Methods

Participants

We examined a group of twelve male pathological gamblers (ten strictly right handed, all smokers) aged 37.3 ± 7.4 yrs (mean ± sd) and a group of twelve, age matched, healthy males, aged 32.3 ± 5.6 yrs (all strictly right handed, nine smokers). The groups were statistically not different with respect to age (t(22) = 1.9, p = n.s.) and smoking (t(22) = 1.9, p = n.s.) We focused our study on male volunteers, as the prevalence of PG in men is 2-3 times higher compared to women, and it has been suggested that women have increased endogenous striatal dopamine concentration\(^1\). PGs were recruited through advertisements and through the behavioural therapy clinic of the Department of Psychiatry and Psychotherapy, University Hospital Hamburg-Eppendorf. All pathological gamblers played mainly slot machines, but not card games or traditional casino games. All controls were free of active substance abuse and known psychiatric, neurological or medical disorders. All subjects underwent a structured psychiatric interview\(^2\) performed by an experienced psychiatrist (T.R.). No active axis I disorders apart from depression in 4 PG subjects was present. Depression was further assessed using the Beck Depression Inventory\(^3\). The mean depression score was 2.5 ± 2.6 for the controls and 11.5 ± 7.5 for the PG group. Therefore, all imaging analyses accounted for higher depression scores in the PG group using an Ancova. The gamblers had been gambling for 13.4 ± 6.8 years. In addition we performed additional analyses without the depressed patients (Supplementary Table 4 and Supplementary Figure 4)

All PG subjects had a diagnosis of pathological gambling according to DSM IV. Furthermore all individuals were assessed with a more detailed German gambling questionnaire “Kurzfragebogen zum Glücksspielverhalten” (KFG)\(^4\), which is a modified German version of the 20 questions developed by “gamblers anonymous” and greatly
overlaps with the questions of the South Oaks Gambling Screen\(^5\). In a validation study including 558 pathological gamblers this instrument has shown a high consistency (Cronbach’s alpha = 0.79) and high test-retest reliability (r=0.80) as shown by the same group in an additional sample of 44 PG subjects assessed over 2 weeks\(^4\). This questionnaire contains 20 items on a 4-point Likert scale (0 to 3 points). The threshold for pathological gambling on this scale is set to 16 points. On this scale, gamblers in our sample scored between 21 and 53 points (35.6±9.7; mean ± sd). Controls scored between 0 and 9 points (2.9±2.9; mean ± sd). PG subjects and volunteers consumed alcohol, but did not fulfil the criteria for alcohol-abuse or -dependence according to DSM IV. Three controls had previous experience with marijuana. All participants and PG subjects were medication free and instructed not to use any substance of abuse other than cigarettes and coffee on the day of the scan.

This study was approved by the Ethics Board of the Board of Physicians in Hamburg, Germany. All subjects gave written informed consent prior to inclusion into this study.

**Task**

To control for the familiarity of the stimulus material, an abstract guessing task unknown to all participants was chosen. In the task participants were shown the backside of two playing cards (Figure 1a) and were asked to choose either the right or the left card with a button press. After 2s the selected card was turned over and displayed for another 2s (ITI = 4s). Depending on the color of the card the participant either won (red card) or lost (black card) € 1.00. After a total presentation time of 2s, the chosen card was turned over At the beginning of the session each participant started with a balance of € 15.00. All subjects were specifically informed that they were to receive the entire balance in cash at the end of the scanning-session and the balance was continuously displayed. The determined sequence
of wins and losses over a total of 237 guessing trials (identical for all subjects) followed a noisy sine-wave with 16 cycles over three sessions of 5.5 minutes with a positive linear trend to end the game with a total win of € 8.00 (i.e. a total balance of € 23.00) (Figure 1b). This procedure enabled us to fully control the sequence of wins and losses, and yet give the participant the impression of choice. Given that participants gained € 8.00 over the whole experiment, the probability in each trial for winning and losing was not exactly 50:50%, but 49:51% i.e. there was a slightly greater probability in each trial to win than to lose.

**Imaging**

Functional MRI was performed on a 3T system (Siemens Trio) with a gradient–echo EPI T₂* sensitive pulse sequence in 38 axial slices (2 mm thickness with 1 mm gap, TR 2.2 s, TE 25 ms, flip angle 90°, field of view 192 x 192 mm², matrix 64 x 64). Thin slices reduced susceptibility related signal dropout in the vicinity of the ventral striatum and the ventral prefrontal cortex.

The paradigm involved 3 sessions with 79 guessing trials in each session. Each trial began with the presentation of the backside of two playing cards. The volunteer was told that one of the cards was red, and he/she had to guess which card is red, by pressing a left or right button. After a total presentation time of 2s, the chosen card was turned over and displayed for another 2s. Thus a complete trial lasted 4s. Each correct guess led to a reward of €1.00, each incorrect guess to a penalty of €1.00. In trials where no response was made, the card was turned over automatically.

Stimuli were presented by a PC that ensured synchronization with the MR-scanner using the software “Presentation” (http://nbs.neuro-bs.com). An LCD projector projected the stimuli on a screen positioned within the bore of the magnet which was viewed by the subjects through a mirror (10 x 15° field of view).
Image processing and statistical analysis were carried out using SPM02 (http://www.fil.ion.ucl.ac.uk/spm). All volumes were realigned to the first volume, spatially normalized to a standard EPI template (SPM2), and smoothed using a 10 mm full-width at half-maximum (FWHM) isotropic Gaussian kernel. Visual inspection of the data (movie) was performed to rule out gross image artifacts. All volunteers showed less than 3mm total head movement for each session. Data analysis was performed by modeling winning and losing events separately as delta functions convolved with a hemodynamic response functions. To exclude session onset effects the first 10 scans of each session were discarded. Regression coefficients for all regressors were estimated using a general linear model. Given the very low number (<1%) of trials without response for each volunteer it was not possible to model these trials separately. For the group, a second level analysis was performed, treating inter-subject variability as a random effect. In addition to the group effect (two-sample t-test) we included regressors modeling depression (Beck score) and gambling severity as assessed with the KFG into this analysis. Both covariates were fitted in a group specific manner thus allowing for a covariate by group interaction. In the first analysis we determined voxels showing a main effect of winning versus losing. In a second analysis we tested for voxels that show higher activity in controls than in PG subjects. To restrict the search volume to voxels showing a significant (p<0.001) main effect of winning > losing (first analysis), the second analysis was masked with this contrast. The threshold adopted was p<0.05 (corrected for multiple comparisons). In regions with an a priori hypothesis (ventral striatum\(^6-10\) and ventromedial prefrontal cortex\(^11-14\)) a small volume correction (SVC) was performed (ventral striatum: 20 mm diameter sphere = 3564 mm\(^3\) volume centered on x, y, z: ±24, 9, -6; VMPFC: ROI Frontal_Mid_Orb (segments 25 & 26) provided by the AAL project at http://www.cyceron.fr/freeware/).
Results

Behavioral data

Participants successfully completed all three runs. Due to a technical defect of the response box, insufficient responses were acquired in the second session in one pathological gambler. Overall compliance was high, on average both the pathological gamblers and the controls only missed two out of 237 responses (PG: 2.4±0.91; controls: 1.5±0.34 (mean±sem); t(22) = 0.9, p=n.s.). Although pathological gamblers were slower to respond, this difference did not reach significance (PG: 787±71ms (mean±sem); controls: 696±36ms; t(22)=1.1, p=n.s.). Furthermore we tested for categorical differences in guessing behaviour, e.g. individual preferences for a certain stack of cards. For each participant we estimated the absolute deviation from choosing both stacks equally often and compared the average deviation between groups. This revealed no significant difference in guessing behaviour (deviation from expected 50% PG: 8±2% (mean±sem); controls: 12±2%; t(22)=1.5, p = n.s.).

References
