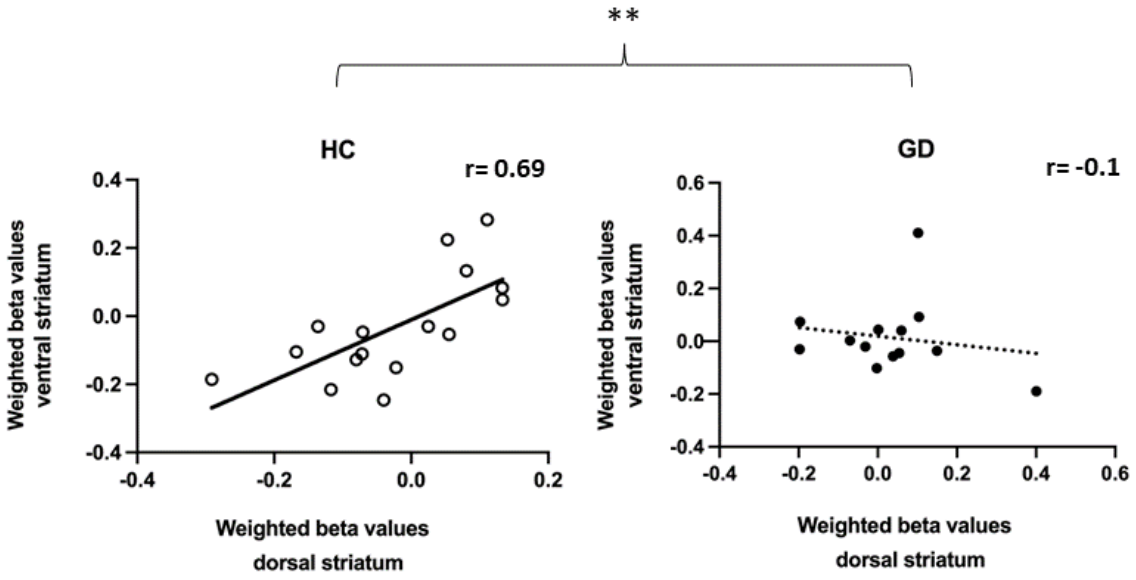

Figure 3. Correlations between ventral and dorsal striatum BOLD response to gambling videos
Correlations in HC and GD group
*** represents a significance level of $p \leq 0.01$.*

Neurotransmitter function in the striatum

The ventral and dorsal striatum ROI tracer binding/uptake values are presented in **Table 2**. There were no significant group differences in the dorsal and ventral striatum binding in any of the tracers (**Table 2**).

TABLE 2. Group comparisons within tracer binding in ventral and dorsal striatum

Tracer and region (mean ± SD)	*GD*	*HC*	*p value*
11C-MADAM (BP_{nd})	n=13	n=16	
Dorsal striatum	0.91 ± 0.13	0.98 ± 0.12	0.15
Ventral striatum	1.14 ± 0.21	1.21 ± 0.15	0.31
18F-fluorodopa (K_i)	n=13	n=16	
Dorsal striatum	0.012 ± 0.002	0.012 ± 0.001	0.68
Ventral striatum	0.01 ± 0.001	0.01 ± 0.001	0.8
11C-carfentanil (BP_{nd})	n=15	n=16	
Dorsal striatum	1 ± 0.22	1.16 ± 0.28	0.13
Ventral striatum	1.7 ± 0.27	1.84 ± 0.22	0.13

SD: Standard deviation; GD: Gambling disorder; HC: Healthy controls; 11C-MADAM

In individuals with GD, ^{11}C-carfentanil BP_{ND} correlated significantly with BOLD response to gambling contrasted to neutral videos in the dorsal, but not ventral, striatum (r=0.81, p<0.001) (**Fig. 4A**). The significance of the correlation did not change when excluding the subject with highest BP_{ND} and BOLD response. There were no other significant correlations between any of the other measured neurotransmitters and striatal BOLD response in the dorsal or ventral striatum (**Fig. 4**). In healthy controls, there were no significant correlations between neurotransmitters and BOLD response in the ventral or dorsal striatum.

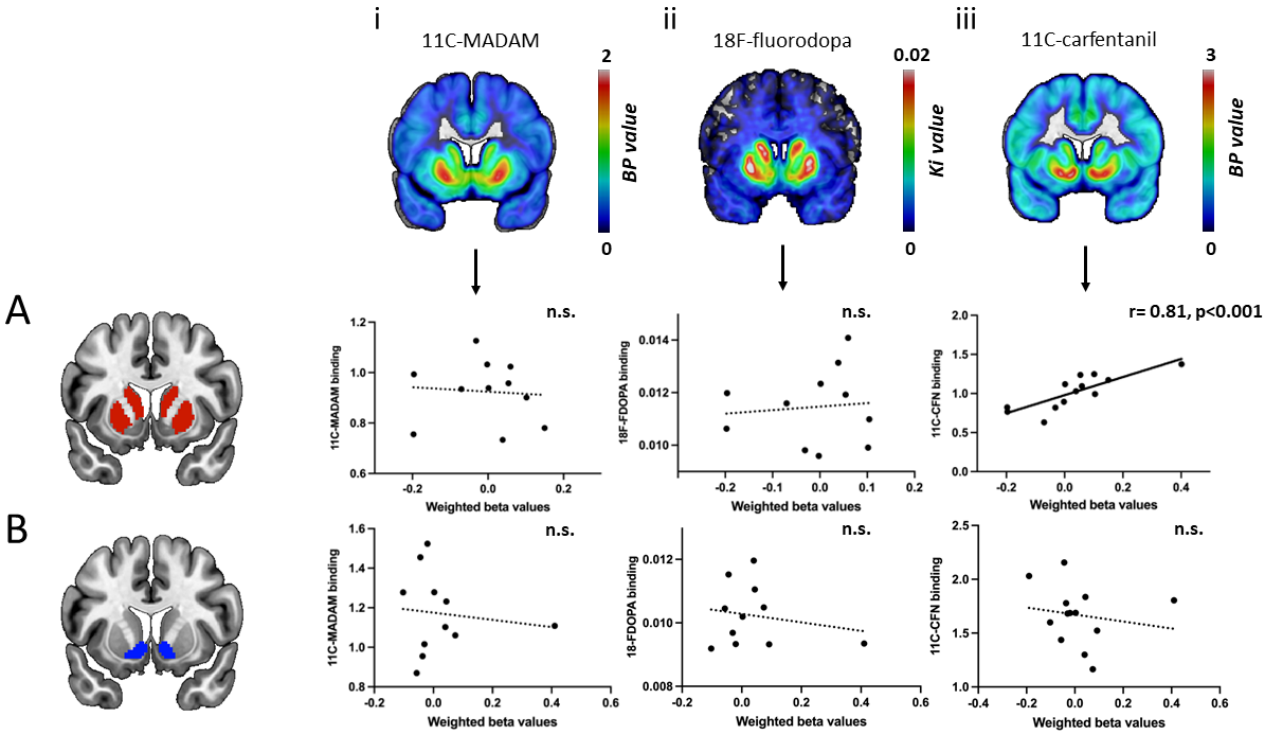

Figure 4. Correlation between cue-induced BOLD response and neurotransmitters.
Dorsal (A) and ventral striatum (B) fMRI BOLD signal alongside dorsal and ventral striatum binding of ^{11}C-MADAM (serotonin transporter ligand) (i), ^{18}F-FDOPA (presynaptic dopamine synthesis capacity) (ii) and ^{11}C-carfentanil (mu-opioid receptors) (iii) in individuals with gambling disorder
Significant correlation between the ^{11}C-carfentanil BP_{ND} and fMRI BOLD signal in dorsal striatum (r = 0.81, p < 0.001)
n.s. = non significant

DISCUSSION

This study has several key findings. First, we observed increased cue-reactivity in the dorsal striatum in GD compared to healthy controls, specifically in response to gambling-related stimuli. Second, unlike in healthy controls, the dorsal striatum responses to gambling cues were decoupled from those in the ventral striatum, suggesting a distruption in the typical functional connectivity between these regions. Lastly, the cue-induced dorsal striatum responses were significantly associated with mu-opioid availability, but not with presynaptic dopamine synthesis capacity or serotonin transporter binding, highlighting the unique involvement of the opiod system in mediating these responses.

Prior studies of cue-reactivity in GD have been heterogeneous in terms of methodology and produced mixed results (Balodis et al., 2012; Choi et al., 2012; Crockford et al., 2005; Kober H et al., 2016; Limbrick-Oldfield EH et al., 2017; Potenza et al., 2003; Sescousse, Barbalat, et al., 2013; van Holst et al., 2012). Our study used gambling-related videos, similar to what has been used to verify increased striatal cue-induced dopamine responses in SUDs (Volkow et al., 2006). In GD, there are three prior studies that have used gambling-related videos to study cue-reactivity with fMRI. Potenza et al. (2003) and Kober H et al. (2016) both compared gambling-related content against baseline conditions (gray screens) shown before and after the videos, and reported both increased and decreased BOLD responses in GD compared to healthy volunteers in several brain regions. Crockford et al. (2005) compared gambling-related videos to nature videos, similarly reporting widespread increases in BOLD responses in several brain regions. However, contrary to the findings of the present study, none of these studies reported increased BOLD responses specifically in the striatum. The findings from these prior studies seemingly contradict the observations in SUDs (Cousijn et al., 2013; Engelmann et al., 2012; Koob & Volkow, 2010; Sjoerds et al., 2014; Zhou et al., 2019). However, the previous cue-reactivity studies in GD have investigated the effects across the whole brain, but our approach of specifically targeting cue-induced responses in the striatum enabled us to increase statistical power to detect these changes, despite a relatively small sample size, which a limitation shared by practically all GD functional neuroimaging studies. Another strength of our study was the inclusion of natural reward cues (erotic videos) to assess specificity of the findings to gambling-related cues. Including this control condition strengthens the validity of our findings by reducing the likelihood that the observed striatal responses were simply due to a generalized sensitivity to cues associated with any rewarding stimuli.

We observed a coupling between the ventral and dorsal striatum BOLD response in healthy individuals, but not in individuals with GD. This highlights a potential mechanism underlying impaired decision-making and reward processing in addiction. In HC, the ventral striatum plays a crucial role in processing reward prediction and value, which is then passed to the dorsal striatum to guide action selection and habit formation (Everitt & Robbins, 2005). In GD, the decoupling between these regions could indicate a disruption in this process, where the DS may become overactive and more independent from ventral striatum function, leading to compulsive gambling behaviors. This decoupling aligns with models of addiction hypothesizing a shift from ventral striatum-dominant (goal-directed) to dorsal striatum-

dominant (habitual) behavior, driven by alterations in corticostriatal circuitry (Everitt & Robbins, 2005).

In this study, dorsal striatum BOLD response to gambling videos was associated with mu-opioid binding in this region. The association between dorsal striatum BOLD response to gambling-cues and mu opioid binding was significant in subjects with GD but not in healthy controls, suggesting specificity for gambling disorder. This finding aligns with the prior observations demonstrating an association between mu opioid receptor binding potential and craving in individuals with SUDs (Gorelick et al., 2005), a state commonly elicited in cue-reactivity paradigms (Antons et al., 2020; Kauer & Malenka, 2007). Accordingly, opioid antagonists have shown to reduce cue-induced responses and reward impulsivity, supporting the role of the opioid system in cue-reactivity and craving (Weber et al., 2016). In addition, naltrexone, an opioid antagonist, reduced cue-reactivity by enhancing the functional connectivity between the dorsal striatum and prefrontal regions during methamphetamine cue processing (Courtney et al. (2016)).

In contrast to mu opioid binding, we did not find an association between presynaptic dopamine synthesis capacity or serotonin transporter binding and striatal cue-induced BOLD responses. Prior research has demonstrated that cue-reactivity in SUDs is associated with striatal dopamine D2/D3 receptor binding (Volkow et al., 2006), but these findings may not generalize to behavioral addictions, as SUDs are associated with reduced striatal dopamine function, but GD is not (Boileau et al., 2013; Clark et al., 2012; Joutsa et al., 2012; Linnet et al., 2012; Majuri, Joutsa, Johansson, Voon, Alakurtti, et al., 2017). However, striatal dopaminergic function is modulated by the opioid system (Colasanti et al., 2012; Jalabert et al., 2011; Mick et al., 2014; Soderman & Unterwald, 2009; Tuominen et al., 2015) and both presynaptic dopamine synthesis capacity and dopamine D2/D3 receptor binding have been shown correlate with mu opioid receptor binding in the dorsal striatum (Colasanti et al., 2012; Majuri et al., 2018; Mick et al., 2014; Tuominen et al., 2015). Thus, although striatal cue-reactivity does not directly correlate with striatal dopamine function in GD, cue-induced striatal responses may still be dopaminergic but abnormal cue-reactivity in GD is mediated via abnormalities of the opioid function. To our knowledge, this is the first neuroimaging study investigating neurotransmitter correlates of cue-reactivity in GD.

There are some limitations in the present study that should be considered when interpreting the results. First, as the sample size was low for an fMRI study, independent confirmation is warranted. However, the number of participants is comparable to the previous studies investigating cue-reactivity in GD. Second, the cross-sectional design of the study limits the ability to establish causality, as the findings are correlational in nature. Finally, we only studied specific components of the opioid, dopamine and serotonin systems, and negative findings with any of these should not be considered to exclude any abnormalities in these neurotransmitter systems.

In summary, this study underscores the involvement of the dorsal striatum and its association with the endogenous opioid system in processing gambling-related cues in individuals with

GD. These findings provide novel information about the underlying neurobiology of individuals with GD processing gambling-related cues. This knowledge may inform future therapeutic interventions targeting the opioid system to reduce cue-induced cravings and relapse in GD.

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