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Neural Correlates of Cue Reactivity in Slot-Machine Gambling

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Abstract

Gambling disorder (GD) shares key features with substance use disorders, including craving, withdrawal, tolerance, loss of control, as well as decision-making impairment, and involves overlapping neurobiological mechanisms. GD provides a unique opportunity to investigate addiction-related neural processes without substance-related confounds. In this fMRI cue-reactivity study, we compared neural responses to gambling-related (slot-machine) and neutral images in 11 male patients with GD at a specialized outpatient clinic for behavioral addictions and 20 male healthy controls in Linz, Austria. Both cue types, relative to a resting baseline, elicited widespread activation across visual processing pathways, oculomotor regions, frontal control areas including the insula, and subcortical structures such as the superior colliculus, reflecting a distributed network supporting visual stimulus processing. Direct comparison of gambling-related versus neutral cues revealed no robust whole-brain group differences. However, voxel-of-interest analyses revealed a significant four-way interaction involving stimulus category, insular subregion, hemisphere, and group. While healthy controls exhibited significantly greater bilateral anterior insula activation in response to neutral cues compared to slot-machine stimuli, patients demonstrated a non-significant trend toward the inverse pattern specifically within the left anterior insula. These results point toward highly localized, region- and hemisphere-specific variations in insular cue processing that require further investigation.

Keywords: behavioral addictions; gambling; cue reactivity; fMRI; slot machines

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1. Introduction

The relevance of behavioral addictions (BAs) is growing constantly, not only in clinical practice, but also as a topic in addiction research. Gambling disorder (GD) is considered the prototypical behavioral addiction [1] and is the first to be included as a diagnosis in the section of substance-related and addictive disorders in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders [2]. It has been argued that behavioral addictions and substance use disorders (SUDs) overlap on a causal level, and that the same treatment strategies might be successful [3]. Furthermore, behavioral addictions in general were found to share similar neurobiological mechanisms with SUDs [3,4]. Obviously, some of the key addiction-related characteristics relevant in SUDs are also evident in behavioral addictions. Specifically, GD shares many (diagnostic) features with SUDs like craving, withdrawal, and tolerance. Therefore, studying the mechanisms of GD is of great scientific interest, since

addiction mechanisms can be investigated independently from substance-use confounds [4]. Further investigation of GD not only assists in determining neural mechanisms of shared addictive features, but also in identifying unique features. As a consequence, recent studies were able to isolate brain activations as a distinct response to GD independently of the neurotoxic effects of substance use itself: specific frontal cortex gray matter alterations were detected in individuals suffering from GD without comorbid SUDs [5].

1.1. Cue Reactivity in Gambling Disorder (GD)

Neuroimaging research has identified key neural substrates involved in cue reactivity and craving, implicating dopaminergic reward pathways in the maintenance of gambling behaviors [6,7]. The anticipatory dopamine response, in particular, has been proposed as a shared mechanism underlying both gambling and substance addictions [8]. Cue reactivity is a widely used procedure to study subjective, peripheral physiological and neural responses to the exposure of cues associated with the addictive substance or behavior [9]. For instance, images of alcoholic beverages can induce a strong desire to drink alcohol in individuals with alcohol use disorder, reflected in increased brain activation in parts of the reward system and regions specifically involved in craving. The concept of craving is not only one of the diagnostic criteria in the DSM-5, but is also strongly associated with future substance use and relapse [10].

The underlying theoretical concept of the cue reactivity paradigm is often explained by Pavlovian conditioning [11]. In this case, the repeated pairing of addiction-related cues (for instance, the sight, smell, or taste of a substance) with the subsequent consumption of that substance leads to conditioned reinforcement after multiple recurrences. As a consequence, these addiction-related cues might trigger reward-associated motivational responses (i.e., cue-reactivity) as conditioned stimuli, which can in return elicit craving [9,11]. Importantly, cue reactivity responses not only occur in SUDs, but also in behavioral addictions: meta-analyses of studies on gambling, gaming, and buying disorders confirmed significant brain activation in patients when compared to healthy controls, specifically in the caudate nucleus, median cingulate cortex, subgenual cingulate, inferior frontal gyrus, and precentral gyrus [9]. Among individuals suffering from behavioral addictions, the comparison between addiction-related and neutral cues further revealed significant neural responses during cue-exposure, including the caudate nucleus, inferior frontal gyrus, angular gyrus, precuneus, and the inferior network [9].

1.2. Neuroimaging Studies in Gambling Disorder (GD)

Neuroimaging studies applying the cue reactivity paradigm in GD are still sparse and have produced mixed results so far. A recent review on this topic found that gambling-related cues consistently activate reward, memory, salience, and executive control regions, with the insula serving as a hub integrating these signals, thereby supporting GD's classification as a behavioral addiction [12]. An early study by Potenza and colleagues found increased activation in frontal, paralimbic, and limbic brain structures, while observing a relative decrease in structures involved in impulse regulation for individuals with GD compared to healthy controls, when exposed to gambling-related cues [13]. Increased brain activity associated with GD was found in the right dorsolateral prefrontal cortex (DLPFC), the right parahippocampal gyrus, and the left occipital cortex (including the fusiform gyrus) after gambling-related cue exposure [14]. A further study with long-term problem gamblers was able to reproduce some of these findings (e.g., increased activation in the parahippocampal gyrus), but also detected the involvement of the amygdala, as well as associations between insular cortex and caudate nucleus activations with craving [15]. An fMRI-study investigating cocaine use disorder (CUD) and GD found both similarities

and differences in brain activations for the two diagnostic groups: activation within the dmPFC/dACC network was elevated in response to addiction-related cues across both groups, whereas a distinct upregulation of brain activation encompassing the posterior putamen and the insula was observed exclusively in female patients with GD, but remained absent in their male counterparts [16].

Naqvi and colleagues observed that damage to the insula resulted in an abrupt cessation of smoking addiction: individuals were able to quit effortlessly, immediately, without relapse, and without experiencing cravings [17]. These findings indicate that the insula plays a crucial role as a neural substrate underlying nicotine addiction. A later review by Naqvi and colleagues discussed the general, important role of the insula in addiction, particularly in craving and drug-seeking behaviors [18].

1.3. Slot Machine Gambling

The current study focused exclusively on slot-machine gambling. Experimental and cognitive neuroscience studies have further explored how reinforcement cues and game design features (such as 'losses disguised as wins') enhance incentive salience and sustain engagement with electronic gaming machines [19,20]. Recent research using eye-tracking and behavioral paradigms has also shed light on the immersive and affective experiences of slot machine gambling, revealing distinct cognitive and emotional states as well as reward reactivity [21,22]. Collectively, these insights highlight the intricate interplay of neurobiological, cognitive, and emotional factors that perpetuate maladaptive gambling behavior.

A study similar to the current investigation used the cue reactivity paradigm and presented images of gambling-related, neutral and highly appetitive (food) cues to patients diagnosed with GD and healthy controls [7]. In that study, three different forms of gambling were investigated: electronic roulette, sports betting, and slot machines. Gambling-related cues of the two most preferred forms for each participant were then used to further investigate neural responses to these images. In their study, only five participants were presented with slot machine images (alongside roulette cues). In general, the authors reported a significant association between craving and activation in the insula and nucleus accumbens for the whole GD group [7].

1.4. Aims and Hypotheses

In the current study we investigated neural activation in response to cue exposure of gambling-related cues by showing images of slot-machines in comparison to neutral cues (e.g., ticket machines). We aimed to identify brain regions specific to slot-machine-gambling-related images, as well as specific brain structures involved in GD. To that end, the activation contrast was investigated between these stimulus categories (gambling-related vs. neutral) for both patients suffering from GD who mainly played slot-machines as well as for healthy controls (HCs). Activation patterns evident in both groups could reflect shared slot-machine elicited responses, while potential differences between groups require further investigation.

2. Materials and Methods

2.1. Participants and Procedure

In this fMRI-study ($N = 31$), two groups were compared. The patient group ($n = 11$) consisted of patients suffering from GD, who most frequently gambled with slot machines. On average, they were 40.4 years old (SD = 10.2 years; range 20–55 years). For comparison, a healthy control (HC) group with 20 participants was included (mean age = 39.9 years, SD = 11.2 years; range 24–58 years). Only male participants were included in the study,

as the vast majority of patients diagnosed with GD at our institution was male, and the healthy control group was selected accordingly.

The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Upper Austria, Kepler University Hospital Linz (Study No. E2714). Written informed consent was obtained from all participants.

Prior to the study, both groups were pre-evaluated. Handedness was determined using the Edinburgh Handedness Inventory [23], and all participants were confirmed to be right-handed. To rule out psychiatric comorbidities, patients were evaluated with the German version of the Structured Clinical Interview for DSM-IV [24], and individuals meeting criteria for substance use disorders (SUDs), major depressive disorder, schizophrenia, bipolar disorder, obsessive–compulsive disorder, anxiety disorders, or eating disorders were excluded from further testing. Additional exclusion criteria related to medical conditions and MRI safety applied for both groups and included a history of brain injury, psychosis, claustrophobia, and the presence of metallic implants. Nicotine dependency was not an exclusion criterion.

It should be noted that the authors aimed to obtain a homogenous patient sample to test our hypotheses concerning slot machine gambling. Patients meeting these rigorous criteria were rare, especially since comorbid psychiatric conditions are often prevalent in GD. Additionally, several further participants of both groups had to be excluded at some point from further analysis for medical or technical reasons, resulting in a total sample size of $N = 31$.

Individuals were instructed to carefully observe the images, before undergoing the fMRI measurements. Following the fMRI scan, participants provided self-reports of thoughts and emotions during the task to evaluate subjective differences. All participants received monetary compensation.

2.2. Cue-Reactivity Paradigm

During the fMRI scan, participants viewed a total of 40 images across two stimulus categories (see Figure 1 for example stimuli). Gambling-related cues consisted of photographs of slot machines, while images of ticket machines for parking or transportation served as neutral cues. Stimuli were presented in a block-wise fashion, alternating in a pseudorandomized order with resting periods in between. Each condition was repeated four times. Every block comprised five images shown for 6 s, resulting in a total duration of 30 s per block. Resting periods (indicated by a fixation cross) also lasted 30 s, yielding a total paradigm duration of 8 min.

2.3. Image Acquisition

Imaging was conducted on 3-Tesla MRI scanners (Siemens Magnetom Verio and Siemens Magnetom Skyra, Erlangen, Germany) using a 64-channel head coil. An axial T2-weighted sequence was acquired to rule out any unexpected neuropathology. In addition, before the fMRI task one sagittal 3D MP-RAGE (T1 weighted, TR 1900 ms, TE 2.26 ms, voxel resolution 1 × 1 × 1 mm^3, 192 slices, image matrix size 256/256) was performed for subsequent co-registration of the functional MR data. For the cue task, whole-brain gradient-echo planar imaging (EPI) data were acquired to measure BOLD contrast, with slice orientation aligned to the AC-PC line (intercommissural plane) (TR 3000 ms, TE 30 ms, 42 slices, slice thickness 3 mm, in-plane resolution 3 × 3 mm^2, slice gap 0.3 mm, flip angle 90°). The paradigm consisted of 160 volumes (10 volumes per block) resulting in an overall duration of 8 min for the paradigm.



Figure 1. Gambling-related and neutral cues. Depicted are examples of stimuli presented in the cue reactivity paradigm. (**Left**) panel: a picture of a slot machine illustrates the gambling-related stimulus cue. (**Right**) panel: images of ticket vending machines were presented as neutral cues.

2.4. Analysis

Image postprocessing was performed using BrainVoyager version 21.4.5 (Brain Innovation B.V., Maastricht, The Netherlands) [25]. First, differences in slice acquisition timing were corrected using sinc interpolation. Motion correction was performed by shifting and rotating each image volume using trilinear interpolation. Additional motion artifacts (exceeding a 1 mm displacement or 1 degree rotation between successive volumes) were manually identified and excluded from further analysis. Linear and nonlinear drifts in the time courses were removed via a temporal high-pass filter of two cycles per time course. Then, the functional data were realigned with the T1 volumes and transformed into the standard anatomical space of Talairach and Tournoux [26]. Finally, a spatial smoothing with a 4 mm Gaussian filter was applied on the 3D volume time course. The stereotaxic coordinates of the center of gravity of the functional clusters were determined. Clusters were identified using the Talairach Client available online at http://talairach.org (accessed on 5 October 2024) [27,28]. For the anatomical VOI analysis, we used an atlas provided by Neuromorphometrics, Inc. (Somerville, MA, USA), available online at http://neuromorphometrics.com (accessed on 5 October 2024) under academic license which is also included in SPM12 [29]. The "label map" was transformed into Talairach space using the transformations developed and validated by Jack Lancaster et al. [30]. We selected the anterior and posterior insula in both the left and right hemispheres as "regions of interest" (VOIs). Each participant's brain activation during each phase was checked for plausibility and unusual activation.

Active Condition versus Rest Condition: This type of contrast is calculated to determine the entire network of brain areas showing higher activation during a task in comparison to simply resting; i.e., it demonstrates which brain areas are involved in a certain task. Activation on the positive scale (shown in yellow/orange) indicates that these brain areas are more active during the active condition than during the rest condition. This contrast calculation was used to analyze general activation during the observation of slot machines or ticket machines.

Active Condition 1 versus Active Condition 2: If the interest is to determine the differences in brain activation between two tasks performed, while the activations common in both conditions are not of interest, two active conditions are compared. Activation on the positive scale (yellow/orange) then indicates that these areas are more active in one active state, while activation on the negative scale (green/blue) indicates areas that are more active in the other active state.

Second-level VOI analysis: Based on the first-level contrasts, an additional voxel-of-interest (VOI) analysis focusing on the insula was conducted. VOIs were defined from activations observed in the HC group using images of both stimulus categories (i.e., slot machine and ticket machine images). Statistical maps were adjusted using a false discovery rate (FDR) of $p < 0.1$ with automatic thresholding, and clusters were manually refined to exclude activations outside the insula. This procedure yielded four VOIs corresponding to the anterior and posterior insula in each hemisphere. Mean beta values were extracted for each participant and analyzed using a 2 × 2 × 2 × 2 repeated-measures ANOVA with the within-subject variables stimulus category (slot vs. neutral), insula subregion (anterior vs. posterior), hemisphere (left vs. right), and the between-subject variable group (patients with GD vs. HC). Importantly, because these functional VOIs were delineated based strictly on activations within the healthy control cohort utilizing the collapsed images of both stimulus categories, they remain orthogonal to and independent of the clinical group factor. Consequently, this selection pipeline avoids circular analysis (double dipping) regarding the direct slot-machine versus neutral contrast within the patient population.

3. Results

3.1. BOLD Contrast "Both Stimulus Categories Versus Rest"

BOLD contrast for both stimulus categories involving gambling-related as well as neutral cues (i.e., images of slot and ticket machines) was compared to the rest condition indicated by a fixation cross. This BOLD contrast is used to depict the entire network when viewing images of slot and ticket machines. It provides an overview of all regions involved in this process and also serves as a control for data quality. Numerous significant clusters of activation were found after the described analysis procedure. These are primarily the well-known regions involved in the processing of visual stimuli. This activates both of the two visual processing pathways: the ventral pathway for processing color information through the viewing of colorful images via the early visual areas V1/V2/V3 to V4 around the fusiform gyrus; and the dorsal pathway, which is triggered by motion pattern and in this case due to eye movements when scanning the images. This pathway runs via areas V1/V2/V3 to the movement-processing area V5 (hMT) and from there via the parietal cortex to frontal regions. There are also areas located for processing eye movements, like the FEF and SEF. Other multifunctional areas, such as the insula, are active in the frontal part of the cortex. Furthermore, cognitive processing of the presented content of the visual stimuli takes place there. And finally, subcortical structures such as the colliculus are involved. Table 1 shows these clusters averaged over the two conditions of slot and ticket machines and both groups ranked by their volume.

In Figure 2 the activation pattern of the BOLD contrast is shown separately for the healthy control group and the patients with gambling disorder. With the same statistical threshold of FDR $p < 0.05$, the activation in the patient group is lower, which can be attributed to the smaller subsample size. Importantly, the neural network does not show any noticeable differences between the two groups.

Table 1. Activation clusters for different brain regions. Talairach coordinates relating to activation clusters for both stimulus categories (gambling related and neutral cues, i.e., images of slot machine and ticket machines) versus rest condition (i.e., fixation cross), averaged over both groups: healthy control group (HC) and patients with gambling disorder (GD).

Areas	x	y	z
V1/V2/V3	−3	−91	1
V4	±27	−36	−13
V5 (hMT)	±39	−61	−5
Cuneus	±28	−83	10
Parietal cortex	±29	−63	42
FEF	±41	−1	35
SEF	−2	1	54
Insula	±39	26	1
Colliculus	±23	−23	−2

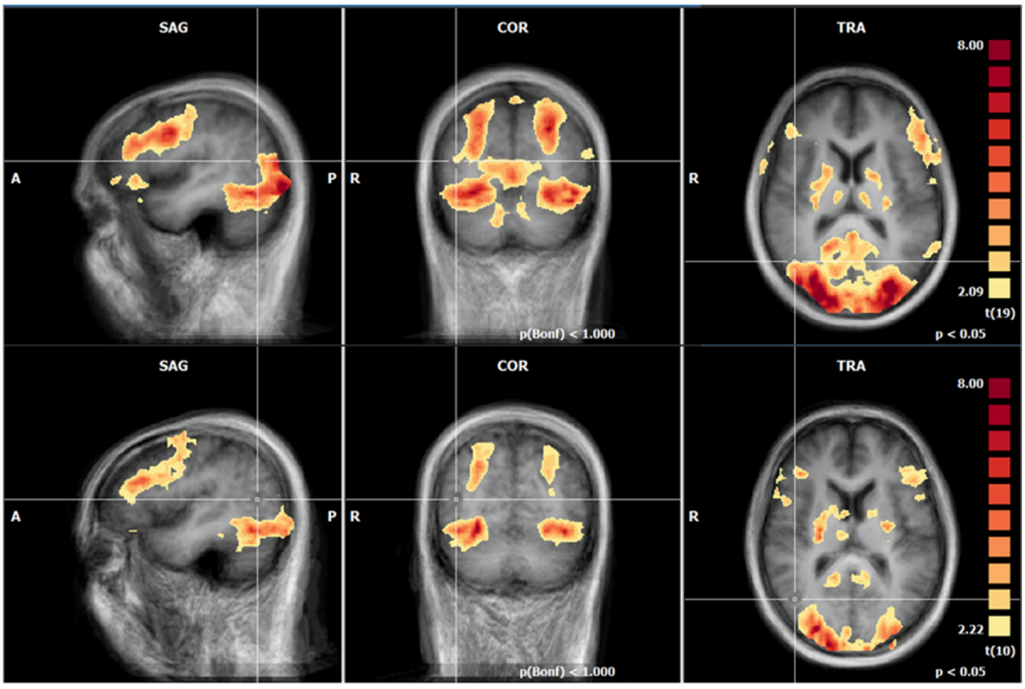


Figure 2. BOLD contrast for all stimuli (gambling-related and neutral cues) versus rest condition. Activations are plotted for both stimulus categories (i.e., images of slot machine and ticket machines) versus rest condition. Coordinates: x = +43 y = −63 z = +13. (**Top**) row: depicted are results of the healthy control group (HC). (**Bottom**) row: results for patients with gambling disorder (GD).

3.2. BOLD Contrast "Gambling-Related Versus Neutral Cues"

This illustrates the BOLD contrast when viewing images of slot machines in direct comparison to neutral ticket machines. With this contrast, all areas with differences are mapped when looking at gambling-related versus neutral cues (see Figure 3). In both groups, there is increased activation for the ticket machines (green/blue) in the early visual areas, suggesting more diverse visual features in the neutral stimuli. This effect is equally present in both groups and therefore serves as a good reference regarding the different activation strengths in other brain regions.

Visual inspection of the activation maps further suggested differences in insular activation patterns between groups, which motivated a more detailed voxel-of-interest analysis.

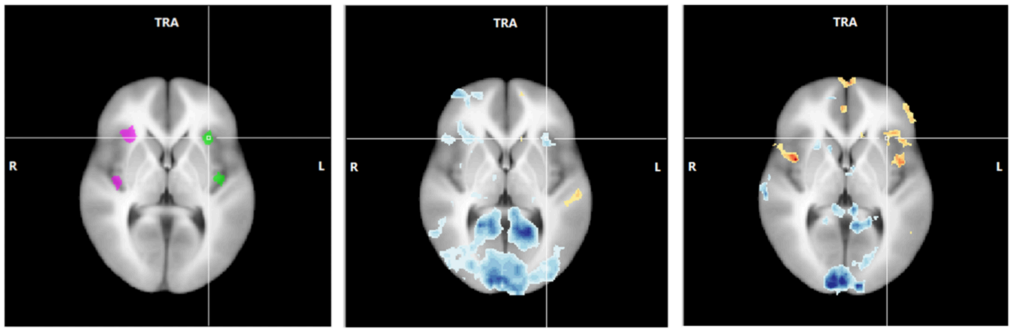


Figure 3. BOLD contrast for gambling-related versus neutral cues. (**Left**) panel: Four different VOIs for the anterior and posterior insula in the right (pink) and left (green) hemispheres. Activations are plotted for gambling-related cues (i.e., slot machines) versus neutral cues (i.e., ticket machines) in the left anterior insula (coordinates: x = −32 y = +22 z = +5), yellow/orange indicates stronger activation for gambling-related cues, and blue indicates stronger activation for neutral cues. (**Mid**) panel: healthy control group (HC). (**Right**) panel: patients with gambling disorder (GD).

3.3. VOI Analysis of the Insula

To further examine activation patterns within the insula, a voxel-of-interest (VOI) analysis was conducted focusing on the anterior and posterior insula as defined by the VOIs (see Table 2). A 2 × 2 × 2 × 2 ANOVA with the within-subject factors stimulus category (slot vs. neutral), insular region (anterior vs. posterior), hemisphere (left vs. right), and the between-subject factor group (patients vs. healthy controls) revealed the following results (see also Figure 4 and Table 3).

Table 2. Insular clusters showing volume (in voxels) and Talairach coordinates. Mean beta values per participant were extracted from four VOIs corresponding to the anterior and posterior insula in each hemisphere.

Insular Region	Volume	x	y	z
Right anterior	917	30	24	5
Right posterior	535	39	−13	4
Left anterior	336	−32	22	5
Left posterior	326	−40	−12	6

Note. VOI = Volume of interest. VOIs correspond to insular regions.

No significant main effect of group was observed ($p = 0.321$). A main effect for insular region was confirmed ($F(1, 29) = 26.1$, $p < 0.001$, $\eta_p^2 = 0.47$) with stronger activation in the anterior (mean = 0.074) compared to posterior regions (mean = −0.074). The stimulus × group interaction was not significant, $F(1, 29) = 0.89$, $p = 0.353$, partial $\eta_p^2 = 0.030$, indicating that the overall difference between gambling-related and neutral stimuli did not significantly differ between groups. Furthermore, a significant four-way interaction between stimulus category, insular region, hemisphere, and group was observed ($F(1, 29) = 14.4$, $p < 0.001$, $\eta_p^2 = 0.33$). Post-hoc Bonferroni-adjusted *t*-tests comparing neutral vs. gambling-related (slot) stimuli separately for the two groups revealed significant differences in the anterior insula for both hemispheres in the HC group (left anterior: $p = 0.017$; right anterior: $p = 0.009$), with stronger activation for neutral compared to slot stimuli (neutral > slot). Patients showed a reversed pattern for the left anterior insula (slot > neutral), which nearly reached significance ($p = 0.053$).

Table 3. ANOVA results.

	F (1, 29)	p	η_p^2
Stimulus	0.16	0.693	0.005
Hemisphere	0.34	0.562	0.012
Insula	26.13	<0.001	0.474
Group	1.02	0.321	0.034
Stimulus × Group	0.89	0.353	0.030
Hemisphere × Group	0.06	0.805	0.002
Insula × Group	1.11	0.300	0.037
Stimulus × Hemisphere	2.70	0.111	0.085
Stimulus × Hemisphere × Group	0.48	0.495	0.016
Stimulus × Insula	5.17	0.031	0.151
Stimulus × Insula × Group	4.17	0.050	0.126
Hemisphere × Insula	1.96	0.172	0.063
Hemisphere × Insula × Group	1.79	0.191	0.058
Stimulus × Hemisphere × Insula	3.10	0.089	0.096
Stimulus × Hemisphere × Insula × Group	14.42	<0.001	0.332

Note. F = F statistic; p = p-value; η_p^2 = partial eta squared. Stimulus refers to the two stimulus categories, hemisphere to the left and right hemispheres, insula to the insular regions examined, and group to the clinical gambling-disorder (GD) and healthy-control (HC) groups.

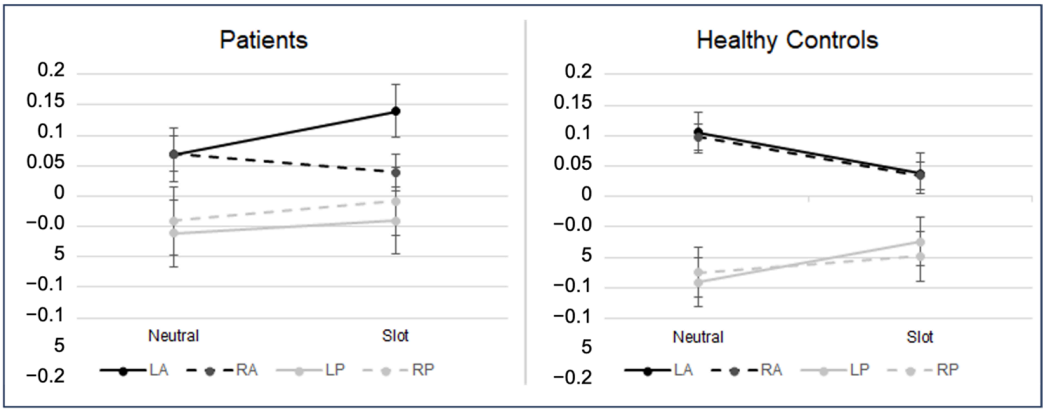


Figure 4. Insular activation patterns in response to gambling-related and neutral cues. Mean beta values extracted from anterior (black) and posterior (grey) insular VOIs in left (solid lines) and right (dotted lines) hemispheres are shown for patients with gambling disorder ((**left**) panel) and healthy controls ((**right**) panel). Within each panel, neutral cues are displayed on the left and gambling-related (slot) cues on the right. Error bars represent standard errors of the mean (SEs).

3.4. Observed Insular Activation Pattern

In addition to the VOI-based results, visual inspection of the activation maps suggested an additional pattern in patients with GD (see Figure 5). Specifically, while HCs showed activation primarily in the anterior insula for both gambling-related and neutral cues, patients with GD exhibited stronger activation in the middle part of the insula when viewing gambling-related stimuli, with little activation for neutral cues. Because the VOI analysis was restricted to anterior and posterior insular regions defined from the control group, this mid-insular pattern was not formally tested. This observation is considered exploratory and is discussed further in the Discussion Section.

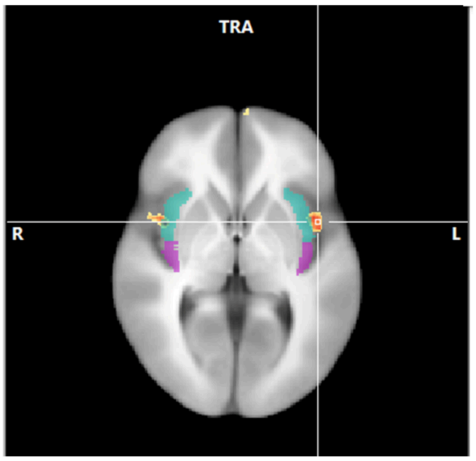


Figure 5. Exploratory visualization of activation in the central region of the insula (peak coordinates: x = −44, y = +6, z = +3). The anatomic atlas overlay (Neuromorphometrics, Inc., Somerville, MA, USA) indicates anterior (turquoise) and posterior insula (purple). This location was identified by visual inspection of the activation maps and was not included as a separately predefined or statistically tested VOI in the anterior/posterior insular analysis. The observation should therefore be considered exploratory.

4. Discussion

The present fMRI study investigated neural responses to gambling-related and neutral visual cues in patients with gambling disorder (GD) and healthy controls (HC). Using a cue-reactivity paradigm with slot-machine and ticket-machine images, we aimed to identify neural mechanisms associated with gambling cue processing and to examine whether individuals with GD exhibit different responses within brain regions implicated in addiction compared to HC. Two main findings emerged. First, both groups showed comparable activation of visual and attentional networks. Second, a voxel-of-interest (VOI) analysis revealed differential insular activation patterns between groups, suggesting altered stimulus processing within insular subregions in GD.

4.1. BOLD Contrast "Both Stimulus Categories Versus Resting State"

The contrast between both stimulus conditions and rest showed widespread activation in visual networks, reflecting engagement of both ventral and dorsal visual pathways and coordinated processing of complex visual stimuli. Differences in low-level visual salience likely contributed to attentional capture, consistent with prior findings [31]. The ventral pathway's involvement (V1–V4 and fusiform gyrus) aligns with its role in processing color, form, and object identity [32,33]. Concurrent dorsal pathway activity might reflect engagement in sensorimotor transformation supporting visually guided actions [34]. Activation of frontal and supplementary eye fields further reflects oculomotor control networks [35].

Beyond visual processing regions, activation was also evident in the insula, a structure implicated in attention, salience detection, interoception, and cognitive-affective evaluation of stimuli [36,37]. Finally, subcortical involvement, particularly in the superior colliculus, likely reflects its role in integrating sensory inputs to guide orienting and sensorimotor responses, complementing cortical processing of visual stimuli [38]. These findings indicate comparable task engagement across groups.

4.2. VOI Analysis of Anterior and Posterior Insula

To examine these insular differences more systematically, we conducted an additional VOI analysis focusing on anterior and posterior insular regions. The insula has repeatedly been implicated in addiction-related processes, including salience attribution, craving, and

interoceptive awareness [18,37,39]. This functional distinction is consistent with evidence linking anterior insular regions to emotional salience and hedonic evaluation via extensive limbic and reward-related connectivity [40]. The VOI analysis demonstrated a strong main effect of insular subregion, with greater activation in anterior compared with posterior insular regions across participants. This finding is in line with neurobiological models proposing a posterior-to-anterior functional gradient within the insular cortex [39,41], where posterior regions process interoceptive states and anterior regions integrate these signals with limbic–prefrontal input to support decision-making [37,39,42].

The significant four-way interaction involving stimulus category, insular subregion, hemisphere, and group indicates that stimulus-related activation patterns varied across these factors. Hence, the current data do not provide evidence for a generalized, overarching difference in neural cue reactivity between patients with gambling disorder and healthy controls in the neural response to gambling-related versus neutral cues.

Healthy controls showed significantly greater anterior insula activation for neutral than for gambling-related cues in both hemispheres (left: $p = 0.017$; right: $p = 0.009$, Bonferroni-adjusted). In patients with GD, the left anterior insula showed a numerically reversed pattern, with greater activation for gambling-related than for neutral cues; however, this difference did not reach statistical significance ($p = 0.053$). Crucially, this localized within-group trend must not be misconstrued as evidence of a statistically robust between-group divergence.

Taken together, the findings suggest that insular response patterns may vary according to stimulus category, subregion, and hemisphere. Given the small sample size and the absence of a significant stimulus × group interaction, these observations should be interpreted cautiously and investigated in adequately powered independent studies.

4.3. Exploratory Observations Regarding Anterior and Mid-Insula

In addition to the predefined VOI analysis, visual inspection of the activation maps suggested that patients with GD showed activation extending beyond the anterior insula into a more central insular region during gambling-cue exposure (Figure 5). Consequently, this localized activation cluster must be treated strictly as an exploratory, hypothesis-generating observation rather than a statistically confirmed mid-insular effect. Speculatively, this emergent pattern might align with theoretical models proposing an enhanced integration of interoceptive and motivational signals during cue exposure [39].

Previous cue-reactivity studies have linked activation of mid- and posterior insular regions to craving and urge-related states in addiction. In particular, Limbrick-Oldfield et al. [7] reported associations between gambling craving and insular activation in patients with GD. In this context, the visually observed central insular activation may be relevant to hypotheses concerning the integration of interoceptive and motivational signals during cue exposure. However, the present study does not allow such a functional interpretation to be established. Future studies using larger samples and finer anatomical parcellation are needed to determine whether this region plays a distinct role in gambling-related cue processing.

4.4. Implications for Gambling Disorder

The insula has been implicated in behavioral addictions and in processes related to salience, interoception, and motivational evaluation. Gambling-related cues may engage networks involved in reward, salience, memory, and control, with the insula potentially contributing to the integration of these processes [12]. Previous research has linked insular activation during gambling-cue exposure to craving [7], while lesion studies suggest that the insula can play a role in addictive behaviors [43]. In the present study, the observed

variation in insular response patterns across subregions and hemispheres highlights the importance of considering the functional heterogeneity of the insula. However, the findings do not establish a general increase in gambling-cue reactivity in GD, and interpretations concerning motivational salience remain tentative.

The present study extends previous gambling cue-reactivity research by focusing specifically on slot-machine gambling in a clinically characterized group of patients whose predominant gambling activity involved slot machines. Unlike studies combining several gambling modalities or selecting cues according to individual preferences across different activities, the present design used a consistent category of gambling-related stimuli and neutral ticket-machine images. This approach allowed us to examine stimulus-related activation patterns in a relatively specific gambling context.

Taken together, the study provides a focused contribution to the investigation of insular cue processing in slot-machine gambling. Its findings underscore the value of examining regional and hemispheric response patterns while distinguishing higher-order interactions from evidence of a general group difference. Replication in larger and more diverse samples is needed to determine the robustness and generalizability of these patterns.

4.5. Limitations

Several limitations should be considered when interpreting the present findings. First, the sample size was relatively small, particularly in the gambling disorder (GD) group ($n = 11$), which limits statistical power and the precision of effect estimates. Consequently, the absence of significant whole-brain group differences should not be interpreted as evidence of equivalence between groups. Second, only male participants were included, limiting the generalizability of the findings to women and to the broader population of individuals with GD.

Third, the study focused exclusively on slot-machine cues, which allowed us to investigate responses to a specific and commonly encountered form of gambling but limits generalization to other gambling activities. Gambling-related slot-machine images and neutral ticket-machine images may also differ in visual complexity, familiarity, and emotional salience. These stimulus characteristics were not independently matched or quantitatively assessed in all relevant dimensions, and may therefore have contributed to the observed responses.

Furthermore, subjective craving and other stimulus-related ratings cannot be directly linked to neural activation unless the corresponding measures are available for analysis. Finally, the mid-insular activation pattern was identified through visual inspection and was not formally tested in the predefined VOI analysis. It should therefore be considered exploratory and requires independent replication in larger samples.

5. Conclusions

The present study shows that gambling-related and neutral cues engage widespread visual and attentional networks in both groups. The VOI analysis revealed a significant interaction involving stimulus category, insular region, hemisphere, and group, suggesting that insular response patterns varied across these factors. However, the non-significant stimulus × group interaction does not support a general group difference in cue reactivity. Exploratory findings suggest possible involvement of mid-insular regions, which require further investigation.

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