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Effects of GABA, Sex, and Stress on Reward Learning in Current and Remitted Major Depression

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ABSTRACT

BACKGROUND: Neurocognitive factors including aberrant reward learning, blunted GABA (gamma-aminobutyric acid), and potentiated stress sensitivity have been linked to anhedonia, a hallmark depressive symptom, possibly in a sex-dependent manner. However, previous research has not investigated the putative associations among these factors or the extent to which they represent trait- or state-based vulnerabilities for depression.

METHODS: Young adults with current major depressive disorder (MDD) (n = 44), remitted MDD (n = 42), and healthy control participants (HCs) (n = 44), stratified by sex assigned at birth, underwent magnetic resonance spectroscopy to assess macromolecular contaminated GABA (GABA+) and then a reward learning task before and after acute stress. We assessed changes in reward learning after stress and associations with GABA+.

RESULTS: Results revealed blunted baseline reward learning in participants with remitted MDD versus participants with current MDD and HCs but, surprisingly, no differences between participants with current MDD and HCs. Reward learning was reduced following acute stress regardless of depressive history. GABA+ in the rostral anterior cingulate cortex, but not the dorsolateral prefrontal cortex, was associated with reduced baseline reward learning only in female participants. GABA+ did not predict stress-related changes in reward learning.

CONCLUSIONS: To our knowledge, this is the first study to investigate associations among GABA, reward learning, and stress reactivity in current versus past depression. Hypothesized depression-related differences in reward learning did not emerge, precluding claims about state versus trait vulnerabilities. However, our finding that blunted GABA was associated with greater reward learning in female participants provides novel insights into sex-selective associations between the frontal GABAergic inhibitory system and reward processing.

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The prevalence of major depressive disorder (MDD) has been rising (1), with 17% of young adults reporting at least one past-year depressive episode (2). Anhedonia, or the loss of interest or pleasure in activities, is a hallmark symptom of MDD that is associated with poor prognosis, predicting treatment resistance, functional impairment, and suicidal ideation above and beyond other depressive symptoms (3). As such, elucidating behavioral or neurochemical mechanisms that underlie anhedonia is a priority. Furthermore, the extent to which mechanisms of anhedonia and MDD are state or trait based remains an unanswered question. Studies examining whether correlates of MDD persist after full remission are needed to identify putative targets for preventing future episodes. Finally, testing for sex-dependent mechanisms is needed given elevated rates of depression in females (4).

Reward Learning in Depression

Reduced reward learning is a well-established behavioral manifestation of anhedonia (5,6), but it is unclear whether these differences are state based or represent a more persistent vulnerability. For example, adults with current MDD developed a lower response bias toward a more frequently rewarded stimulus than control participants, and this effect was correlated with anhedonia severity (7,8). Blunted reward learning also emerged in remitted MDD (rMDD), as well as in never-depressed, asymptomatic offspring of parents with MDD, suggesting a possible trait-level vulnerability (9–11). Studies that include past and current depression in the same sample are needed to clarify the state- versus trait-like nature of reward deficits in MDD.

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The Role of Stress. Stress is a well-established contributor to depression and anhedonia (5,12), and altered reward learning is strongly implicated in the relationship between stress and depression (5). Chronic stress reliably reduces reward motivation in animals and humans and may sensitize an organism to the effects of subsequent stressors (12–16). Meanwhile, research on the effects of acute stress on reward learning has produced mixed results, with studies reporting both acute stress-induced increases (17,18) and decreases in reward learning (12,19–21). Thus, while aberrant reward learning is clearly linked to chronic stress and depression, the specific relationship between acute stress and reward learning merits further investigation.

Furthermore, few studies have examined whether reward response deficits under stress represent a state- or trait-dependent marker for depression. Greater stress-induced blunting in reward learning was associated with current anhedonia in a community sample (20). To our knowledge, the association between reward learning and stress in rMDD has not been tested. Reward response deficits may moderate the effects of stress on depression (a trait-level marker), develop alongside depression (a state-dependent marker), or mediate the relationship between stress and depression (13).

The Role of GABA. Alongside stress, atypical signaling of GABA (gamma-aminobutyric acid) has been implicated in depression (22) and anhedonia more specifically (23,24). Studies have described blunted GABA in both the anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (dlPFC) in current and past MDD (23–28). However, the findings have been mixed overall (29–32) and generally based on small sample sizes (all but one group analysis utilized $N < 81$) (23–32).

Some early preclinical and clinical evidence has supported the notion that GABA deficits may contribute to depression via impaired reward processing (33). While the precise neural circuitry that underlies this effect remains poorly understood, prefrontal cortical projections are known to innervate classic reward areas including the striatum and ventral tegmental area (34). Moreover, preclinical findings have shown that infusion of a GABA antagonist into the PFC interferes with reward sensitivity and valuation, which may be due to downstream effects in the nucleus accumbens (35). Meanwhile, the results of initial studies of ACC GABA and reward have been mixed. In a study of 37 healthy participants, higher ACC GABA during the baseline condition of a reward task predicted better subsequent learning performance (36); in contrast, another study found that higher ACC GABA at baseline predicted poorer learning on a reward task in 30 healthy participants (37). Clearly, additional studies are needed.

Furthermore, whether this putative association between GABA and reward learning is modulated by sex remains largely unexplored, although early research indicates a nuanced relationship. Females with depression have been found to have larger reductions in ACC GABA interneurons than males, while males with depression show a greater reduction in synthesizing enzymes in the ACC (38). GABA signaling has also been shown to be suppressed by estrogens in ex vivo and animal work (39). Furthermore, there is initial preclinical evidence of sex differences (i.e., stronger in females) in the associations between alterations in cortical GABAergic signaling and differences in learning, although the directionality of GABAergic effects on learning have been mixed (40,41).

Despite evidence connecting both GABA and stress to aberrant reward learning, the role of GABA in stress-induced reward response deficits has received little study. Czéh *et al.* (42) found that rats that developed anhedonic-like behavior under chronic stress showed fewer neuropeptide Y-positive GABAergic neurons in the infralimbic cortex than stress-resilient rats, possibly related to stress-induced alterations in reward learning. Sex differences have been found in the structure and function of brain regions that are involved in stress and reward circuitry in humans (43–46), but to our knowledge, they have not been assessed in the context of GABA and stress-induced changes in reward learning. Research is needed directly probing the relationship between acute stress, sex, reward, and GABA.

The Current Study

To address these gaps, the current study examined associations among reward learning, GABA, sex, and acute stress sensitivity in current and past depression. Specifically, we hypothesized the following: 1) Reward learning would be blunted in participants with lifetime depression compared to healthy control participants (HCs) (indicating a trait-level vulnerability), but particularly among those with current compared to remitted MDD (a state-dependent change), regardless of sex. 2) Reward learning would decrease after a stress induction across all participants, and especially in those with lifetime depression (i.e., either current or remitted MDD) versus HCs, indicating a trait-level vulnerability, regardless of sex. 3) Higher rostral ACC (rACC) and dlPFC macromolecular-contaminated GABA (GABA+) would be associated with greater reward learning across all participants, with the strongest association among females. 4) Higher rACC and dlPFC GABA+ would buffer against potential changes in reward learning after a stress induction across all participants, with the strongest association among females.

METHODS AND MATERIALS

Participants

A sample of 130 young adults (ages 18–25 years [mean = 21.3, SD = 2.2]; balanced by sex assigned at birth) was recruited from the greater Boston area. The sample comprised 3 groups: current depression (MDD, $n = 44$), rMDD ($n = 42$), and HCs with no psychopathology history ($n = 44$). See Table 1 for demographics and the Supplement for more detail.

Procedures: Screening Session

Participants provided informed consent in compliance with the Mass General Brigham Human Research Protection Program. Participants were assessed for lifetime psychopathology via the Structured Clinical Interview for DSM-5 (47), clinician-rated Quick Inventory of Depressive Symptomatology (48), and Hamilton Depression Rating Scale (49) by a master- or Ph.D.-level clinician. See Table 2 for clinical characteristics of the sample and the Supplement for detailed eligibility criteria, interrater reliability metrics, and quality control steps. Participants who met criteria for nondepressive psychiatric disorders were excluded, except for those with anxiety (when secondary to MDD) and cannabis use disorders due to high rates of comorbidity (50,51). Participants were excluded if they were taking psychoactive medications.

Table 1. Demographic Characteristics of Study Participants

	MDD, *n* = 44	rMDD, *n* = 42	HC, *n* = 44	Total, *N* = 130
Age, Years	20.8 (2.1)	21.6 (2.0)	21.4 (2.3)	21.3 (2.2)
Sex				
Assigned female at birth	50.00%	52.38%	47.73%	50.00%
Assigned male at birth	50.00%	47.62%	52.27%	50.00%
Ethnicity				
Hispanic or Latine	25.00%	9.52%	11.36%	15.38%
Non-Hispanic or Non-Latine	75.00%	90.48%	88.64%	84.62%
Race				
American Indian or Alaskan Native	0.00%	0.00%	0.00%	0.00%
Asian	15.91%	11.90%	27.27%	18.46%
Black or African American	11.36%	4.76%	11.36%	9.23%
Native Hawaiian or Other Pacific Islander	0.00%	2.38%	0.00%	0.77%
Multiracial	6.82%	11.90%	9.09%	9.23%
White	56.82%	66.67%	50.00%	57.69%
Undisclosed	9.09%	2.38%	2.27%	4.62%
Education				
High school	20.45%	2.38%	20.45%	14.62%
Some college	50.00%	54.76%	20.45%	41.54%
4-year college degree	18.18%	30.95%	27.27%	25.38%
Postgraduate	9.09%	11.90%	25.00%	15.38%
Undisclosed	2.27%	0.00%	6.82%	3.08%
Family Annual Income				
<$10,000	11.36%	11.90%	18.18%	13.85%
~$10,000–$24,999	18.18%	9.52%	9.09%	12.31%
~$25,000–$49,999	27.27%	16.67%	18.18%	20.77%
~$50,000–$74,999	15.91%	9.52%	13.64%	13.08%
~$75,000–$99,000	18.18%	16.67%	15.91%	16.92%
>$100,000	9.09%	35.71%	22.73%	22.31%
Undisclosed	0.00%	0.00%	2.27%	0.77%

Values are presented as mean (SD) or %. The table shows descriptive statistics for self-identified demographic information split by diagnostic group. Education was measured using number of years completed rather than degrees obtained and was operationalized as 12 years = high school, 13 to 15 years = some college, 16 years = 4-year college degree, and 17+ years = postgraduate. If financially independent, participants were instructed to report only personal income; if participants were financially dependent, they were instructed to include parental or guardian income.

HC, healthy control participant; MDD, major depressive disorder; rMDD, remitted major depressive disorder.

Table 2. Clinical Characteristics of Study Participants

Characteristic	MDD, *n* = 44	rMDD, *n* = 42	HC, *n* = 44	Total, *N* = 130
Depression Metrics				
SHAPS	33.9 (5.0)	18.4 (4.0)	19.2 (4.6)	23.8 (8.4)
QIDS	14.2 (2.7)	0.7 (1.1)	0.4 (0.8)	4.3 (6.3)
HDRS	16.6 (4.0)	1.1 (1.5)	0.3 (0.8)	6.1 (7.9)
Number of depressive episodes	2.3 (1.3)	1.6 (0.9)	–	1.9 (1.2)
Age of depression onset, years	16.9 (3.5)	17.7 (2.4)	–	17.2 (3.0)
Secondary Comorbidities				
Generalized anxiety disorder	22.73%	–	–	7.69%
Social anxiety disorder	15.91%	–	–	5.38%
Specific phobia	9.09%	–	–	3.08%
Cannabis use disorder	2.27%	4.76%	–	2.31%

Values are presented as mean (SD) or %. The table shows clinical characteristics of the participants split by diagnostic group. Participants could report a maximum of 5 depressive episodes (>5 episodes or "too many to count" were coded as 5 to facilitate computing the mean and SD). All anxiety disorders were required to be secondary to MDD to meet eligibility requirements. rMDD and HCs were ineligible if they met criteria for a current anxiety disorder, and HCs were also ineligible if they met criteria for a cannabis use disorder. Participants with any current psychiatric comorbidities that are not listed above (e.g., panic disorder, posttraumatic stress disorder, agoraphobia, obsessive-compulsive disorder, eating disorders) were ineligible for the study regardless of diagnostic group.

HC, healthy control participant; HDRS, Hamilton Depression Rating Scale; MDD, major depressive disorder; QIDS, Quick Inventory of Depressive Symptomatology; rMDD, remitted MDD; SHAPS, Snaith-Hamilton Pleasure Scale.

Procedures: Scanning Session

Scanning session procedures included 1) resting-state magnetic resonance spectroscopy, 2) subsequent functional magnetic resonance imaging with a psychosocial stressor (44,52), and 3) 2 runs of a reward learning paradigm (probabilistic reward task [PRT]) (53) completed outside the scanner before and after a second psychosocial stressor. Figure 1 depicts the flow of study procedures.

Spectroscopy. Voxels were placed in the rACC (17.5 mL; 35 × 20 × 25 mm^3) and left dlPFC (18.75 mL; 25 × 30 × 25 mm^3) using a T1-weighted structural image. GABA+ was acquired via a Meshcher-Garwood point resolved spectroscopy sequence. Current macromolecular suppression techniques suffer from notable limitations (e.g., frequency drift) (54) and thus were not employed. GABA+ was fit from the difference spectrum using LCModel (55). See Duda *et al.* (56) for further description of magnetic resonance spectroscopy methods and reliability analyses and the Supplement for quality assurance procedures. Group differences in rACC and dlPFC GABA+ are reported separately (57). Results showed significantly higher rACC GABA+ in HCs than in participants with a lifetime history of depression, consistent with a trait-level vulnerability, and no significant group differences in dlPFC GABA+.

Initial Stress Induction. Next, participants underwent a hybrid psychosocial stressor in the scanner comprising the Montreal Imaging Stress Task (MIST) (58) and the Maastricht Acute Stress Test (MAST) (59). For details regarding functional magnetic resonance imaging findings and the hybrid stressor, see the Supplement and Ironside *et al.* (52).

PRT and Second Stress Induction. Before completing the PRT, participants engaged in neutral activities (reading or writing) for 90 minutes after the onset of the MIST/MAST stressor. To assess baseline reward learning, participants completed a first run of the PRT [prestress onset (53)] (see the Supplement for detailed task information). Next, stress was reintroduced by experimenters falsely informing participants that they would need to complete the MIST/MAST stressor a second time due to inferior performance (PRT stressor). Participants then completed a second PRT run (poststress onset). Stress induction via the threat of an upcoming stressor such as a cold pressor task has been used in previous literature and shown

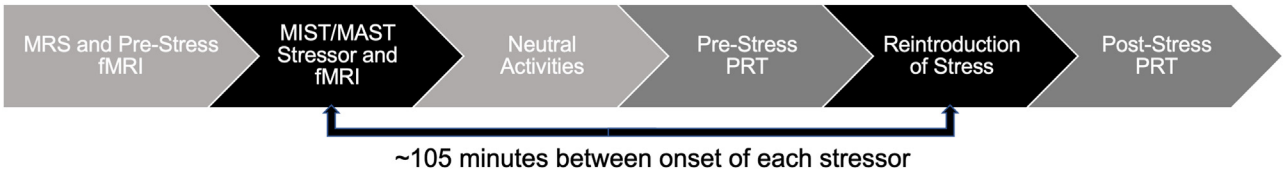

Figure 1. A schematic outlining study procedures at the scanning session. Neuroimaging scans began with the acquisition of GABA+ (gamma-aminobutyric acid with macromolecular contamination) in the rostral anterior cingulate cortex and dorsolateral prefrontal cortex at rest followed by functional magnetic resonance imaging (fMRI) scans while participants completed a hybrid psychosocial stressor. After exiting the scanner, participants sat quietly while either reading or writing neutral materials to allow cortisol to return to baseline. Participants then completed the first administration of the probabilistic reward task (PRT) (approximately 105 min after the initiation of the Montreal Imaging Stress Task and Maastricht Acute Stress Test [MIST/MAST] stressor), after which stress was reintroduced when a study team member informed them that they would need to repeat the MIST/MAST stressor due to poor performance. A second, poststress administration of the PRT was then completed before participants were debriefed and the study visit concluded. MRS, magnetic resonance spectroscopy.

to reliably induce a stress response [for a summary, see (60)]. Furthermore, it has been shown that repeated administrations of a socially evaluated cold pressor test continued to induce a stress response (utilizing a 24-hour delay between administrations) [see (61)]. A response bias metric for the PRT was computed consistent with previous studies (7) to assess reward learning (6). PRT response bias has been shown to have modest test-retest reliability over a much longer period of 3 weeks (r = 0.59, $p < .004$) (D. Dillon, Ph.D., *et al.*, unpublished data, December 2023).

Cortisol. Serum cortisol was collected at 9 time points via a saline-lock intravenous line (see the Supplement for details). Stress-induced cortisol reactivity was calculated using area under the curve with respect to increase (AUC_i) (62) as a manipulation check.

Affective Ratings. As a further stress manipulation check, Visual Analog Mood Scale measuring tenseness versus relaxedness, hostility versus friendliness, and sadness versus happiness were administered at 6 time points. Changes in affect were calculated with AUC_is.

Analysis

Analyses were performed in RStudio. Continuous predictors were mean centered, and categorical variables were contrast coded, which yielded 2 clinical contrasts: healthy versus clinical (combined MDD and rMDD) and MDD versus rMDD. Alternate group contrasts (HC vs. MDD; rMDD vs. combined HC and MDD) were also run for the reward learning models to test for hypothesized differences between currently depressed and healthy participants. All mixed-effects models included fixed effects of sex and a random intercept for subject, and stress models also included a fixed effect of condition and random slope for condition (pre- vs. poststress onset). Outliers were removed based on Cook's distance. For specific details of each model and additional results based on dimensional symptoms of depression, see the Supplement.

RESULTS

Manipulation Checks: Affect and Cortisol

One-sample t tests showed that cortisol (AUC_i) increased following the MIST/MAST stressor (t_{91} = 7.08, $p < .001$) and returned to baseline after 90 minutes (i.e., no difference from baseline (t_{97} = −0.25, p = .800). In contrast, following the second introduction of stress (PRT stressor), participants showed a significant decrease in cortisol over 90 minutes (t_{87} = −5.80, $p < .001$) despite reporting heightened tension (t_{108} = 9.46, $p < .001$), sadness, (t_{109} = 3.71, $p < .001$), and hostility (t_{108} = 5.20, $p < .001$).

To better understand the diminished cortisol reactivity in response to the second stressor, an exploratory linear model assessed the association between cortisol responses (AUC_is) to the MIST/MAST and PRT stressors and revealed a significant negative association, t_{86} = −4.04, $p < .001$ (Figure S1). These data indicate a possible cortisol habituation effect, with greater cortisol responses to the initial stress induction predicting lower cortisol reactivity during stress reintroduction (see the Supplement for group analyses of cortisol findings).

Hypothesis 1: Reward Learning and Depression

A mixed-effects model predicting baseline reward learning with fixed effects of block, group, sex, the interactions of group and sex, and a subject-specific random intercept revealed no significant reward learning differences between the current MDD group and HCs (t_{108} = 0.67, p = .504) or between the MDD group and HCs (t_{108} = 0.47, p = .641), in contrast to our hypotheses. Interestingly, participants with rMDD had significantly blunted reward learning compared to those with current MDD and HCs (t_{108} = −2.12, p = .036). An unexpected interaction between sex and the HC versus MDD contrast emerged (t_{108} = 2.30, p = .023), but post hoc tests run separately for males and females revealed no significant group differences in reward learning.

Hypothesis 2: Reward Learning After Stress

A similar mixed-effects model was run predicting pre- and poststress reward learning, adding a fixed effect of condition (pre- or poststress onset), its interactions with group and sex, and a random slope for condition (Figure 2). Results revealed that participants' response bias was significantly lower poststress onset versus prestress (t_{107} = −2.85, p = .005), consistent with our hypotheses. Contrary to our hypotheses, group did not interact with condition to predict response bias ($ps > .087$). An unexpected simple effect of sex emerged, with female participants showing greater reward learning, averaging across stress (t_{105} = 2.05, p = .043). Finally, results also revealed an effect of block (t_{224} = 5.45, $p < .001$), reflecting improvements in performance during the task.

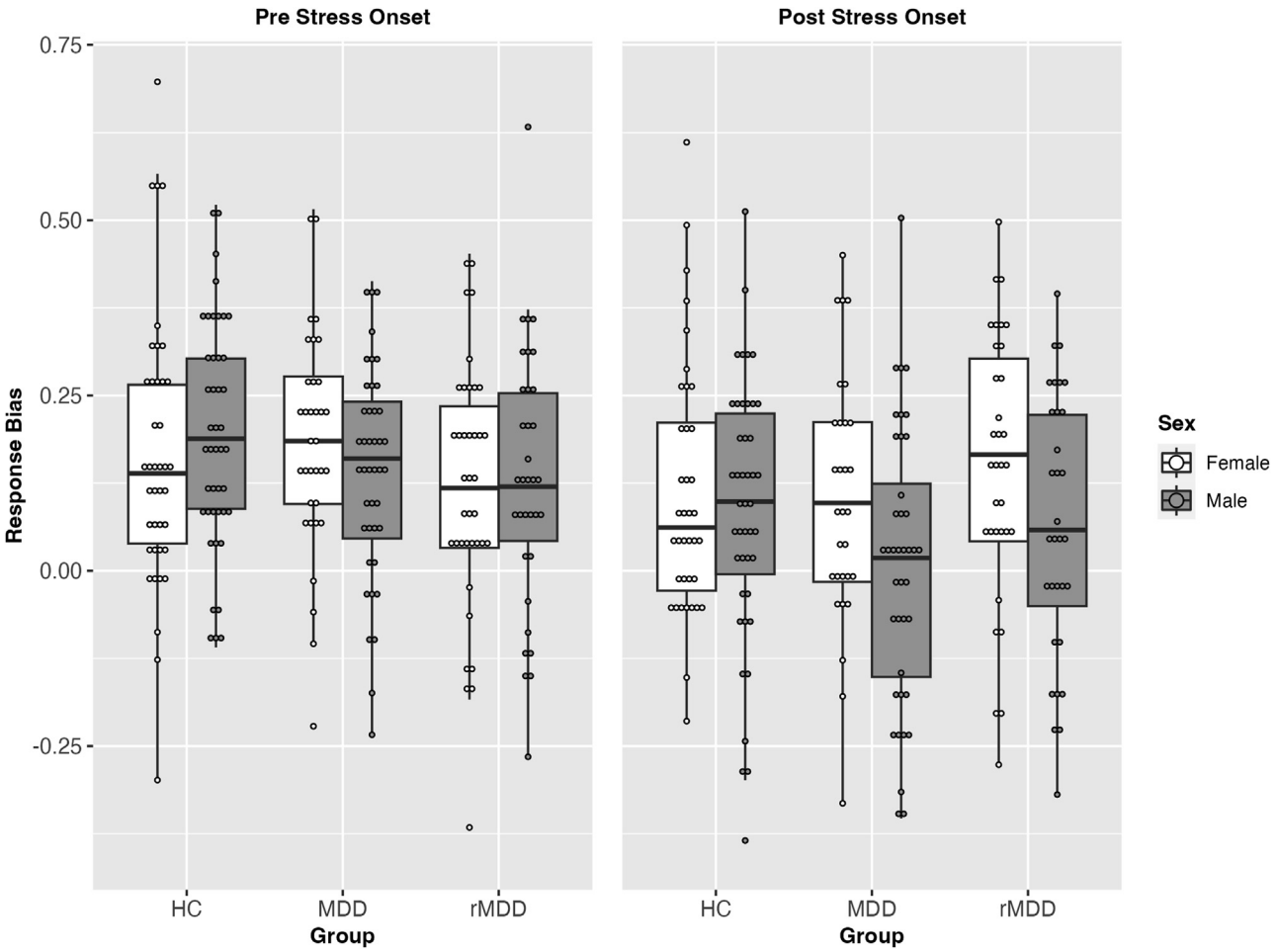

Figure 2. Box plots of pre- and poststress reward learning operationalized as response bias on the probabilistic reward task, by clinical group and sex assigned at birth. Outliers were removed based on Cook's distance values > 4/*n*. HC, healthy control participant; MDD, major depressive disorder; rMDD, remitted MDD.

Hypothesis 3: GABA+ and Reward Learning

Next, a similar mixed-effects model to hypothesis 1 was run predicting baseline reward learning, but with GABA+ (rACC or dlPFC, run separately) replacing clinical group[1]. Results revealed an interaction between sex and rACC GABA+ ($t_{92} = -2.30, p = .024$) and a simple effect of block ($t_{95} = 2.44, p = .017$) (Figure 3). Post hoc mixed-effects models run separately by sex revealed no relationship between rACC GABA+ and baseline reward learning among male participants ($t_{48} = 0.48$, $p = .634$); however, contrary to our hypotheses, there was a significant negative relationship between rACC GABA+ and reward learning for female participants ($t_{44} = -2.45$, $p = .018$). Notably, an exploratory linear model revealed no overall sex differences in rACC GABA+ ($t_{101} = 0.18$, $p = .855$). Contrary to our hypotheses, a similar model with dlPFC replacing rACC GABA+ revealed no significant effect of dlPFC GABA+ on baseline reward learning ($t_{97} = -0.62, p = .535$) or a sex × dlPFC GABA+ interaction ($t_{97} = -0.27$, $p = .786$).

[1]We evaluated the relationship between GABA+ and reward learning across all participants (without group as a covariate) because we did not hypothesize that the strength of the relationship between GABA+ and reward learning would differ by diagnosis and were concerned about limited power as well as collinearity between GABA+ and group. Additionally, given that we predicted that GABAergic-related impairments in reward processing might be one mechanism by which depression is maintained, there was concern that controlling for depression might mask this effect.

Hypothesis 4: GABA+ and Reward Learning After Stress

Next, the mixed-effects model predicting reward learning pre- and poststress onset was estimated with GABA+ levels replacing clinical group. Contrary to our hypotheses, results revealed no significant interaction between condition and rACC GABA+ ($t_{95} = -0.31, p = .758$) or dlPFC GABA+ ($t_{105} = -0.23, p = .823$), and sex did not moderate these relationships (*p*s > .399).

DISCUSSION

In the current study, we examined—to the best of our knowledge, for the first time—putative associations among GABA, reward learning, and stress sensitivity in a moderately large

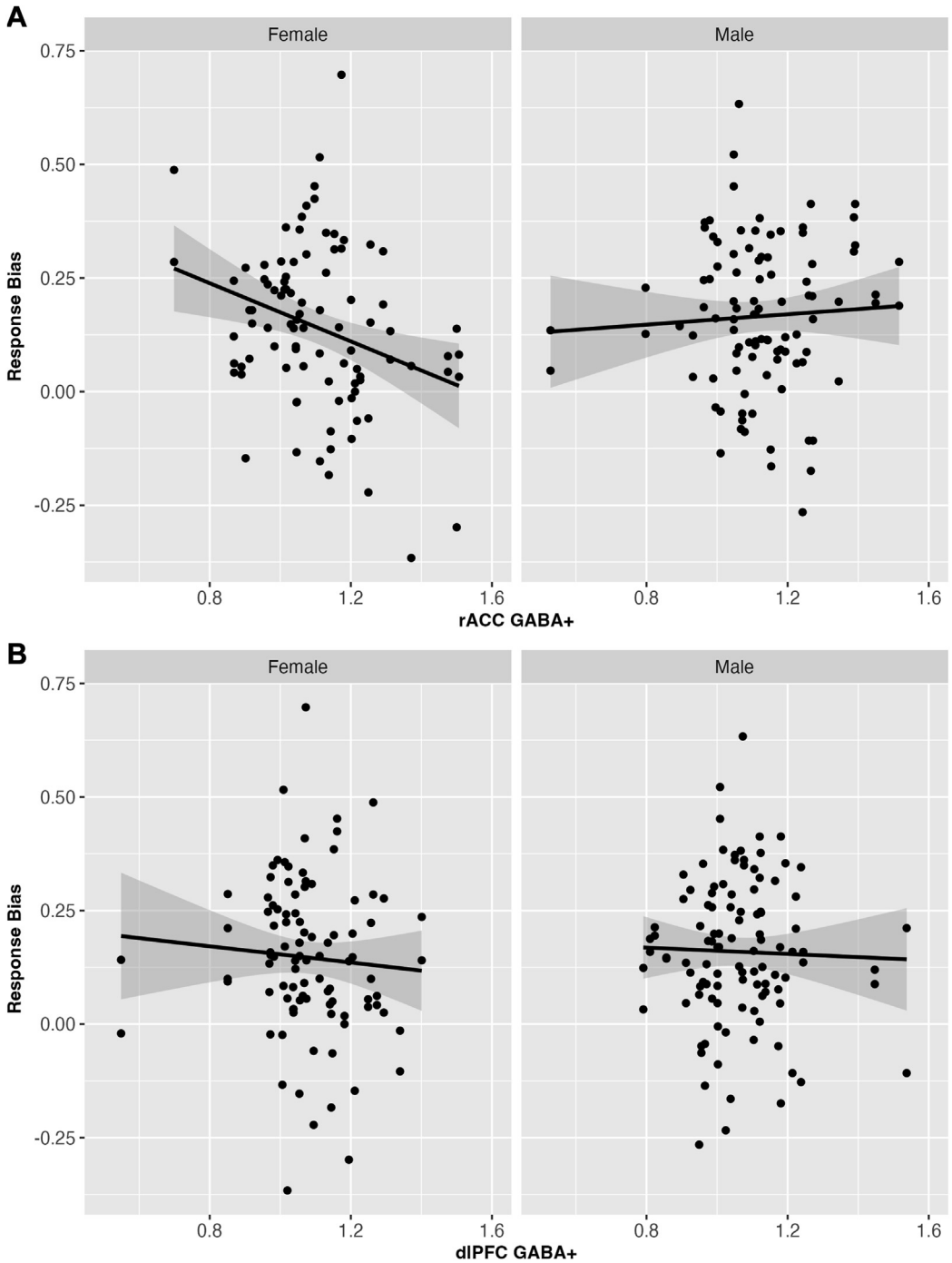

Figure 3. Prestress response bias by sex and **(A)** rostral anterior cingulate cortex (rACC) GABA+ (gamma-aminobutyric acid with macromolecular contamination) or **(B)** dorsolateral prefrontal cortex (dlPFC) GABA+. Reward learning was operationalized as response bias on the probabilistic reward task. Outliers were removed based on Cook's distance values $> 4/n$. Females showed a significant negative relationship between rACC GABA+ and baseline response bias. The relationship between rACC GABA+ and response bias was not significant for males. There were no significant associations between dlPFC GABA+ and response bias.

sample of young adults with current and past depression. Contrary to hypothesis 1, we found no baseline differences in reward learning between participants with current depression and HCs. However, we observed reduced baseline reward learning in rMDD compared to current depression or no history of psychopathology. This finding is consistent with previous

research that has shown blunted response bias in rMDD (10,11), although these earlier studies did not include a currently depressed comparison group. Previous studies interpreted diminished response bias in rMDD as evidence of a trait-level abnormality, and the current results partially support this hypothesis. However, the lack of blunted response bias in our current MDD group complicates the interpretation, and thus this question remains unanswered. The reasons that we did not replicate blunted reward learning in the MDD group are unclear, although several explanations are possible. First, our MDD group may have experienced less severe depressive episodes than our remitted group for 2 reasons: 1) individuals may have been less motivated to enroll in studies during a severe depressive episode, whereas remitted participants with past severe episodes may have been more likely to participate; and 2) recent psychiatric medication use was grounds for exclusion, and thus participants experiencing severe current depression that required medication could not participate. Second, our MDD sample may have less severe depression than samples from previous studies with the PRT that have utilized inpatient samples [e.g., (8)]. Third, only 27.3% of our participants met criteria for the melancholic subtype of depression, which has been specifically associated with reduced response bias on the PRT (63). In addition to differing sample characteristics, our failure to replicate blunted reward learning in current MDD could also relate to the heterogeneity of depression, which may reflect multiple phenotypes (5).

In support of hypothesis 2, we found that reward learning was lower after stress, replicating results of past studies (19,20,64). This could reflect transient stress-induced anhedonic behavior across the sample. Contrary to our hypotheses, we found no significant differences in stress-related changes in reward learning between participants with lifetime depression and control participants (20,65), and thus, claims cannot be made about whether stress-related changes in reward learning represent a state versus trait vulnerability for depression. The lack of a cortisol response to the second stressor should be considered when interpreting these results; however, the elevated self-reported negative affect after stress suggests that the stress induction was successful, and the finding of a cortisol habituation effect (i.e., greater early cortisol responsiveness predicting subsequently blunted cortisol reactivity) may explain the surprising lack of a cortisol response.

Contrary to hypothesis 3, we did not find an overall relationship between GABA+ and baseline reward learning. Interestingly, results revealed a sex × rACC GABA+ interaction on baseline response bias, with GABA+ being negatively associated with reward learning among females but not males, in contrast to our hypotheses. These sex-related functional differences in the rACC mirror structural differences by sex in this region (45). Interpretation of these findings is challenged by a paucity of studies that have probed sex differences in the relationship between GABA and reward learning. In a preclinical study, cortical GABA was associated with greater depressive behavior specifically in females, possibly related to alterations in PFC and amygdala function (40), although reward learning was not assessed. Scholl *et al.* (37) demonstrated a similarly negative relationship between dorsal ACC GABA and the use of learned information in decision making (a measure of reward learning) but did not directly probe response biases or examine sex differences. Future studies are needed to replicate a negative relationship between ACC GABA and reward learning in female participants. Finally, claims about trait versus state vulnerabilities cannot be made with these models, because group predictors were omitted due to an expectation that GABA would serve as a mechanism of blunted reward learning (and thus that this association would not depend on depression status). We expected reduced rACC GABA to be associated with lower reward learning, because both blunted rACC GABA and reward learning have been implicated in depression. However, our findings that lower rACC GABA was associated with greater reward learning suggests that these associations may be more complex than was previously thought.

In contrast to hypothesis 4, GABA levels did not explain variability in reward learning under stress, and sex did not moderate these effects. While GABA has been connected to anhedonic behavior under chronic stress in rats (42), studies have not yet examined the relationship between GABA and acute stress-induced changes in reward learning in humans. Thus, additional research is needed to substantiate these findings, especially with a larger sample size for testing 3-way interactions.

The current study has a number of strengths, including a moderately large unmedicated sample with current or past depression, which allowed for the examination of possible state or trait effects. Additionally, participants who were assigned male and female at birth were recruited in equal numbers to test possible sex effects. Female participants were scanned during the follicular phase of their menstrual cycles to control for cyclical changes in neural GABA levels (66), and the visits consistently began in the early afternoon to account for diurnal variation in cortisol levels (67). Finally, inclusion criteria were strict to limit confounds.

However, several important limitations should be considered. A study utilizing a larger sample would have better power to capture interaction effects, and it is possible that some of our null findings (especially the 3-way interaction between sex, stress, and GABA+) are due to a lack of power. Additionally, the initial MIST/MAST stressor may have impacted subsequent prestress PRT results, although there was a >90-minute gap between these procedures to allow cortisol levels to return to baseline. The lack of a comparison condition for the second stressor also prevents ruling out practice or fatigue effects on reward learning, although participants reported increased tension after the stress induction, and our results are consistent with previous studies that have shown stress-induced reductions in response bias (12,20). The ecologic validity of laboratory-based stressors also limits generalizability, and we did not collect measures of participant life stress. The current study also relied on a single measure of reward learning (the PRT); applying a battery of tasks would likely result in a more generalizable assessment of reward processing. Furthermore, the use of categorical measures of depression may have masked more specific associations between depressive symptoms and reward learning (although see the Supplement for nonsignificant dimensional analyses). Additionally, acquiring GABA+ via magnetic resonance spectroscopy is technically challenging, with evidence of only moderate test-retest reliability in the rACC (56). Finally, findings of sex

differences may stem from biology or the social environment and often reflect overlapping distributions of traits (68). Research should disentangle the effects of sex at birth, the social environment, and diverse gender identities on these factors.

Conclusions

The current study is a novel investigation into relationships among 3 key neurocognitive correlates of depression (GABA, reward learning, and stress sensitivity) in both symptomatic and remitted young adults with depression. Contrary to our expectations, we found reduced reward learning at baseline only in participants with remitted, but not current, depression and no group differences in changes in reward learning following stress. Given the mixed nature of these findings, we cannot answer the question of whether baseline or stress-induced reward learning deficits in depression are state or trait.

Nevertheless, our results contribute several important findings to the literature. First, we replicated findings of blunted reward learning after stress (11,19) using a within-subject design and a relatively large sample, thereby adding to the body of literature indicating that stress may alter reward-related behavior. We also found a sex-specific association between GABA and reward learning, with lower rACC GABA being associated with greater reward learning only in female participants. Results challenge previous hypotheses [e.g., (69)] that blunted GABA is associated with anhedonia via impaired reward processing but are consistent with preclinical work indicating an association between cortical GABA and depressive behaviors in female mice (40). Notably, this study is one of the first to translate preclinical results of sex-dependent associations between GABA and depressive behaviors to a human population.

Our results raise a number of questions, including possible sex differences in the relationship between GABA and reward learning. Replication is needed to substantiate these findings, particularly in a larger sample, and to further explore the relationships between GABA, sex, stress, and reward learning in depression. Better understanding the associations among these neurocognitive mechanisms may be an important step toward elucidating the etiology of depression.

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Previous publications utilized data from the current sample and reported results based on separate hypotheses and analyses (44,52,56).

JMD and ADM were responsible for formal analysis. JMD, ADM, MI, KEN, CSZ, SME, SP, CER, RL, and MA were responsible for investigation. JMD, ADM, MI, KEN, CSZ, FD, SME, XC, SP, CER, RL, and MA were responsible for data curation. JMD and ADM were responsible for writing the original draft. JMD, ADM, MI, KEN, LMH, CSZ, FD, SME, XC, SP, CER, RL, MA, MM, JMG, and DAP were responsible for the review and editing of the manuscript. MI was responsible for software. MI, LMH, MM, JMG, and DAP were responsible for supervision. MI, LMH, MM, JMG, and DAP were responsible for project administration. JMG and DAP were responsible for conceptualization, methodology, and funding acquisition.

Preliminary results related to these hypotheses were presented in a poster presentation at the Anxiety and Depression Association of America Annual Conference March 17–20, 2022, Denver, Colorado. The hypotheses and analyses in the current manuscript have not been published previously.

Data from the current study have been uploaded to the National Institute of Mental Health Data Archive (Collection ID 2485) and are available following guidelines from the National Institutes of Health.

Over the past 3 years, DAP has received consulting fees from Boehringer Ingelheim, Compass Pathways, Engrail Therapeutics, Neumora Therapeutics (formerly BlackThorn Therapeutics), Otsuka Pharmaceuticals, Sage Therapeutics, Sama Therapeutics, and Takeda Pharmaceuticals; honoraria from the American Psychological Association, the Psychonomic Society, and Springer (for editorial work) as well as Alkermes; and research funding from the Bird Foundation, Brain and Behavior Research Foundation, Dana Foundation, DARPA, Millennium Pharmaceuticals, the National Institute of Mental Health, and Wellcome Leap. In addition, he has received stock options from Compass Pathways, Engrail Therapeutics, Neumora Therapeutics, and Neuroscience Software; he has a financial interest in Neumora Therapeutics, which has licensed the copyright to the probabilistic reward task through Harvard University. DAP's interests were reviewed and are managed by McLean Hospital and Partners HealthCare in accordance with their conflict of interest policies. JMG is on the scientific advisory board and has an equity interest in Cala Health, a neuromodulation device company. MM has served on the advisory board of Ipsen and AbbVie and served as a consultant for AbbVie and Sanofi. She also receives royalties from UpToDate. There are no conflicts of interest with the work conducted in this study. No funding from these entities was used to support the current work, and all views expressed are solely those of the authors. All other authors reported no biomedical financial interests or potential conflicts of interest.

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SUPPLEMENTARY INFORMATION

Effects of GABA, Sex, and Stress on Reward Learning in Current and Remitted Major Depression

Duda *et al.*

Supplemental Information

Methods

Eligibility Criteria

To be eligible for the major depressive disorder (MDD) group, participants were required to meet criteria for a current depressive episode according to the Structured Clinical Interview for DSM-5 [SCID-5 (1)], have a minimum Beck Depression Inventory-II [BDI-II; (2)] score of 13, and either a Quick Inventory of Depressive Symptomatology [QIDS-C (3)] score above 10 or a Hamilton Depression Rating Scale (4) score above 15. As the SCID-5 assesses symptoms in the past month, these additional score requirements were designed to ensure symptoms persisted at the time of the session. To be eligible for the remitted MDD (rMDD) group, participants were required to meet criteria for one fully-remitted depressive episode lasting at least two months (or two prior episodes lasting at least two weeks) in the past five years, given concerns around possible biases in retrospective reporting. RMDD participants were required to be in remission for at least 8 weeks (i.e., with no anhedonia or low mood and with no more than two sub-threshold symptoms among the other seven diagnostic criteria of depression). On average, rMDD participants' most recent episode had remitted 1.5 years prior (range = 2 months-4.5 years). Healthy control (HC) participants were required to have no history of psychopathology according to the SCID-5. Both rMDD and HC participants were additionally required to have a current BDI-II score of less than or equal to 10, QIDS-C score of less than or equal to 5, and HDRS score of less than or equal to 7. Scores were entered into REDCap by trained research assistants and entries were checked for accuracy by a second research assistant. Diagnostic (SCID) interviews were performed by masters- or PhD-level clinicians, who underwent various calibration processes. An inter-rater reliability analysis for the current study demonstrated high diagnostic agreement on the SCID-5 depression diagnosis (kappa coefficient=0.94), HDRS score (ICC = 0.95), and QIDS score [ICC=0.96; see supplement of (5)].

Participants were excluded for recent recreational substance use aside from marijuana, greater than five lifetime alcohol-related blackouts, or an alcohol use disorder, given the well-established relationship between alcohol and neural GABA levels (6).

Scanning Session

The scanning session with spectroscopy was completed within a month of the initial screening visit. Female participants completed their visit during the follicular phase of their menstrual cycle (1-11 days after cycle onset, $M = 4.5$ days), given known effects of the menstrual cycle on neural GABA levels (7) and stress-induced cortisol secretion (8)].

Spectroscopy

GABA+ datapoints were excluded based on signal-to-noise ratios < 20 and linewidth values > .07 ppm. Two additional participants were excluded for severe baseline distortion based on visual inspection by MR physicists (XC and FD). After quality assurance, we had a sample of $N_{\text{dlPFC}} = 114$ and $N_{\text{rACC}} = 108$.

Initial Stressor

Participants completed the Montreal Imaging Stress Test [MIST (54)] and the Maastricht Acute Stress Task [MAST (55); together "MIST/MAST"] (See **Figure 1** in main text). The MIST involved blocks of arithmetic problems (some timed and some untimed) with real-time

feedback provided about performance. Between the first and second block, participants completed the MAST stressor while lying on the scanner table, in which two experimenters acting as "doctors" asked participants to perform blocks of mental arithmetic (counting backward from a large number in steps of 17) interspersed with placing their hand in ice water. Participants then completed three more blocks of the MIST. Between the third and fourth block of the MIST, a "doctor" told the participant via the intercom that their performance was below average to induce further stress. Affective ratings (see **Affective Ratings** section of main text) and serum cortisol (see **Cortisol** section below) were utilized to assess stress response.

Probabilistic Reward Task (PRT)

Participants completed the 2-block version of the PRT (9), with 100 trials per block. On each trial of the PRT, participants were briefly (150 ms) shown one of two highly similar faces and were asked to identify which stimulus was shown by pressing a corresponding key. To induce a response bias (serving as a measure of reward learning), some correct responses yielded a reward of $0.20 based on an asymmetric reward ratio, with correct identifications of one face (*rich* stimulus) rewarded 3x more frequently than the other face (*lean* stimulus). Participants were informed that only some correct responses would be rewarded but were not told of the asymmetric reward ratio. Participants completed the task twice, before and after undergoing a psychosocial stressor ("PRT Stressor", in which they were falsely informed they would need to redo the earlier hybrid MIST/MAST stressor in the scanner due to inferior performance). To avoid practice effects, separate versions of the task were administered pre vs. post stress onset (counterbalanced across participants): a "mouth" version with two highly similar mouths (11mm vs. 10mm) and a "nose" version with noses of similar lengths (5.3mm vs. 5.0mm). To ensure data quality, trials with response times of less than 150 ms or greater than 2500 ms were excluded from analyses. Runs were excluded if at least one block had 1) >20 invalid trials (e.g., due to outlier response times), 2) fewer than 20 *rich* rewarded trials or six *lean* rewarded trials, or 3) a ratio of rewards between *rich* and *lean* trials of less than 2:1.

As a measure of reward learning, a response bias metric was calculated for each block as:

$$\text{Response bias: } \log b = \frac{1}{2} log \left(\frac{Rich_{correct} * Lean_{incorrect}}{Rich_{incorrect} * Lean_{correct}} \right),$$

with 0.5 added to each "rich" and "lean" parameter in the formula, such that response bias could be computed even when one of the parameters was equal to zero.

Cortisol

The IV was placed at the start of the session at least one hour prior to the beginning of the magnetic resonance spectroscopy scan. A baseline blood draw was collected immediately before the onset of the MIST/MAST stressor (during fMRI scanning). Subsequent draws occurred every 15 minutes following stress onset for 60 minutes, and again at 90 minutes post-stress, with the goal of tracking cortisol's return to baseline (10). The draw occurring 90-minutes post-MIST/MAST stressor was used as the baseline cortisol value for the second stressor (PRT stressor). Blood was then collected every 30 minutes for the 90 minutes following stress reintroduction (again allowing cortisol responses to return to baseline).

A maximum of 35cc of blood was collected at each draw. Samples sat for 30 minutes to allow clotting before centrifuging, and were then aliquoted and stored at -80 °C. Given occasional difficulties maintaining a clear IV line while participants moved throughout their

visit, imputed values were calculated for missing serum cortisol data. These imputed values were based on quadratic mixed effects models that allowed for the estimation of each participant's cortisol levels by time point.

Analysis Plan

First, as a manipulation check, one-sample *t*-tests evaluated whether tension, sadness, and hostility rating AUCis and cortisol AUCis were elevated after the PRT stressor. Second, a linear mixed effects model predicted baseline reward learning by clinical group, sex, and their interactions, controlling for task block (Hypothesis 1). Third, the mixed effects model was re-run to predict reward learning across *both* runs of the task, adding fixed effects of condition (pre or post stress onset) and its interactions with group and sex (Hypothesis 2). Fourth, the mixed-effects model predicting baseline (pre-stress onset) reward learning was re-run, substituting GABA+ (either dlPFC or rACC, run separately) for clinical group (Hypothesis 3). Fifth, the mixed-effects model predicting overall reward learning (pre and post stress onset) was re-run substituting GABA+ (either dlPFC or rACC, run separately, in place of clinical group) and its interaction with condition and sex as predictors (Hypothesis 4).

Results

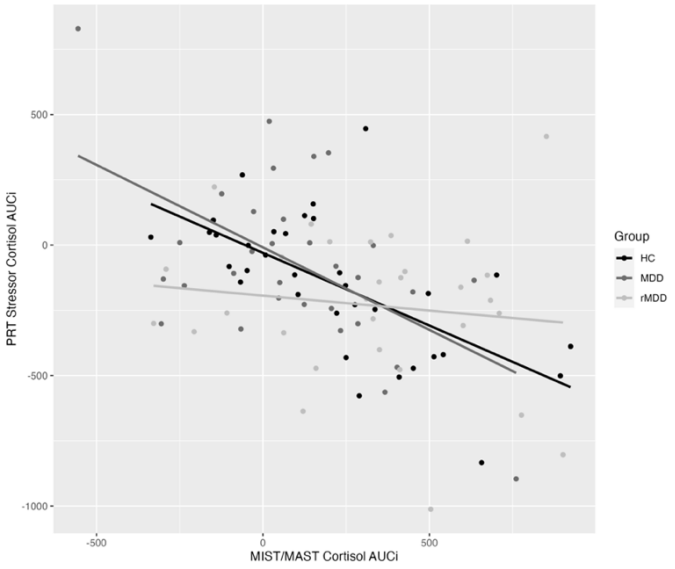
Demographics

The three groups (HC, MDD, and rMDD) did not differ significantly by sex assigned at birth, $X^2(2) = 0.19$, $p = .911$, age, $F(2,127) = 1.40$, $p = .249$, race, two-tailed Fisher's exact test, $p = .404$, or ethnicity, two-tailed Fisher's exact test, $p =$. 809. See **Table 1** in the main text for more information.

Manipulation Checks & Habituation Effect

A regression model predicting cortisol AUCis after the PRT stressor with sex and group was not significant, $F(3,85) = 2.22$, $p = .092$, nor was a model allowing sex by group interactions, $F(5,82) = 1.31$, $p = .270$.

Figure S1. Cortisol Responses to Repeated Stressors



Note. AUCi, Area Under the Curve with respect to increase; HC, Healthy Control; MDD, Major Depressive Disorder; MIST/MAST, Montreal Imaging Stress Task and Maastricht Acute Stress Test; PRT, Probabilistic Reward Task; rMDD, Remitted Major Depressive Disorder. The above graph depicts the significant negative correlation between participants' cortisol responses to the initial stressor (MIST/MAST) and the subsequent stressor (PRT stressor), $t(86) = -4.04$, $p < .001$, with separate lines of best fit by group. There was an interaction such that rMDD participants showed a significantly weaker negative relationship in cortisol response to the two stressors than the other groups, $t(81) = 2.26$, $p = .027$. Thus, those in the MDD and HC groups showed steeper "habituation" than those in the rMDD group. Outliers were removed based on Cook's D values greater than 4/n.

Dimensional Measures of Depressive Symptoms and Neurocognitive Factors

Reward Learning

To specifically investigate possible associations between reward learning and dimensional measures of depression, including current anhedonia, we also ran secondary analyses utilizing the Snaith-Hamilton Pleasure Scale (SHAPS), QIDS, and HDRS total scores in analyses in lieu of diagnostic group. Across diagnostic groups, current anhedonia was not significantly associated with baseline reward learning, $t(103) = 0.83$, $p = .408$, nor was either dimensional measure of overall depression symptoms (QIDS-C and HDRS), ps $> .545$.

Reward Learning Under Stress

None of the dimensional measures of depressive symptoms interacted with condition (pre- versus post-stress) to predict response bias before and after stress (SHAPS, $t(107) = -0.76$, $p = .447$; QIDS, $t(103) = -0.95$, $p = .343$; HDRS, $t(111) = -1.91$, $p = .058$).

GABA

We also tested whether anhedonia was associated with altered GABA+ in the rostral anterior cingulate cortex (rACC) or dorsolateral prefrontal cortex (dlPFC). Anhedonia (SHAPS) did not predict GABA+ in the rACC, $t(96) = -1.21$, $p = .229$, or dlPFC, $t(102) = 0.88$, $p = .380$. The HDRS depression measure was not associated with rACC GABA+, $t(98) = -1.45$, $p = .150$, nor with dlPFC GABA+, $t(104) = 0.10$, $p = .924$. The QIDS depression measure was not associated with GABA+ in the rACC, $t(89) = -1.10$, $p = .274$, or dlPFC, $t(92) = 0.10$, $p = .919$.

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