Supplemental Information

Summary of Published Studies with the Affective Go/No-Go Task

Currently depressed patients have demonstrated *negative biases* on the affective go/no-go (AGNG) task, in the form of slower reaction times for happy than sad targets in two studies (1, 2), and additionally a higher number of omission errors during happy than sad word blocks in one of the studies (2). By contrast, studies in *healthy volunteers* with a negative family history of psychiatric disorders in first-degree relatives have shown a *positive bias*, but the specific results varied depending on the study, with faster reaction times for happy than sad targets reported by Erickson *et al.* (2), and a higher number of errors in response to happy distracter words by Robinson & Sahakian (3).

Three acute tryptophan depletion (ATD) studies using the affective go/no-go have been conducted in healthy volunteers, and one in remitted depressives. While the three studies in *healthy volunteers* found a *reduction in positive bias* during ATD, the studies differed in either the type of dependent measure that showed this reduction, or in participant selection criteria. In the first study, ATD abolished the faster reaction time to happy words seen during placebo in healthy females (4), but this study did not assess family history of psychiatric disorders. In the second study, ATD abolished the higher number of errors in response to happy distracters seen during placebo in healthy females without psychiatric disorders in first-degree relatives (3). In the third study (which included brain imaging), the treatment-by-valence interaction in the analysis of behavioral data fell short of significance ($p = 0.13$); however, planned comparisons showed a significantly higher number of inappropriate responses to happy than to sad distracters during the placebo condition, but not during ATD, in healthy volunteers without mood or anxiety disorders in first-degree relatives (5).

The only published study examining responses on the affective go/no-go in *remitted depressive subjects* had unexpected behavioral findings, in that remitted depressives showed significant *positive biases* in their responses to distracters during both ATD and placebo conditions, a result that did not support the authors' prediction that ATD would be associated with negative emotional biases (6). In this brain imaging study, the authors reported an association between increasing amygdala blood flow and increasing negative bias across both remitted depressives and healthy controls (6).

In summary, published studies using the affective go/no-go task have found evidence of negative bias in currently depressed patients, positive bias in healthy volunteers with a negative family psychiatric history, and abolition of positive bias in healthy volunteers during ATD. The specific dependent measures revealing emotional bias (i.e., omission errors, distracter errors, reaction times), however, have varied across studies. Finally, the only published ATD study in remitted depressives reported positive bias both during ATD and placebo, an unexpected finding. To our knowledge, there are no published studies comparing performance on the affective go/no-go task in healthy volunteers at high and low risk for depression.

Participants

Healthy volunteers were assessed with the Structural Clinical Interview for DSM-IV (SCID), administered by a trained masters-level clinician, and a clinical interview by a psychiatrist. Participants had no personal history of any Axis I psychiatric disorders. Participants also had no current or chronic medical illness (assessed by medical history, and physical and laboratory examinations), and no self-reported lifetime exposure to MDMA (ecstasy). The high-risk (HR) group comprised participants with a positive family

history of MDD, and the low-risk (LR) group comprised participants with a negative family history of any Axis I disorders that have been genetically linked to MDD, e.g., schizophrenia, mood disorders, substance use disorders, and anxiety disorders. A positive family history of MDD in the HR group was defined as at least one first-degree relative (parent or sibling) with recurrent or chronic unipolar MDD, according to DSM-IV criteria, with at least one of the episodes starting or persisting beyond the age of 18. HR participants and, whenever possible, one other family informant (54% of HR cases) were also asked to confirm by history that the affected first-degree relative had been formally diagnosed with MDD by a physician and had been prescribed antidepressant medication.

For most HR participants, age of onset of MDD in the affected relative was < 30 (92%), and MDD was recurrent (92%). For one HR participant, the affected relative had early-onset chronic MDD. For three HR participants, a diagnosis of bipolar disorder (in lieu of unipolar MDD) in the affected first-degree relative could not be ruled out with certainty. A negative family history in the LR group was defined as the absence of any lifetime Axis-I disorders that have been genetically linked to MDD, in all first-degree relatives. Individuals with family histories intermediate between the two groups, for example with a first-degree relative with non-recurrent or non-chronic MDD, were excluded from the study.

Depletion Procedures

Subjects were enrolled into a double-blind, placebo-controlled crossover ATD study, and were randomly assigned to undergo either ATD first and sham depletion second or sham depletion first and ATD second, in a counterbalanced order. To avoid carryover effects, a period of at least 6 days between each test day was established. Each depletion day

was followed by a brief assessment the following morning. We used a modified methodology for ATD and sham depletion (7). This method is equally effective in lowering plasma tryptophan levels and inducing a mood-lowering response (8), with acceptable tolerability (very low incidence of nausea). ATD procedures began at 8:00 AM, by administration of 70 capsules containing an amino acid mixture consisting of L-isoleucine (4.2 g), L-leucine (6.6 g), L-lysine (4.8 g), L-methionine (1.5 g), L-phenylalanine (6.6 g), L-threonine (3.0 g), and L-valine (4.8 g). During sham depletion, at 8:00 AM, participants received 70 capsules containing microcrystalline cellulose (used instead of lactose in order to allow enrollment of participants who might be lactose-intolerant). Participants fasted for at least 8 hours before capsule ingestion and until approximately 4:30 PM on each study day. During the study day, participants stayed awake in a private hospital room, and were allowed to read and watch TV (uncensored content) when they were not participating in assessments or study tasks. After receiving a meal at approximately 4:30 PM, participants were discharged home, and came back for a brief assessment the following morning.

Data from three low-risk participants who completed only one of the two depletion sessions was not included in the analyses: a female participant who dropped out due to her busy schedule; another female participant who dropped out due to needle phobia surfacing during study procedures; and a male participant who was exited from the study due to inconsistent answers on study questionnaires.

Measurement of Plasma Total and Free Tryptophan Concentrations

Plasma total and free tryptophan levels were measured on each study day at baseline, 6 hours, 8 hours and 24 hours after intake of the capsules. Collected blood was immediately centrifuged for 15 minutes at 4°C and 3000 rpm. Plasma was frozen at

-70°C until analyzed. Immediately after thawing, plasma proteins were precipitated by adding 20 µl of 70% perchloric acid to 400 µl plasma, followed by centrifugation for 30 minutes at 20,000 g at 4°C. For detection of total tryptophan, 100 µl of the supernatant were injected into the high performance liquid chromatography (HPLC) system, leaving another 100 µl for a second injection. For detection of free tryptophan, samples were filtered through a 10 kDa Chemicon filter (Millipore, Billerica, MA) before injection. A calibration of the system was performed by an external standard solution of tryptophan, solved in a phosphate buffer solution (PBS) containing 0.5 mg bovine serum albumin (BSA) per ml. The standard solutions contained tryptophan concentrations ranging from 0.31-20.0 µg/ml for total tryptophan and 0.125–10 µg/ml for free tryptophan. For calibration, 10 µl of 70% perchloric acid was added to 200 µl of standard solution, thawed immediately before use and handled in the same way as the plasma samples. The HPLC system consisted of a Waters 2690 Separations Module. The operational isocratic chromatographic conditions for this HPLC system were set as follows: column temperature 25.0°C; flow-rate 1.0 ml/min. The mobile phase consisted of 2.5 g sodium acetate, 100 mg disodium EDTA and 50 mg sodium octyl sulfonate, which were dissolved in 2500 ml deionized water and 150 ml acetonitrile. A pH of 4.50 was reached by the addition of acetic acid to the buffer before acetonitrile was added. This solution was filtered through a 0.47 µm membrane filter and degassed before use. The analytical column was a 250 mm x 4 mm Supersphere 60 RP-select B, packed with C8 (MERCK LiChroCART 250-4, Darmstadt, Germany).

Approximate run time after injection until detection of tryptophan was 10 minutes. A Waters 474 Scanning Fluorescence Detector (l Ex = 300 nm, l Em = 350 nm) was used to detect tryptophan. The amount of a substance was obtained by the ratio of the peak height to the peak height of the calibration curve of the external standards. The

tryptophan recovery evaluated by the amount of spiked vs. non-spiked plasma after extraction was expected to be 90-100%. Results from our previous studies have shown that intra-assay and inter-assay variations were less than 5% (8).

Mood Measure

Mood-lowering response was assessed by self-report with Visual Analogue Scales (VAS). These included two 1-10 scales on which participants circled how "euphoric/happy" and how "sad" they felt at the moment, respectively.

Neurocognitive Tasks

Participants were administered the AGNG and the Tower of London (TOL) tasks. In between these two tasks, participants underwent a functional brain imaging study, the results of which will be reported separately.

AGNG: Participants viewed ten word blocks (including two practice and eight test blocks) on a laptop computer, each displaying nine happy (e.g., joyful, success) and nine sad (e.g., hopeless, failure) words. For each block, happy (H) or (S) words were specified as targets, presented in a HHSSHHSSHH order. During H target blocks, participants were instructed to press the space bar for happy, but not sad words, with the reverse instructions during S blocks. Four test "blocks" are called shift blocks because they introduce a shift in target valence (H-to-S or S-to-H); the other four are "non-shift" blocks because they maintain the same target valence as the previous block.

TOL: Participants were shown pictures of two boards, each depicting an array of three pegs and three colored beads. Participants were instructed to state the minimum number of moves required to match the bead configuration of the second board with the

one depicted on the first board. Difficulty level ranged from one to six moves. Participants were administered levels five to six only if they completed levels one to four without any errors. Totals for difficulty levels four to six were thus collapsed into one total during data analysis. For the TOL task, *n* = 24 due to missing data for one participant.

Group Characteristics

Group characteristics, which did not differ significantly between the groups, are summarized in Table S1.

Table S1. Group Characteristics

	High-risk group (*N* = 13)	**Low-risk group (*N* = 12)**
Female gender, N (%)	10 (76.9)	9 (75.0)
Age, mean (SD)	26.5 (4.5)	25.3 (4.4)
Race/ethnicity, N (%)		
White	11 (84.6)	8 (66.7)
African American	1 (.08)	2 (.17)
Asian	1 (.08)	2 (.17)
IQ[a,b]	120.7 (9.7)	113.5 (10.1)
Baseline HDRS-24 score, mean (SD)	.78 (.95)	.69 (.81)

[a]Based on the WASI matrices and vocabulary sections.
[b]*N* = 22 due to incomplete data.
HDRS, Hamilton Depression Rating Scale; SD, standard deviation; WASI, Wechsler Abbreviated Scale of Intelligence.

Additional Analysis

Mood effects: As described in the main article, there were no significant treatment x time x group interactions on the VAS happy/euphoric or sad scales. There was a significant main effect of depletion on the VAS sad scale [$F(1,23) = 4.77$, $p = .04$], with lower mean score (less sad) during ATD [.22 (standard error = .09)] and higher mean score during placebo [.59 (SE = .18)]. There was also main effect of time (T_0, T_5 and T_8) approaching significance on the VAS happy/euphoric scale [$F(2,22) = 3.08$, $p = .07$], with

highest mean score (happier/more euphoric) at T_0 [6.74 (SE = .34)] and lowest mean score at T_8 [6.15 (SE = .35)]. There were no significant or trend-level treatment-by-risk, treatment-by-time, or time-by-risk interactions.

References

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