Data supplement for Admon et al.,Dopaminergic Enhancement of Striatal Response to Reward in Major Depression. Am J Psychiatry (doi: 10.1176/appi.ajp.2016.16010111)

Supplementary Methods

Participants and Procedure: General exclusion criteria for all participants included: pregnancy, use of oral contraceptives or hormone therapy in the previous six months, a serious or unstable medical illness (e.g., cardiovascular, hepatic, renal, respiratory, endocrine, neurologic or hematologic disease), history of seizure disorder, history of cocaine or stimulant use (e.g., amphetamine, cocaine, methamphetamine), history of dopaminergic drug use (including methylphenidate), history or current diagnosis of dementia, or a score of < 26 on the Mini Mental Status Examination (1), or a history of adverse drug reactions or allergy to amisulpride. Failure to meet standard MRI safety requirements, renal insufficiency, clinical or laboratory evidence of hypothyroidism, severe concussion, or loss of consciousness longer than two minutes also resulted in exclusion. Exclusion criteria specific to depressed participants included: suicidal ideation, any psychotropic medication in the past two weeks (six weeks for fluoxetine; six months for dopaminergic drugs or neuroleptics), a lifetime history of electroconvulsive therapy, and a history or current diagnosis of any of the following DSM-IV psychiatric illnesses: organic mental disorder, schizophrenia, schizoaffective disorder, delusional disorder, psychotic disorders not otherwise specified, bipolar disorder, mood congruent or mood incongruent psychotic features, lifetime substance dependence, substance abuse within the last 12 months (with the exception of cocaine or stimulant abuse, any use of which would lead to exclusion). Simple phobia, social anxiety disorder and generalized anxiety disorders were allowed only if secondary to major depression.

Prospective candidates underwent a clinical evaluation that included: (A) an interview to assess relevant psychiatric, medical and neurological history; (B) administration of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient

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Edition (SCID-I/NP) (2); (C) administration of the 21-item Beck Depression Inventory (BDI-II) (3) to assess depression severity, and the Mood and Anxiety Symptom Questionnaire (MASQ) (4) and the Snaith–Hamilton Pleasure Scale (SHAPS) (5) to assess anhedonia (Table 1, main text). Patients meeting criteria for major depressive disorder and healthy controls were invited for a second session in which neuroimaging and behavioral data were collected (Figure 1, main text). To avoid craving effects, subjects were asked to consume their usual amount of caffeine and/or nicotine on the study day, a procedure that is widely used in imaging studies (6). Groups did not differ with regard to caffeine consumption and smoking status (Table S2).

Pharmacological intervention: The dopamine D2 receptor antagonist amisulpride was administrated due to its high affinity for D_2/D_3 dopamine receptors, especially in the mesocorticolimbic dopaminergic pathway, and its low affinity for other receptors (7). Animal studies have shown that, at low doses, amisulpride preferentially blocks presynaptic dopamine autoreceptors, leading to increased striatal dopamine release and prohedonic effects. For instance, low doses of amisulpride have been shown to (A) increase dopamine release in the Nacc (8); (B) potentiate food-induced place preference (9); (C) reverse performance deficits in positively-reinforced operant behavior caused by a hypodopaminergic state (10, 11); and (D) reverse stress-induced decreases in sucrose consumption (12). In humans, a single low dose (50 mg) administration of amisulpride has no explicit effects on mood or sensory-motor coordination (13), which is crucial to maintaining a blind design. Conversely, sustained administration of 50 mg amisulpride has been found to have antidepressant and anti-anhedonic effects in depressive disorders (14-16).

The Monetary Incentive Delay Task: Each trial began with a visual cue (0.5 sec) indicating the potential outcome (reward: +$; penalty: –$; no incentive: 0$). After a variable inter-stimulus interval (2.25–3.75 sec), a red target square was briefly presented (0.15 sec). Participants

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responded to the square by pressing a button as quickly as possible. After a second variable delay (2.4–3.9 sec), visual feedback (1.25 sec) was displayed to indicate the trial outcome: reward, penalty, or no change. A variable interval (1.5–4.5 sec) separated the trials. Participants were told that responding rapidly to the red square would maximize their chances of obtaining rewards and avoiding penalties. In order to match task difficulty across participants, the 70th percentile of each participant's reaction time during a practice session was defined as the individual's reaction time threshold for success. In the reward condition, successful trials were associated with monetary gains ($1.96 to $2.34), whereas unsuccessful trials led to no-change. In the penalty condition, successful trials were associated with no-change, whereas unsuccessful trials were associated with monetary penalties (-$1.81 to -$2.19). No-incentive trials always ended with no-change feedback. The task included five blocks of 24 trials (8 reward, 8 penalty, and 8 no-incentive trials). Feedback about cumulative earnings was not provided.

The Probabilistic Selection Task: This widely used reinforcement learning task featured a learning phase and a testing phase. The learning phase included up to six blocks of 60 trials each. Each trial began with a fixation cross (1 sec), followed by presentation (2 sec) of one of three different pairs of Japanese Hiragana stimuli, referred to as pairs AB, CD, and EF. Presentation order was randomized (20 trials per pair per block). Participants were instructed to choose one stimulus in each pair by pressing a key, after which visual feedback ("Correct", "Incorrect", or "No response detected" (if RT> 2 sec)) was provided (1.5 sec). Feedback was probabilistic: choosing stimulus A, C, or E led to positive feedback 80%, 70%, and 60% of the time, respectively, while choosing stimulus B, D, or F led to negative feedback 80%, 70%, and 60% of time. The learning phase ended after participants reached performance criteria (65% accuracy for A, 60% for C, and 50% for E) or after six blocks. Immediately following the learning phase, participants completed the testing phase (1 block of 90 trials). Test trials began with a

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fixation cross (1 sec) followed by a pair of stimuli (3 sec); no feedback was provided. The stimulus pairs included the three pairs used in the learning phase (AB, CD, EF) plus all possible novel combinations. As described in the main text, performance on novel stimulus pairs containing A (e.g., AC, AD) - "Choose A" trials - and novel stimulus pairs containing B (e.g., BC, BD) - "Avoid B" trials - was used to measure learning from rewards and penalties, respectively. "Choose A" was calculated as the proportion of test-phase trials in which the participant chose "A" among all test-phase trials in which "A" was one of the stimuli in the pair presented; "Avoid B" was calculated as the proportion of test-phase trials in which the participant did not choose "B" (i.e., avoided "B") among all test-phase trials in which "B" was one of the stimuli in the pair presented. Hence, higher "Choose A" or "Avoid B" scores represent better learning (from reward or penalty, respectively). Following standard procedures (17, 18), a minimal learning criteria of 50% accuracy on AB test trials was enforced; if participants did not meet this criterion, their Choose A and Avoid B data were regarded uninterpretable. Two participants from the control group (one from each drug group) were excluded from behavioral analyses because they failed to meet this criterion.

MRI acquisition: MRI data were acquired during the Monetary Incentive Delay Task on a Siemens Tim Trio 3T MR scanner with a 32-channel head coil. A T2-weighted spin echo planar imaging sequence was used to collect 461 functional volumes [repetition time (TR) = 3000ms; echo time (TE) = 30ms; field of view (FOV) = 224mm; matrix = 64x64; resolution = 3.5x3.5x2mm; 57 contiguous slices aligned to the AC–PC plane]. High-resolution T1-weighted MPRAGE images were also acquired [TR = 2200ms; TE = 1.54ms; FOV = 230mm; matrix = 192x192; resolution = 1.22mm^3; 144 slices]. The Probabilistic Selection Task was performed behaviorally (i.e., not with fMRI).

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Artifact Detection Tools (ART): ART (http://web.mit.edu/swg/software.htm) was used to identify and exclude outlier time points in the global mean image time series (threshold: 3 standard deviations (SD) from the mean) and movement (threshold: 0.7mm; measured as scan-to-scan movement, separately for translation and rotation) parameters.

Whole-brain analysis: Whole-brain analyses were conducted in order to explore potential effects of dopamine enhancement, or clinical depression, on other neural systems beyond the striatum. A whole brain *Diagnosis* (Depressed vs. Controls) by *Drug* (Amisulpride vs. Placebo) 2X2 factorial ANOVA was conducted separately for the responses to Reward Cue, Penalty Cue, Reward Outcome and Penalty Outcome. In addition, whole brain analyses of the entire sample treated as a single group (n=89) were conducted for each condition (i.e., Reward Cue, Penalty Cue, Reward Outcome and Penalty Outcome). All whole-brain analyses were thresholded and cluster corrected using the same thresholds applied in the psychophysiological interaction (PPI) analysis, peak $p<0.001$, FWE $p<0.05$ (see main text). For additional details regarding thresholding and cluster correction see (19).

Statistical analyses: Following previous findings that depression is associated with differential striatal abnormalities in response to anticipation versus receipt of monetary reward (20), statistical analyses were separately conducted for the cue and outcome phases of the task. For the cue phase, activation was compared between cues (Reward, Penalty, and No-incentive, each relative to fixation baseline). For outcomes, activation was contrasted between the two outcome regressors that followed the same cue, i.e., response to Reward Outcome was calculated as the difference in activation for the contrast of *Win minus No-Win*, and response to Penalty Outcome was calculated as the difference in activation for the contrast of *Loss minus No-Loss*. This analytic approach was implemented in order to mitigate possible spillover effects of cue type on the neural responses to outcomes. In order to account for the potential effects of

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age, current anxiety, and past anxiety, all analyses were repeated with age, current (dummy-coded) comorbid anxiety disorder, and past (dummy-coded) comorbid anxiety disorder as covariates. Notably, adding these covariates did not influence the pattern or significance of results, indicating that our findings were not driven by age, current anxiety diagnosis, or past anxiety diagnosis. Similarly, multiple regression analyses were performed separately for BDI-II, MASQ-AD (Anhedonic Depression sub-scale), and SHAPS scores with *Drug* (Amisulpride coded as +1, Placebo coded as -1), and interaction with *Drug* as independent variables, and either reward learning, striatal response to cue, striatal response to reward outcome, or corticostriatal functional connectivity as dependent variables; these analyses revealed only main effects of *Drug*, stemming from increased striatal activation and connectivity in depressed individuals receiving amisulpride relative to placebo, as reported in the text. No significant effects emerged of depression severity or anhedonia, or interactions between depression severity or anhedonia and *Drug*, suggesting that in the present sample individual differences in anhedonia and depression severity were not associated with reward learning or with neural responses in the placebo vs. amisulpride.

Supplementary Results

Performance on the Probabilistic Selection Task: For the training phase, factorial ANOVA with the number of blocks needed to reach the learning criteria as the dependent variable and *Diagnosis* (Depressed vs. Controls) and *Drug* (Amisulpride vs. Placebo) as between-subject variables revealed no significant effects (all p's > 0.42). Repeated-measures ANOVAs with accuracy or reaction time in the final block of training as the dependent variable, trial *Type* (AB, CD, and EF) as the within-subject variable, and *Diagnosis* (Depressed vs. Controls) and *Drug* (Amisulpride vs. Placebo) as between-subject variables revealed main effects of *Type* on accuracy ($F_{(2,162)} = 15.91$ $p < 0.001$) and reaction time ($F_{(2,162)} = 8.66$ $p = 0.004$), driven by higher accuracy and faster responses on AB trials than CD or EF trials. Importantly however, there

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were no significant effects of *Drug* or *Diagnosis* on accuracy or reaction time (all *p*'s > 0.23), suggesting that groups did not differ in the acquisition of reinforcement contingencies. See the main text for results from the test phase.

Performance on the Monetary Incentive Delay Task: Reaction time in response to the target was investigated using repeated-measures ANOVA with *Cue Type* (Reward, Penalty, No-incentive) as the within-subject variable and *Diagnosis* (Depressed vs. Controls) and *Drug* (Amisulpride vs. Placebo) as between-subject variables. This analysis revealed a main effect of *Cue Type* ($F_{(2,170)} = 70.03$, $p < 0.001$), with no significant effects of *Diagnosis* or *Drug* (all *p*'s > 0.05). As shown in Figure S2B, the main effect of *Cue Type* was driven by longer reaction time to no incentive cues relative to either reward cues ($p < 0.001$) or penalty cues ($p < 0.001$), reflecting motivated responding on reward and penalty trials versus no-incentive trials across all groups. The groups also did not differ in the percentage of reward trials ending in gains or the percentage of loss trials ending in penalties. Specifically, a mixed-effects ANOVA with outcome *Type* (Win, No-Win, Loss, No-Loss, No Change) as within-subject variables, and *Diagnosis* (Depressed vs. Controls) and *Drug* (Amisulpride vs. Placebo) as between-subject variables revealed only a main effect of *Outcome Type* ($F_{(5,510)} = 226.30$, $p < 0.001$), with no significant effects of *Diagnosis* or *Drug*. The main effect of *Outcome Type* was driven by a higher frequency for Win vs. No-Win following the reward cue ($p < 0.001$), as well as a higher frequency for No-Loss relative to Loss following the penalty cue ($p < 0.001$); these results are consistent with our use of individually-titrated reaction time thresholds, which were intended to ensure approximately 70% successful trials – Win or No-Loss – for all participants. Collectively, the analyses of both reaction time and outcome frequency (i.e., "accuracy") data suggest that the fMRI findings were not confounded by group differences in task difficulty. Of note, the lack of amisulpride-related effects in reaction time are consistent with animal studies indicating that amisulpride has higher binding to DA receptors in mesolimbic compared to nigrostriatal regions

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(21, 22), and thus further highlight the specificity of the behavioral and neural effects reported in the main text.

Whole-brain analysis: Whole-brain analyses were conducted to separately investigate activations in response to reward cues, penalty cues, reward outcomes and penalty outcomes. A *Diagnosis* (Depressed vs. Controls) by *Drug* (Amisulpride vs. Placebo) 2x2 factorial ANOVA revealed no significant *Diagnosis* and/or *Drug* effects for any analysis at peak $p<0.001$, whole brain family-wise error (FWE) corrected to $p<0.05$. Next, activations were investigated across the entire sample ($n = 89$). These analyses revealed the expected pattern of activation in response to anticipation and receipt of monetary rewards and penalties. Specifically, in response to reward or penalty anticipation, we observed robust activation across the striatum as well as in motor preparation regions (Figure S4 A & B). In response to receipt of monetary rewards or penalties we observed robust medial prefrontal (mPFC) activation (Figure S4 C & D). Taken together, those activation patterns are highly consistent with previous fMRI studies that implemented the Monetary Incentive Delay Task (see (23) for a recent review).

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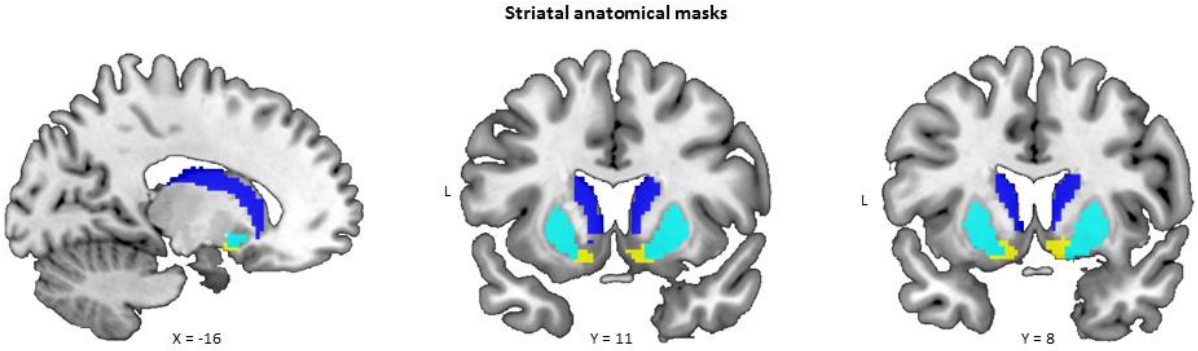
FIGURE S1



Striatal anatomical masks: Location of anatomically defined masks for the Caudate (blue), Nacc (yellow), and Putamen (turquoise). Mask volumes were 169 and 195 voxels for the left and right caudate, respectively; 25 and 34 voxels for the left and right Nacc, respectively; 226 and 239 voxels for the left and right putamen, respectively.

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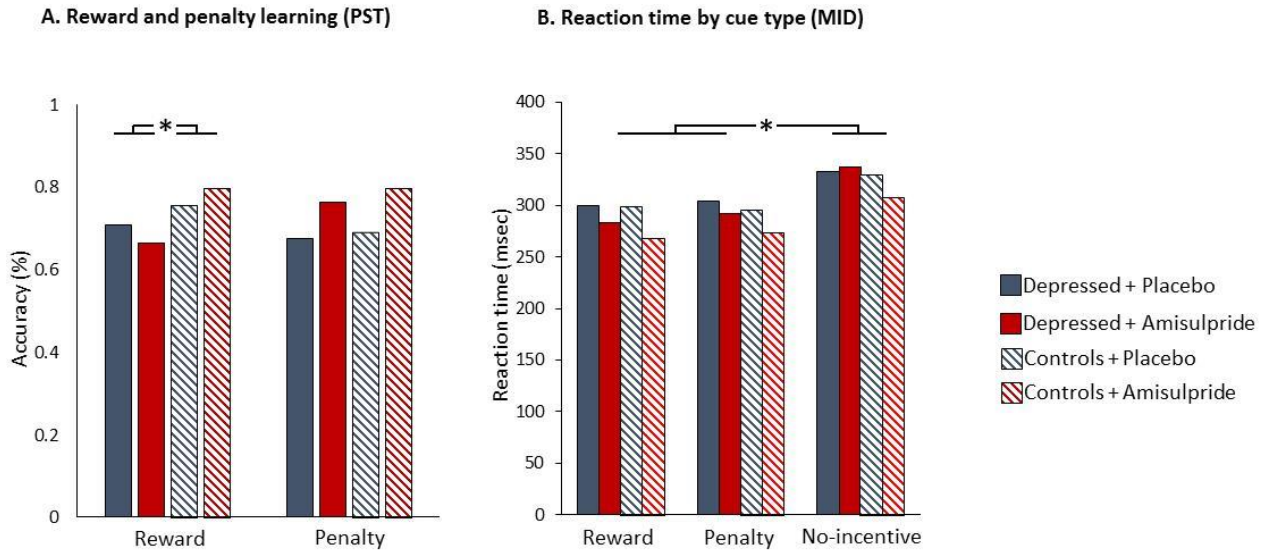
FIGURE S2



Performance on the Probabilistic Selection (PST) and Monetary Incentive Delay (MID) tasks:

(A) In the PST task, a repeated measures analysis of variance (ANOVA) with Learning type ("Choose A" and "Avoid B" accuracy) as the within-subject variable and Diagnosis (depressed versus control group) and Drug (amisulpride versus placebo) as between-subject variables revealed no significant effects. However, when reward learning was tested separately there was a main effect of Diagnosis ($F_{(1,75)} = 6.28$, $p = 0.014$), due to reduced reward learning in depressed compared to control individuals. **(B)** In the MID task, reaction times were longer in response to no-incentive cues than reward ($p < 0.001$) or penalty cues ($p < 0.001$) across all groups, reflecting motivated responding on reward and penalty trials versus no-incentive trials.

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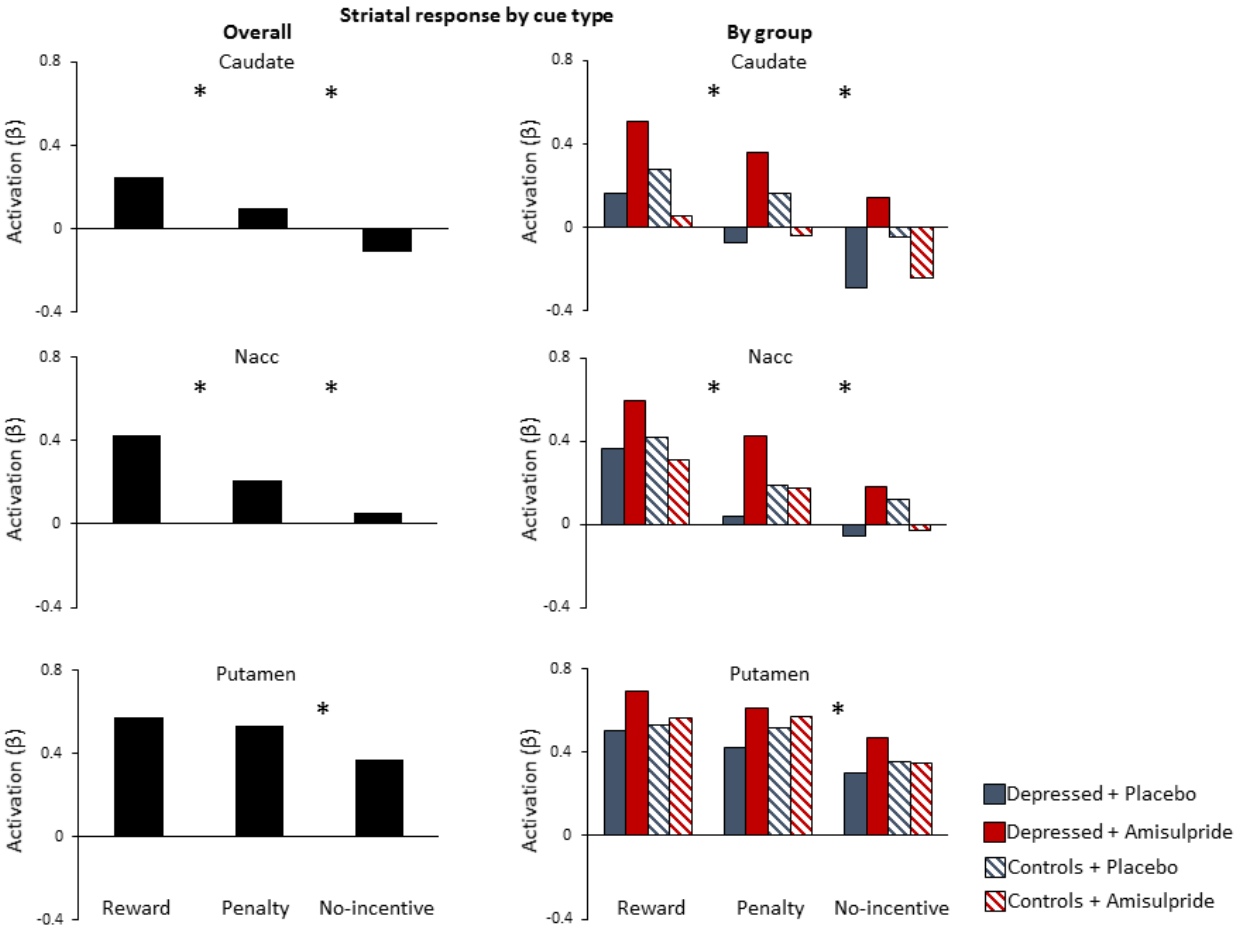
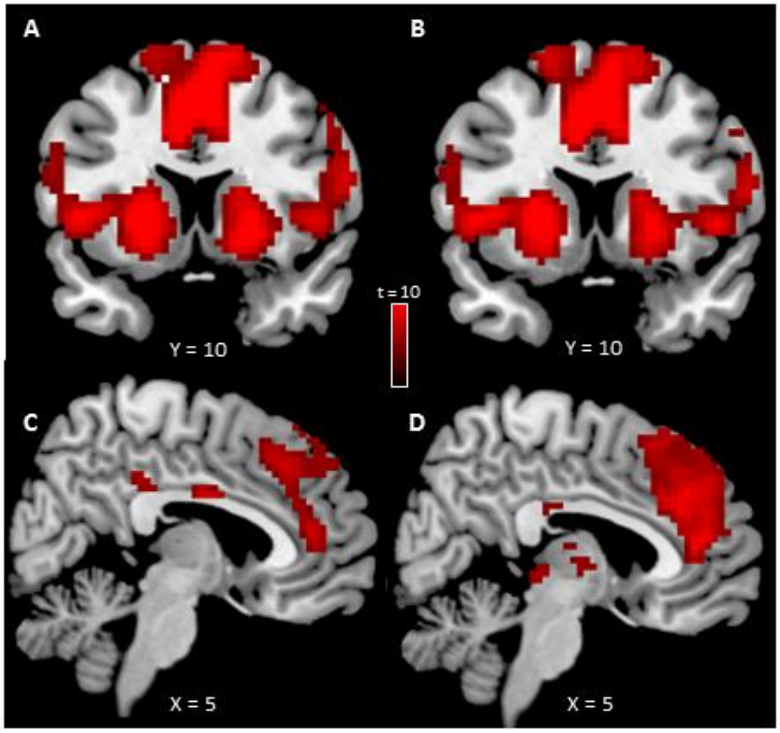
FIGURE S3



Striatal response by cue type: Across all groups and striatal regions, reward cues elicited the strongest striatal response, followed by striatal responses to penalty cues, and finally the weakest striatal responses were observed to no-incentive cues. Specifically, striatal activation was increased in response to reward cues relative to penalty cues (Caudate: $p < 0.001$; Nacc: $p < 0.001$), or no-incentive cues (Caudate: $p < 0.001$; Nacc: $p < 0.001$; Putamen: $p < 0.001$). Striatal activation was also stronger in response to penalty cues relative to no-incentive cues (Caudate: $p < 0.001$; Nacc: $p < 0.001$; Putamen: $p < 0.001$).

FIGURE S4

Whole brain response to reward and penalty cues and outcomes



Whole-brain analysis: Whole-brain analyses across the entire sample (*n* = 89) in response to anticipation and receipt of monetary rewards and penalties revealed the expected pattern of activation. Specifically, in response to (**A**) reward anticipation and (**B**) penalty anticipation, robust activation was observed across the striatum and in motor preparation regions, while in response to receipt of monetary rewards (**C**) and penalties (**D**) participants exhibited robust medial prefrontal (mPFC) activation. All whole-brain analyses were thresholded and cluster corrected using the same thresholds applied in the psychophysiological interaction (PPI) analysis, peak $p<0.001$, FWE $p<0.05$.

TABLE S1. Clusters showing significant increases in functional connectivity with bilateral striatal seeds in response to reward outcomes at peak $p < 0.001$, whole brain family-wise error (FWE) corrected to $p<0.05$. Coordinates are presented in MNI space. Nacc = nucleus accumbens

Seed	Region	# of voxels	*X*	*Y*	*Z*	*Z* score	*P* FWE-corr
Caudate	dorsal anterior cingulate cortex (dACC; BA 32)	22	6	38	23	5.62	>0.001
Nacc	mid-cingulate cortex (MCC; BA 32)	13	6	8	44	5.25	0.004

TABLE S2. Smoking status and caffeine consumption

	Depressed + Amisulpride (N=23)	Depressed + Placebo (N=23)	Controls + Amisulpride (N=23)	Controls + Placebo (N=20)
	n (%)	n (%)	n (%)	n (%)
Current smokers	1 (4.3)	0 (0)	0 (0)	0 (0)
Past smokers	0 (0)	3 (13.0)	2 (8.7)	0 (0)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Caffeine consumption (mg/daily)	103.4 (47.6)	147.7 (93.7)	106.4 (86.2)	88.9 (73.2)

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