Dopaminergic Enhancement of Striatal Response to Reward in Major Depression

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Objective: Major depressive disorder is characterized by reduced reward-related striatal activation and dysfunctional reward learning, putatively reflecting decreased dopaminergic signaling. The goal of this study was to test whether a pharmacological challenge designed to facilitate dopaminergic transmission can enhance striatal responses to reward and improve reward learning in depressed individuals.

Method: In a double-blind placebo-controlled design, 46 unmedicated depressed participants and 43 healthy control participants were randomly assigned to receive either placebo or a single low dose (50 mg) of the D_2/D_3 receptor antagonist amisulpride, which is believed to increase dopamine signaling through presynaptic autoreceptor blockade. To investigate the effects of increased dopaminergic transmission on reward-related striatal function and behavior, a monetary incentive delay task (in conjunction with functional MRI) and a probabilistic reward learning task were administered at absorption peaks of amisulpride.

Results: Depressed participants selected previously rewarded stimuli less frequently than did control participants, indicating reduced reward learning, but this effect was not modulated by amisulpride. Relative to depressed participants receiving placebo (and control participants receiving amisulpride), depressed participants receiving amisulpride exhibited increased striatal activation and potentiated corticostriatal functional connectivity between the nucleus accumbens and the midcingulate cortex in response to monetary rewards. Stronger corticostriatal connectivity in response to rewards predicted better reward learning among depressed individuals receiving amisulpride as well as among control participants receiving placebo.

Conclusions: Acute enhancement of dopaminergic transmission potentiated reward-related striatal activation and corticostriatal functional connectivity in depressed individuals but had no behavioral effects. Taken together, the results suggest that targeted pharmacological treatments may normalize neural correlates of reward processing in depression; despite such acute effects on neural function, behavioral modification may require more chronic exposure. This is consistent with previous reports that antidepressant effects of amisulpride in depression emerged after sustained administration.

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Major depressive disorder is a highly prevalent psychiatric condition characterized by blunted reward processing and diminished positive affect (1). Preclinical research has shown that phasic dopamine signaling, particularly in the striatum, constitutes an important neural mediator of reward-related behaviors, including reinforcement learning (2, 3) and incentive motivation (4). Functional MRI (fMRI) studies in humans have corroborated the central role of striatal function in reinforcement learning (5) and reward processing (6) and demonstrated that these striatal functions are disrupted in depression (7, 8). Accordingly, reduced striatal dopamine functioning is believed to play a key role in the pathophysiology of depression, particularly in the context of impaired reward processing and reward learning (9–11). fMRI studies have further suggested that reward dysfunction in depression is related to disrupted corticostriatal functional connectivity (12, 13), consistent with the notion that altered communication among dopamine-rich striatal regions and cortical regulatory systems is an important substrate of depression (14).

Despite theories implicating striatal dopamine dysfunction in depression, it is unknown whether an acute manipulation thought to transiently increase dopamine signaling might normalize reward processing in depression. In healthy individuals, studies combining fMRI with acute pharmacologically induced dopaminergic enhancements have shown increased reward-related striatal responses and improved reward learning relative to placebo (15–17). For instance, acute administration of amisulpride (200 mg) improved healthy participants' ability to select the better of two rewarding options, purportedly by enhancing reinforcement learning signals in the striatum and ventromedial prefrontal cortex (15). However, no study to date has tested whether

pharmacologically induced enhancement of dopaminergic transmission can improve reward learning or striatal activity and corticostriatal connectivity in response to reward in depression.

To address these important gaps in the literature, we conducted a double-blind randomized placebo-controlled study integrating neural and behavioral measures of reward processing in conjunction with a dopamine pharmacological challenge. To this end, 46 unmedicated depressed individuals and 43 healthy control subjects were randomly assigned to receive either placebo or a single low dose (50 mg) of the D_2/D_3 receptor antagonist amisulpride, which has a particularly high affinity for mesolimbic pathways and is believed to increase dopaminergic transmission by means of presynaptic D_2/D_3 autoreceptor blockade (18, 19) (see also the Supplementary Methods section in the data supplement that accompanies the online edition of this article). After administration of amisulpride or placebo, participants underwent fMRI scanning during a monetary incentive delay task involving anticipation and receipt of monetary rewards and penalties (7). After the scan, participants completed a probabilistic selection task that separately measured the ability to learn from rewards or penalties (20). We selected a 50-mg dose in light of previous reports that a (sustained) 50-mg dosage of amisulpride has antidepressant and antianhedonic effects in depressive disorders (21, 22) and in order to avoid postsynaptic blockade (23), with the goal of maximizing the likelihood of autoreceptor effects. We hypothesized that this pharmacological manipulation would be associated with increased striatal response to reward and improved reward learning, and that such effects would be largest among depressed individuals.

METHOD

Participants

Participants were recruited from the Boston metropolitan community. The depressed and control groups were matched for age, gender, ethnicity, and years of education (Table 1). Inclusion criteria restricted recruitment to right-handed individuals 18–45 years of age with no contraindications to MRI, no lifetime substance dependence, no past-year substance abuse, and no serious medical conditions. For the depression group, participants had to have a diagnosis of major depressive disorder according to the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID) (24). Exclusion criteria for the depressed group included use of any psychotropic medication in the past 2 weeks (6 weeks for fluoxetine, 6 months for dopaminergic drugs or antipsychotics) and a psychiatric history of other major axis I disorders. For the control group, inclusion criteria included medication-free status for at least 3 weeks, absence of current or past psychiatric illnesses (based on the SCID interview), and absence of first-degree familial psychiatric illness. Participants received $15/hour in compensation plus earnings in the fMRI task. All participants provided written informed consent to a protocol approved by Partners Human Research Committee.

Procedure

Participants first completed a clinical evaluation to determine eligibility (based on the SCID interview) and self-report measures of depression and anhedonia (Table 1; see also the Supplementary Methods section in the online data supplement). Eligible participants were invited to take part in the neuroimaging session, and those who participated were randomly assigned to receive amisulpride or placebo under double-blind conditions. Pharmacokinetic data indicate that plasma concentration of amisulpride has two peaks, approximately 1–1.5 hours and 2.5 hours after administration (18, 19). Therefore, the study physician administered either amisulpride or placebo at the beginning of the neuroimaging session, and fMRI scanning of the monetary incentive delay task started 1 hour after amisulpride or placebo administration to coincide with the first plasma concentration peak. The probabilistic selection task was administered after scan completion, approximately 2.5 hours after amisulpride or placebo administration, to coincide with the second plasma concentration peak. Heart rate, blood pressure, and side effects were assessed by the study physician throughout the session (Figure 1).

fMRI Task

The monetary incentive delay task involves anticipation and receipt of monetary rewards and penalties, which have been shown to elicit robust striatal response in healthy individuals (25). Previous studies using this task have revealed reduced striatal activation and reduced corticostriatal functional connectivity in depressed compared with healthy adults during anticipation and receipt of monetary reward (7, 26), making it well suited for the present study (see the Supplementary Methods section in the data supplement).

Behavioral Task

A probabilistic selection task was used to probe learning from positive and negative feedback (20). In the learning phase, participants repeatedly viewed three pairs of stimuli (AB, CD, and EF) and had to integrate feedback over several trials to learn which stimulus in each pair was rewarded most consistently. In the test phase, the most reliably rewarded (A) and penalized (B) stimuli were presented in conjunction with all other stimuli (e.g., AC, AD, AE, AF); participants' ability to "choose A" or to "avoid B" were used as measures of reward or penalty learning, respectively (see the Supplementary Methods section in the data supplement).

MRI Acquisition Parameters

The MRI acquisition parameters are described in the Supplementary Methods section of the data supplement.

fMRI Data Analysis

fMRI data were preprocessed using SPM12 (http://www.fil. ion.ucl.ac.uk/spm/software/). Preprocessing included co-registration of functional and anatomical images, segmentation, nonlinear volume-based spatial normalization (using Montreal Neurological Institute [MNI] space), and spatial

TABLE 1. Demographic and Clinical Characteristics of Participants in a Study of Dopaminergic Enhancement of Striatal Response to Reward in Major Depression[a]

Characteristic	Depression Group				Healthy Control Group			
	Amisulpride (N=23)		Placebo (N=23)		Amisulpride (N=23)		Placebo (N=20)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	27.7	8.2	26.3	5.2	26.5	6.8	25.3	5.6
Education (years)	10.9	5.1	11.4	6.1	13.0	5.0	12.9	5.2
Beck Depression Inventory–II[b]	26.6	8.1	28.0	9.4	1.3	2.1	1.7	2.6
Mood and Anxiety Symptom Questionnaire								
Total score	170.3	15.0	176.6	25.0	91.0	13.5	91.3	14.2
General distress depression subscale score[b]	39.3	7.9	39.3	8.9	14.3	3.1	14.4	2.7
Anhedonic depression subscale score[b]	86.7	9.3	85.2	9.3	43.4	7.9	46.7	9.9
General distress anxiety subscale score[b]	22.2	5.6	25.8	6.9	13.8	2.8	12.3	1.8
Anxious arousal subscale score[b]	22.1	4.2	26.3	8.7	19.4	3.1	17.9	1.3
Snaith-Hamilton Pleasure Scale[b]	32.9	4.5	32.8	6.2	21.5	6.0	22.6	6.8
Duration of current major depressive episode (months)	18.1	16.4	21.3	42.2				
Number of past depressive episodes	3.9	3.0	4.6	3.2				
	N	%	N	%	N	%	N	%
Female	21	91.3	16	69.6	18	78.3	15	75.0
Caucasian	10	43.5	10	43.5	10	43.5	5	25.0
Current comorbid anxiety disorders	3	13.0	2	8.7				
Past comorbid anxiety disorders	3	13.0	2	8.7				

[a] The depression and control groups were matched for age, gender, ethnicity, and years of education. All participants were right-handed, per inclusion criteria.
[b] Main effect of diagnosis in a factorial analysis of variance with diagnosis (depressed versus control group) and drug (amisulpride versus placebo) as between-subject variables. No effects of drug or diagnosis-by-drug interactions were significant.

smoothing with a Gaussian filter (6 mm full width at half maximum).

Hemodynamic responses were modeled using a canonical hemodynamic response function that was convolved with the onset times of task regressors in order to compute a general linear model at the single-subject level. The general linear model included nine task-related regressors: three cues (reward, penalty, no incentive), the target, and five outcomes (win [reward outcome following reward cue], no win [no-change outcome following reward cue], loss [penalty outcome following penalty cue], no loss [no-change outcome following penalty cue], and no change [no-change outcome following no-incentive cue]). The general linear model also included high-pass temporal filtering (0.008 Hz), seven rigid-body movement parameters, nuisance regressors accounting for no-response trials, and outlier time points (see the Supplementary Methods section in the data supplement).

To test a priori hypotheses regarding striatal responses to reward (7), we conducted a region-of-interest analysis in which activations (beta weights) were extracted from anatomical masks of the caudate, the nucleus accumbens, and the putamen for each participant and for each task regressor (relative to baseline). To avoid any biases, masks were defined using a manually segmented MNI-152 brain and implemented as overlays on the SPM12 canonical brain (see Figure S1 in the data supplement; see also reference 27). Activations reported throughout the analyses were quantified by averaging beta weights from all voxels within a mask. Exploratory whole brain analyses were also conducted (see the Supplementary Methods and Results sections of the data supplement).

Psychophysiological interaction analyses were performed to examine the effects of reward and penalty outcomes on striatal functional connectivity. Because hemispheric effects on task activation were nonsignificant, striatal masks were collapsed across hemispheres, yielding three bilateral seeds (caudate, nucleus accumbens, putamen). Analyses retained the subject-level general linear models described above, adding regressors corresponding to the seed time course and the interaction of the seed time course with the task condition of interest (separately for reward and penalty outcome). Single-subject connectivity maps for the interaction between each seed time course and the regressor of interest were entered into second-level whole brain random-effects analysis. Effects were thresholded at a peak p value of <0.001, whole brain family-wise error corrected to p<0.05 at the cluster level.

Statistical Analysis
The methods for statistical analysis are detailed in the Supplementary Methods section of the online data supplement.

RESULTS

Behavioral Results
Accuracy in "choose A" and "avoid B" trials of the probabilistic selection task test phase were used as measures of

FIGURE 1. Procedure and Timeline for a Study of Dopaminergic Enhancement of Striatal Response to Reward in Major Depression[a]
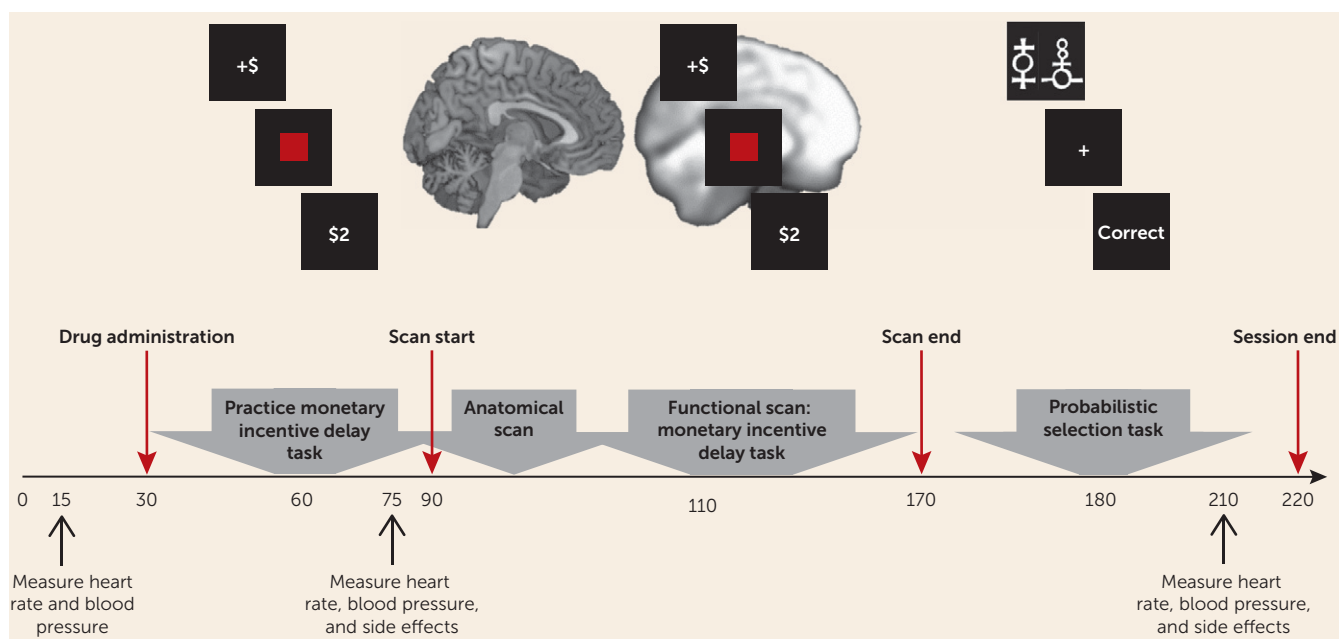
[a] On arrival to the scanning session, participants completed a pre-MRI safety screening form and provided a urine sample for drug testing and for pregnancy testing in female participants. Participants' heart rate and blood pressure were then measured by the study physician. Next, the study physician administered a capsule of either amisulpride or placebo (participants were randomly assigned to receive double-blind amisulpride or placebo). Participants then waited for 1 hour in a quiet room to allow amisulpride plasma concentration to reach its first peak. During the waiting period, participants practiced the monetary incentive delay task. Forty-five minutes after drug administration, the study physician measured participants' heart rate and blood pressure again, and participants completed a questionnaire on drug side effects. Next, participants completed an MRI scan (approximately 1.5 hours) that included structural scans and a functional scan while completing the monetary incentive delay task. After the scan, and approximately 2.5 hours after drug administration, participants completed the probabilistic selection task (administered to coincide with the second peak in amisulpride plasma concentration). After participants completed the probabilistic selection task, the study physician once again assessed their heart rate, blood pressure, and side effects. Participants were then debriefed, paid, and discharged.

reward and penalty learning, respectively. 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 main effects or interactions (see Figure S2A in the online data supplement). Because the primary focus of this study was reward processing, we also performed analyses that separately probed group differences in reward learning (which may be driven by a mixture of reward responsiveness and learning ability) and penalty learning (which may be driven by both penalty sensitivity and learning ability). Factorial ANOVAs were conducted separately with either reward or penalty learning (i.e., accuracy in "choose A" and "avoid B" trials, respectively) as the dependent variable and diagnosis (depressed versus control group) and drug (amisulpride versus placebo) as between-subject variables. For reward learning, there was a main effect of diagnosis (F=6.28, df=1, 75, p=0.014), due to reduced reward learning in the depressed compared with the control group. No significant group differences in penalty learning were observed. Thus, depressed participants exhibited impaired reward learning, but not penalty learning, relative to control participants, and this impairment was not affected by

drug administration. Nevertheless, the lack of a significant type-by-diagnosis interaction ("choose A" and "avoid B" accuracy; depressed versus control group) in the repeated-measures ANOVA precludes any strong inferences about the specificity of these findings. No other significant effects of diagnosis or drug emerged across behavioral analyses of either experimental task (see the Supplementary Results section of the data supplement).

Striatal Response to Cues

A repeated-measures ANOVA was performed for each striatal region with the following factors: hemisphere (left versus right) and cue (reward, penalty, no-incentive) as within-subject variables and diagnosis (depressed versus control group) and drug (amisulpride versus placebo) as between-subject variables. These analyses revealed a main effect of cue in all three regions (caudate: F=56.55, df=2, 170, p<0.001; nucleus accumbens: F=61.33, df=2, 170, p<0.001; putamen: F=40.31, df=2, 170, p<0.001). Consistent with previous studies (7), post hoc analyses indicated that this effect was driven by increased striatal responses to reward cues, followed by penalty cues, followed by no-incentive cues (see Figure S3 in the data supplement). Relevant to the study hypotheses, a diagnosis-by-drug interaction also emerged for

all regions (caudate: F=9.65, df=1, 85, p=0.003; putamen: F=5.84, df=1, 85, p=0.018; the interaction fell short of statistical significance for the nucleus accumbens: F=3.35, df=1, 85, p=0.071). These effects were driven by increased striatal response to cues (regardless of cue type) in depressed participants receiving amisulpride relative to depressed participants receiving placebo (caudate: p=0.022; nucleus accumbens: p=0.036; putamen: p=0.049) and relative to control participants receiving amisulpride (caudate: p=0.017; the interaction fell short of statistical significance for the nucleus accumbens: p=0.063). Together, these results indicate that amisulpride enhanced striatal responses to cues, regardless of cue valance, in depressed but not healthy participants (Figure 2A).

Striatal Response to Outcomes

A repeated-measures ANOVA was performed for each striatal region with the following factors: hemisphere (left versus right) and outcome (reward outcome versus penalty outcome) as within-subject variables and diagnosis (depressed versus control group) and drug (amisulpride versus placebo) as between-subject variables. These analyses revealed a main effect of outcome in the nucleus accumbens (F=11.30, df=1, 85, p=0.001) related to greater nucleus accumbens activation to rewards than to penalties across participants. Critically, all three striatal regions showed an outcome-by-diagnosis-by-drug interaction (caudate: F=4.64, df=1, 85, p=0.034; putamen: F=6.73, df=1, 85, p=0.011; the interaction fell short of statistical significance for the nucleus accumbens: F=3.17, df=1, 85, p=0.078). As shown in Figure 2B, amisulpride administration in depressed participants enhanced striatal response to reward outcomes relative to placebo administration (nucleus accumbens: p=0.007; putamen: p=0.050) and relative to amisulpride administration in control participants (caudate: p=0.044; putamen: p=0.003). Nucleus accumbens response to reward outcome was also greater in control participants receiving placebo than in depressed participants receiving placebo (p=0.026). In contrast, no significant group differences emerged in striatal response to penalty outcome (Figure 2C). In sum, amisulpride selectively enhanced striatal response to reward outcomes, but not penalty outcomes, in depressed (but not healthy) participants.

Striatal Connectivity in Response to Outcomes

Whole-brain psychophysiological interaction analyses were conducted to separately investigate the effects of reward and penalty outcomes on striatal functional connectivity. A whole brain diagnosis-by-drug ANOVA (depressed versus control group; amisulpride versus placebo) revealed no significant group differences for striatal connectivity in response to reward or penalty outcomes at peak p<0.001, whole brain family-wise error corrected p<0.05. Next, striatal connectivity at the whole brain level was investigated across the entire sample (N=89). These analyses revealed that in response to reward but not penalty outcomes, participants

exhibited increased functional connectivity bilaterally between the caudate and a region (k=22 voxels) of the dorsal anterior cingulate cortex, as well as bilaterally between the nucleus accumbens and a region (k=13 voxels) of the midcingulate cortex (Figure 3A; see also Table S1 in the data supplement). Post hoc analyses were conducted to investigate whether depression or amisulpride moderated these reward-related corticostriatal connectivity patterns. To this end, caudate–dorsal anterior cingulate cortex and nucleus accumbens–midcingulate cortex connectivity values were extracted and used as the dependent variables in mixed-effect ANOVAs with diagnosis (depressed versus control group) and drug (amisulpride versus placebo) as between-subject variables. For both analyses investigating caudate–dorsal anterior cingulate cortex as well as nucleus accumbens–midcingulate cortex connectivity, significant diagnosis-by-drug interactions emerged (F=4.26, df=1, 85, p=0.043, and F=6.25, df=1, 85, p=0.015, respectively). Post hoc analyses revealed that control participants receiving placebo exhibited stronger reward-related caudate–dorsal anterior cingulate cortex functional connectivity relative to all three other groups (all p values, <0.033) (Figure 3B). With regard to nucleus accumbens–midcingulate cortex functional connectivity, both control participants receiving placebo and depressed participants receiving amisulpride showed stronger connectivity than depressed participants receiving placebo (p=0.037 and p=0.022, respectively) (Figure 3C).

Striatal Connectivity During Reward Outcomes and Reward Learning

Given the observed effects of amisulpride on nucleus accumbens–midcingulate cortex functional connectivity in depressed participants, multiple regression analyses were conducted to investigate the relationship between reward-related nucleus accumbens–midcingulate cortex connectivity and reward learning. Specifically, diagnosis (depressed group coded as +1, control group as −1), drug (amisulpride coded as +1, placebo as −1), reward learning ("choose A" accuracy from the probabilistic selection task), and their interactions were regressed on reward-related nucleus accumbens–midcingulate cortex functional connectivity. The results revealed a significant diagnosis-by-drug-by-reward learning interaction (F=5.76, df=1, 67, p=0.019). Post hoc simple regression analyses within each group revealed positive relationships between reward learning and reward-related nucleus accumbens–midcingulate cortex functional connectivity in depressed participants receiving amisulpride (r=0.65, p=0.003) and in control participants receiving placebo (r=0.54, p=0.029), but not in depressed participants receiving placebo (r=−0.24, p=0.35) or control participants receiving amisulpride (r=0.08, p=0.74) (Figure 4). These results indicate that amisulpride administration enhanced nucleus accumbens–midcingulate cortex functional connectivity in response to reward outcome in depressed individuals to a level comparable to that exhibited

FIGURE 2. Striatal Response to Cues and Outcomes in Depressed Individuals and Healthy Control Subjects With and Without Dopaminergic Enhancement[a]

A. Striatal Response to (All) Cues   **B. Striatal Response to Reward Outcomes**   **C. Striatal Response to Penalty Outcomes**

■ Placebo   ■ Amisulpride
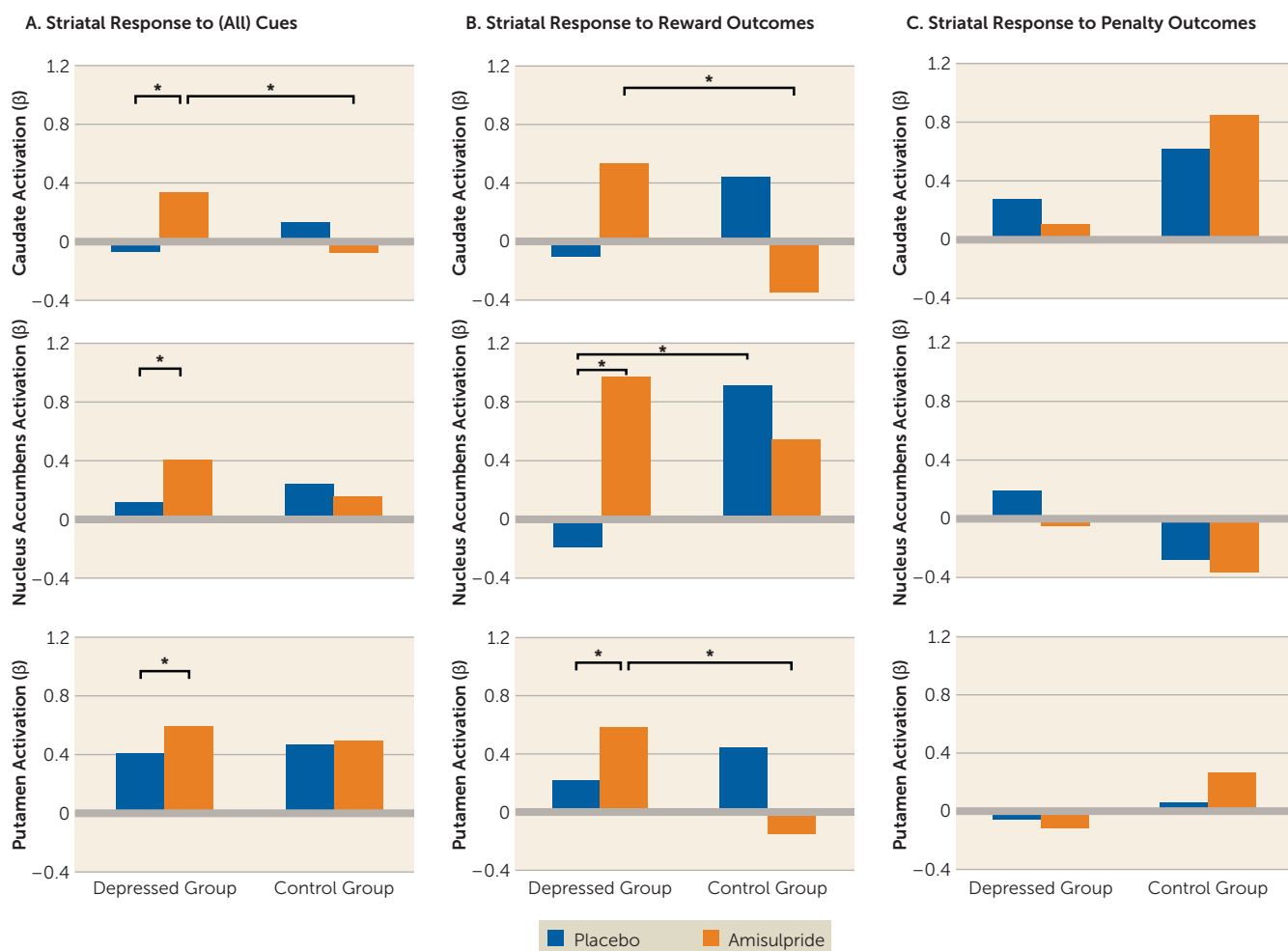
[a] In panel A, striatal response to cues, across all types, was greater in depressed participants receiving amisulpride compared with depressed participants receiving placebo (caudate: p=0.022; nucleus accumbens: p=0.036; putamen: p=0.049), as well as compared with healthy control participants receiving amisulpride (caudate: p=0.017; the group difference in nucleus accumbens fell short of statistical significance: p=0.063). In panel B, striatal response to reward outcomes was greater in depressed participants receiving amisulpride compared with depressed participants receiving placebo (nucleus accumbens, p=0.007; putamen, p=0.050), as well as compared with control participants receiving amisulpride (caudate, p=0.044; putamen, p=0.003); nucleus accumbens activation in response to reward outcome was also higher in control participants receiving placebo relative to depressed participants receiving placebo (p=0.026). In panel C, striatal response to penalty outcomes did not differ significantly across groups and there was no consistent pattern across regions in the penalty condition.
*p<0.05.

by healthy subjects receiving placebo. Furthermore, the magnitude of nucleus accumbens–midcingulate cortex functional connectivity during reward outcome for both depressed individuals receiving amisulpride and control participants receiving placebo was positively associated with reward learning in the behavioral probabilistic selection task.

DISCUSSION

Major depression is a debilitating psychiatric disorder characterized by high rates of relapse and recurrence. Discovering treatment tools that target putative mechanisms of illness in depression—such as blunted response to reward—is therefore a key clinical priority. Findings from this proof-

of-mechanism study suggest that an acute pharmacological challenge transiently increased striatal response to reward among adults with major depressive disorder, putatively via enhancement of dopaminergic transmission owing to autoreceptor blockade. Specifically, depressed participants receiving amisulpride exhibited increased striatal activity in response to cues, and increased striatal activity and corticostriatal functional connectivity in response to reward outcomes. Furthermore, stronger corticostriatal functional connectivity between the nucleus accumbens and the midcingulate cortex in depressed participants who received amisulpride was associated with better reward learning performance, a pattern similar to that observed in healthy control subjects receiving placebo. Together, these results

FIGURE 3. Striatal Connectivity in Response to Reward Outcomes in Depressed Individuals and Healthy Control Subjects With and Without Dopaminergic Enhancement[a]

A. Striatal Connectivity During Reward Outcomes

B. Caudate-Dorsal Anterior Cingulate Cortex

C. Nucleus Accumbens-Midcingulate Cortex
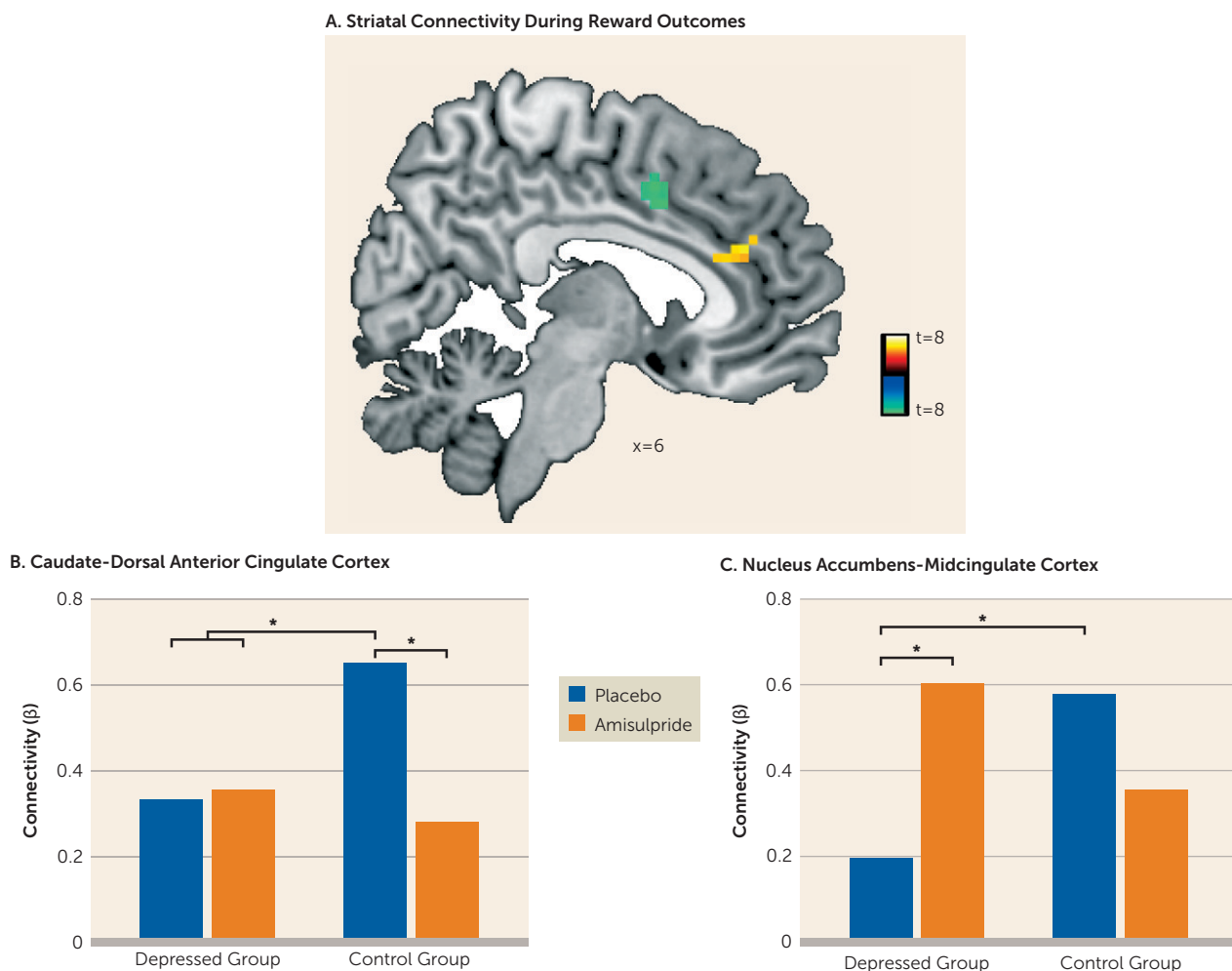
[a] In panel A, whole-brain psychophysiological interaction analyses revealed increased functional connectivity bilaterally between the caudate and regions of the dorsal anterior cingulate cortex (yellow) and bilaterally between the nucleus accumbens and regions of the midcingulate cortex (green) in response to reward outcomes across the entire sample at peak $p < 0.001$, family-wise error corrected $p < 0.05$. No changes in striatal connectivity were found in response to penalty outcomes. In panel B, caudate–dorsal anterior cingulate cortex functional connectivity was significantly higher in control participants receiving placebo relative to all other groups (all p values < 0.033). In panel C, nucleus accumbens–midcingulate cortex functional connectivity was significantly higher in both control participants receiving placebo and depressed participants receiving amisulpride than in depressed participants receiving placebo ($p = 0.037$ and $p = 0.022$, respectively).

provide converging evidence for abnormalities in neural reward systems in depression and highlight the potential of targeted pharmacological treatments to normalize reward processing in depression.

Extensive preclinical research has emphasized the key role of striatal dopamine signaling in mediating reward-related behaviors (2–4) and has postulated links between reduced striatal dopamine function and blunted reward processing and reinforcement learning in depression (9, 10). Interestingly, previous research indicates that dopamine differentially mediates anticipatory and consummatory phases of reward processing (28) and thus may uniquely affect their putative dysfunction in anhedonia and depression (29). In support of this idea, we observed that acute administration of amisulpride enhanced striatal response to cues regardless of valence (e.g., signaling potential rewards, penalties, or null

outcomes), yet in response to outcomes, striatal enhancement was selective to reward.
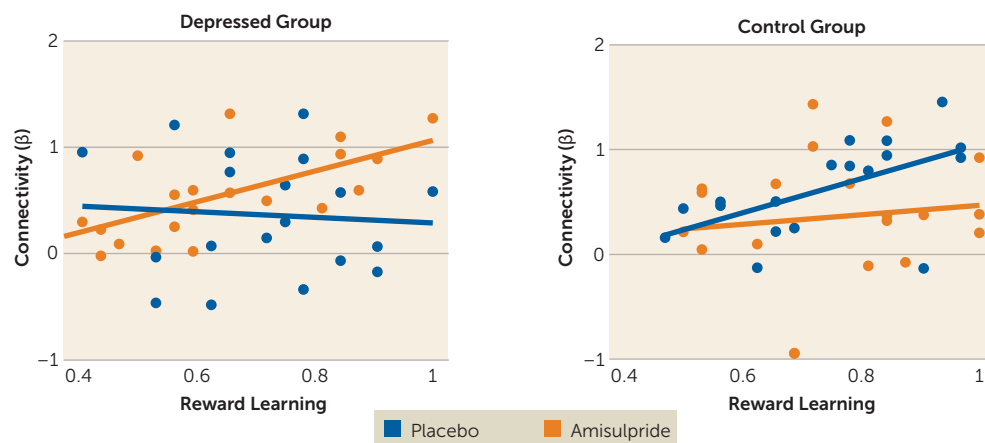
In addition to increasing striatal activity in response to rewards, enhancement of dopamine signaling in depressed individuals was also associated with amplified functional connectivity between the striatum and areas of the midcingulate cortex. This finding is consistent with a model in which abnormal coordinated activity among large-scale brain circuits, including corticostriatal pathways, is central to the pathophysiology of depression (30, 31). Critically, those depressed individuals who exhibited the strongest nucleus accumbens–midcingulate cortex connectivity in response to rewards after amisulpride administration also exhibited better reward learning in an independent behavioral task, and this pattern was not found among depressed individuals who received placebo. Of relevance to the present findings,

increased functional connectivity has been observed between midcingulate and striatal regions (and the insula) during learning (32), supporting the importance of this corticostriatal subcircuit in dopamine-mediated functioning. Coordination between dopamine-rich areas of the striatum and midline regions involved in processing behavioral salience may therefore be an important dimension of healthy reinforcement learning, and dopamine enhancement may help to regulate this functional circuit in depression. In fact, given preclinical evidence that amisulpride has a particularly high affinity for mesolimbic pathways (18, 19),

FIGURE 4. Reward Learning and Nucleus Accumbens–Midcingulate Cortex Functional Connectivity in Depressed Individuals and Healthy Control Subjects With and Without Dopaminergic Enhancement[a]

[a] Regression analyses revealed positive relationships between reward learning and reward-related nucleus accumbens–midcingulate cortex functional connectivity in depressed participants receiving amisulpride (r=0.65, p=0.003) and healthy control participants receiving placebo (r=0.54, p=0.029), but not in depressed participants receiving placebo (r=−0.24, p=0.354) or control participants receiving amisulpride (r=0.08, p=0.740). Reward learning refers to "choose A" accuracy from the probabilistic selection task.

one may speculate that amisulpride may enhance striatal function by affecting regulatory mechanisms beyond the striatum, and in particular in regions of the mesocorticolimbic pathway that communicate with the striatum via dopaminergic signaling to enable reward motivation and reinforcement learning (29). Thus, while in the present study we investigated the effects of amisulpride on striatal functioning, other brain systems that have exhibited abnormal activity or functional connectivity in depression (e.g., prefrontal cortex) may be important targets of dopamine manipulation.

Several additional questions remain open for future investigation. First, evidence from preclinical studies linking reinforcement learning and motivation with phasic dopamine signaling in the striatum suggests that amisulpride enhancement of reward processing in depressed individuals most likely occurs via increased phasic dopamine signaling (2–4). Nevertheless, the mechanisms by which amisulpride may act to enhance striatal response to reward are complex and may involve modifications of phasic and tonic levels of dopamine, as well as of additional neurotransmitters (33, 34). Additional research, especially in humans, investigating the effects of amisulpride on tonic and phasic dopamine release is needed. A second area for future investigation is motivated by differences between our findings and the results of previous investigations in which dopaminergic manipulation in healthy individuals resulted in better reward learning and increased striatal activity. The modest amisulpride dose used in the present study (50 mg as opposed to 200 mg and 400 mg in past studies [15, 35]) may have contributed to these discrepancies. We selected a 50-mg dose based on animal work showing that low doses of amisulpride potentiate striatal dopamine release, have strong hedonic effects, and increase the incentive value of environmental cues (18, 19). In

humans, a 50-mg dose of amisulpride has been associated with reduced blockade of postsynaptic D_2/D_3 receptor in comparison to higher doses of 200–400 mg (23), increasing the likelihood of presynaptic effects. Perhaps more importantly, (sustained) 50-mg amisulpride dosing has been shown to have antidepressant and antianhedonic effects in depressive disorders (21, 22), suggesting that the present pharmacological manipulation may preferentially benefit depressed individuals as compared with healthy subjects. Nevertheless, while the pharmacological manipulation enhanced striatal function in depressed individuals, it had no such effect on behavior (i.e., reward learning). One potential reason for this could relate to the fact that we administered only a single dose of the drug. Thus, while the drug may have an immediate effect on neural function, modifying behavior may require longer and more chronic exposure. In support of this idea, antidepressant effects of amisulpride among depressed individuals have been observed after sustained (but not acute) administration (21, 22).

In conclusion, in depressed individuals, but not healthy subjects, acute pharmacological challenge transiently increased striatal activity and corticostriatal functional connectivity in response to rewards, putatively via enhancement of dopaminergic transmission. These findings suggest that an acute pharmacological manipulation believed to increase dopamine transmission may help normalize reward processing in depressed individuals through the enhancement of key corticostriatal mechanisms.

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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).

<u>Artifact Detection Tools (ART):</u> 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.

<u>Whole-brain analysis:</u> 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).

<u>Statistical analyses:</u> 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).
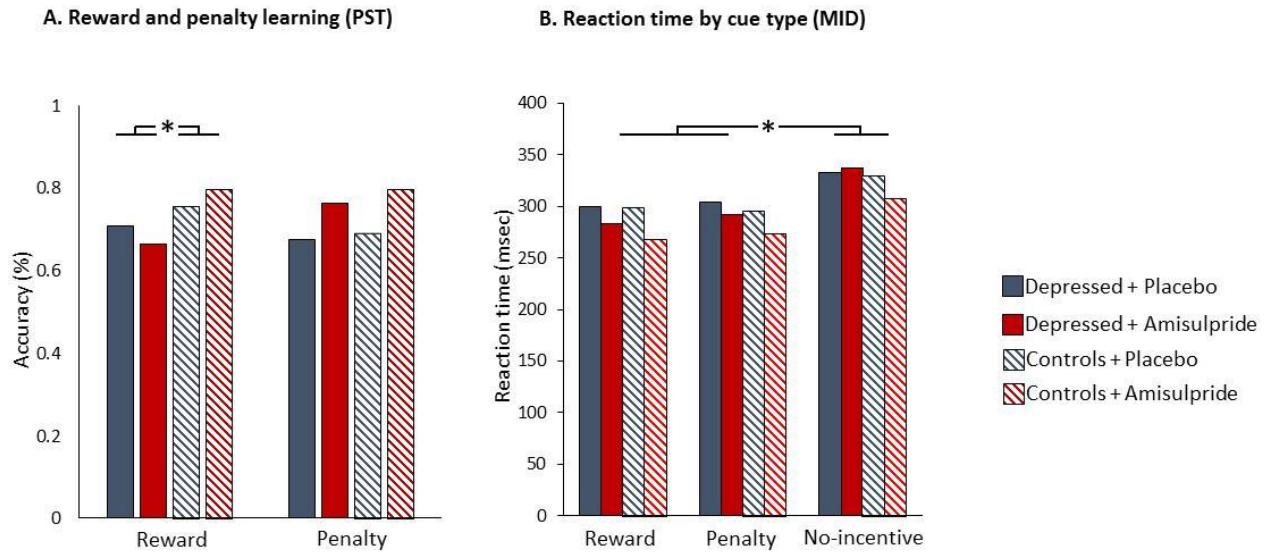
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FIGURE S1

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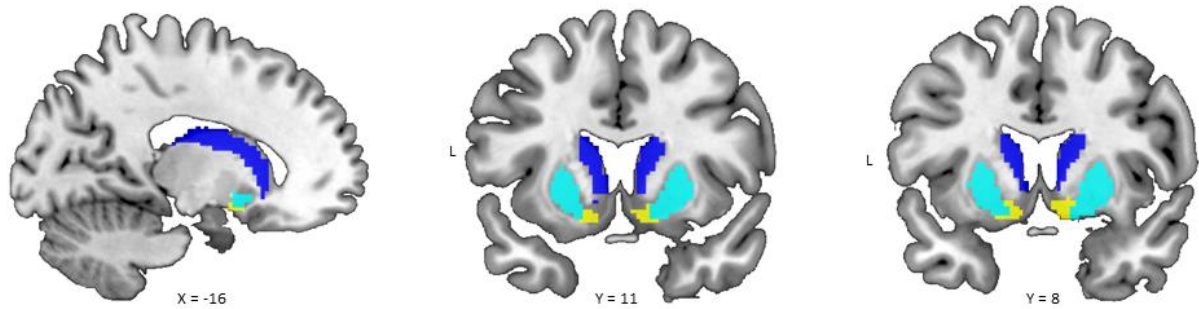
Striatal anatomical masks: Location of anatomically defined masks for the Caudate (blue), Nacc (yellow), and Putamen (turquoise). Mask volumes were 169 and 195 voxels for the left and right caudate, respectively; 25 and 34 voxels for the left and right Nacc, respectively; 226 and 239 voxels for the left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

FIGURE S2

A. Reward and penalty learning (PST)

B. Reaction time by cue type (MID)

Legend:
- Depressed + Placebo
- Depressed + Amisulpride
- Controls + Placebo
- Controls + Amisulpride

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.

Striatal response by cue type
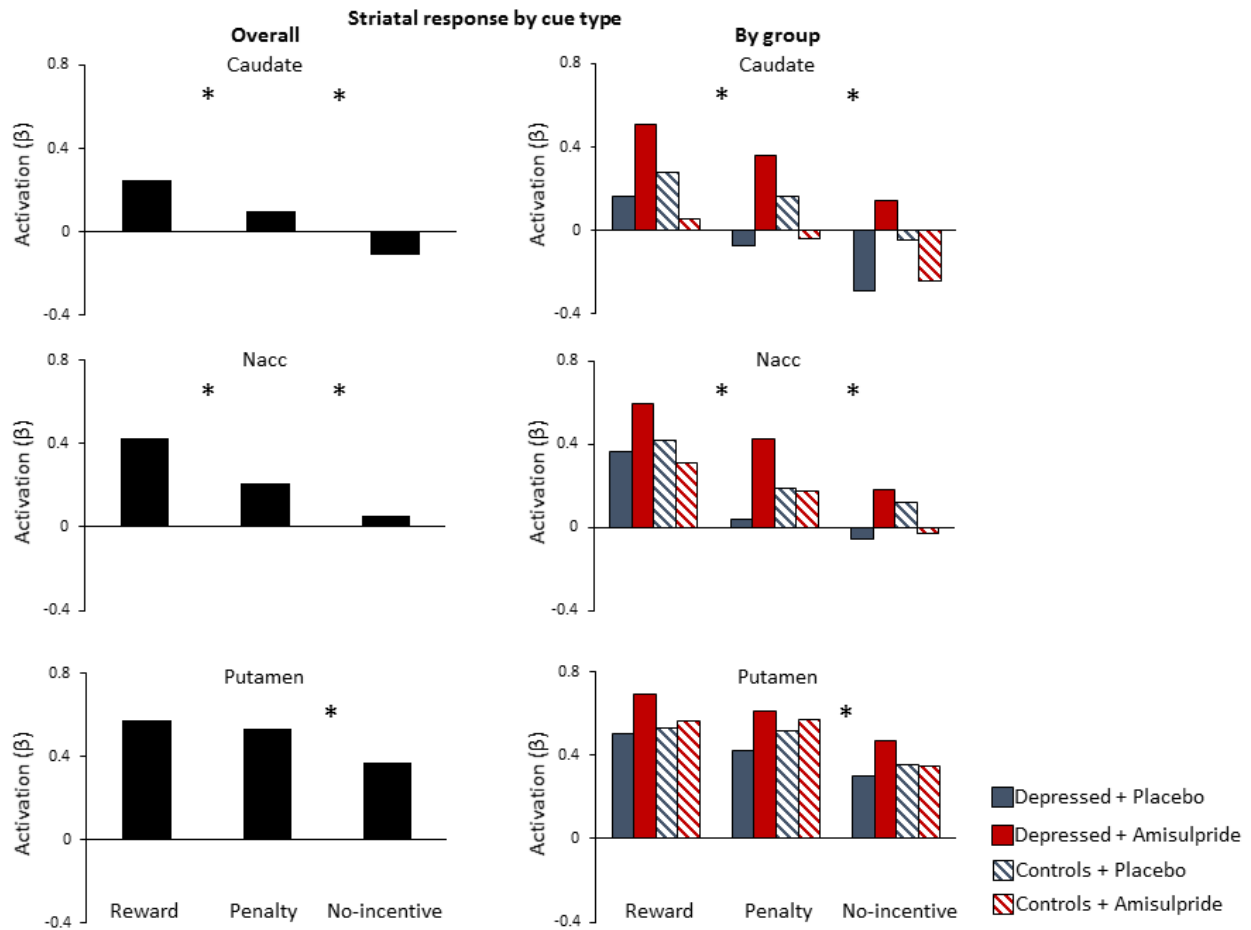
Striatal response by cue type: Across all groups and striatal regions, reward cues elicited the strongest striatal response, followed by striatal responses to penalty cues, and finally the weakest striatal responses were observed to no-incentive cues. Specifically, striatal activation was increased in 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
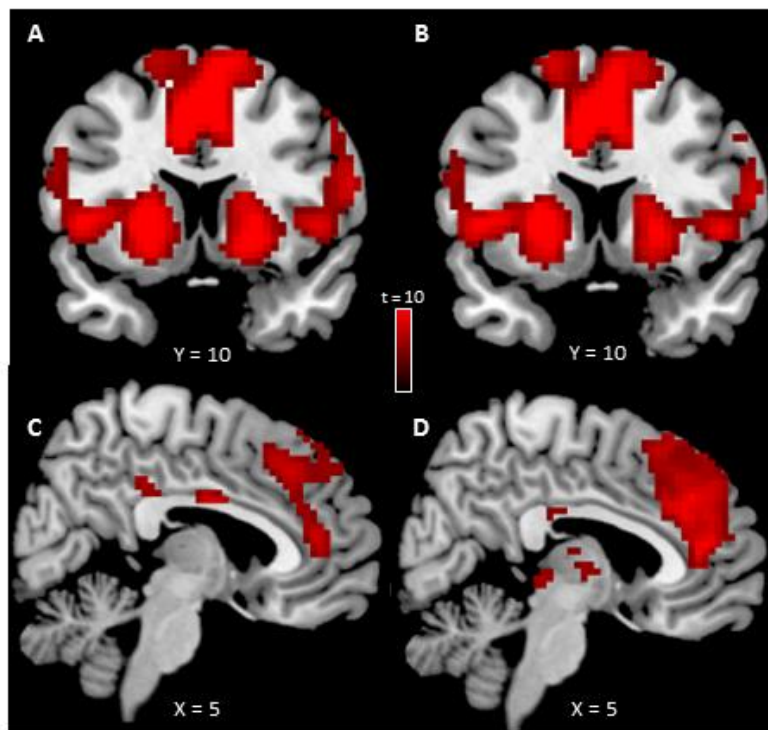
Whole-brain analysis: Whole-brain analyses across the entire sample ($n = 89$) in response to anticipation and receipt of monetary rewards and penalties revealed the expected pattern of activation. Specifically, in response to (**A**) reward anticipation and (**B**) penalty anticipation, robust activation 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.

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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

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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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