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Supporting Online Material for

Serotonin Modulates Behavioral Reactions to Unfairness

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Supporting Online Material

Materials and Methods

The protocol was approved by the Norfolk Research Ethics Committee. Twenty healthy subjects (six males; mean age 25.6) were screened for neurological and psychiatric disorders and gave written informed consent before participating. Participants were financially compensated. Participants attended two sessions, spaced at least one week apart, and were randomized to receive either ATD or placebo on the first session. The UG was administered as part of a larger cognitive assessment (to be reported separately). The ATD procedure was carried out according to an established protocol (*1*, *2*).

Upon arrival (between 0830 and 1000), participants gave a blood sample and ingested either the placebo or ATD drink (75 g). After 5.5 hours (to ensure stable and low tryptophan levels (*3*)), participants gave a second blood sample and completed the cognitive assessment. Mood was assessed using the Positive and Negative Affect Scale (PANAS; (*4*)) at five time points during the session.

To enhance the credibility of the UG task, participants were told that they were part of a large ongoing study in which they would be playing the role of responder with volunteers who had submitted their offers previously. In addition, they were told they would have the opportunity to play the role of proposer with volunteers who would participate in the future, if they would allow their photograph to be taken and used in future sessions, and submit proposals for 12 different stake sizes. Four participants declined having their photograph

taken. In reality, there were no actual proposers, and participants' proposals were not used beyond their function as a cover story. Before the game started, the experimenter required a verbal confirmation that the participant understood the game. Participants were told that they would receive the financial outcomes from two trials that would be randomly selected at the end of the game. During each trial, participants viewed sequentially a photograph of the purported proposer, the amount of the stake (total pie to be shared), and the amount of the offer (Figure 1A). On each session, participants played 48 games, each with a different proposer. There were 16 fair proposals, ranging from 40-50% of the stake; 16 unfair proposals, ranging from 27-33% of the stake; and 16 very unfair proposals, ranging from 18-22% of the stake. Participants received identical offers at each session, but the order of offers was randomized to disguise this. On different trials, the same monetary amount could appear as a large percentage of the total stake and therefore "fair," or as a small percentage of the total stake and therefore "unfair". This design allowed us to observe independent effects of ATD on responding to different levels of fairness versus different levels of monetary reward.

We also sought to replicate previous studies that have found no effects of ATD on basic motor response inhibition (*1,5*). All participants completed a Go/No-go task. Stimuli were 5 x 5 checkerboards composed of random configurations of blue and yellow squares. For half of participants, "Go" stimuli had a majority of blue squares and a minority of yellow squares; "No-go" stimuli had a majority of yellow squares and a minority of blue squares. For the other half of participants, the Go stimuli were yellow-dominant and the No-go stimuli were blue-dominant. During the task, stimuli were presented serially against a

black background, for an average duration of 900ms. Participants were instructed to press a key as quickly as possible in response to Go stimuli, but to avoid responding to No-go stimuli. In total, there were 28 No-go trials and 56 Go trials. Average reaction times for correct Go responses, % correct Go responses (hits), and % incorrect No-go responses (commission errors) were recorded.

Blood samples were analyzed to determine the ratio of tryptophan to other large neutral amino acids (TRP:ΣLNAA), which is strongly associated with the brain tryptophan availability due to non-specific transport of LNAAs across the blood-brain barrier. The ATD procedure resulted in an 85% reduction in the TRP:ΣLNAA ratio, compared to a 25% increase on placebo. This is in line with what has been reported previously (*1, 2*). A repeated-measures ANOVA revealed a significant two-way interaction between treatment and time point, resulting from significant reductions in the TRP:ΣLNAA ratio ($F=45.968$, $P<0.0001$), 5.5h following ATD relative to placebo. None of the participants reported any side effects of ATD, such as nausea or vomiting. Although there was no formal assessment of subjects' blindness to treatment, the experimenter noted during the debriefing whether subjects noticed any differences in how they felt on either study day, and did not find any evidence that subjects were not blind to treatment condition.

Text

Participants were more likely to reject unfair offers (main effect of fairness $F=61.817$, $P<0.0001$). Pairwise comparisons indicated that participants rejected more 20% offers than

30% and 45% offers, and more 30% offers than 45% offers (all *P*'s<0.005, corrected for multiple comparisons). The main effect of offer size (F=2.974, P=0.101), and the fairness by offer size interaction (F=2.633, P=0.109) approached significance. A closer look at the marginal means shows higher rejection rates for low unfair offers (e.g., £1 out of £4), compared to high unfair offers (e.g., £5 out of £20), but no difference in rejection rates between high and low fair offers. The main effect of treatment also approached significance (F=3.510, P=0.077). However, there were no significant interactions between treatment and offer size (F=1.164, P=0.294), or between treatment, offer size, and fairness (F=2.028, P=0.147), indicating that ATD did not affect basic motivation for monetary reward.

Repeated-measures ANOVAs (with treatment as the within-subjects factor) were conducted on rejection rates at each fairness level to assess simple effects. Compared to placebo, following ATD participants rejected a significantly higher proportion of the most unfair (20%) offers (F=7.551, P=0.013). Rejection rates for the 30% offers showed a similar (non-significant) pattern (F=2.333, P=0.143). Rejection rates for the 45% offers did not differ significantly between treatments (F=0.543, P=0.470).

One potential limitation of within-subject designs is the possibility of practice effects that can occur from the first session to the second session. To address this issue, we included treatment order as a between-subjects factor in our model. There was no significant main effect of treatment order on rejection rates (F=0.057, P=0.814). Treatment order did not

interact significantly with fairness (F=0.379, P=0.687), treatment (F=0.151, P=0.703), or fairness X treatment (F=1.558, P=0.225) and entering treatment order into the model did not affect the significant treatment X fairness interaction (F=7.093, P=0.003). We also conducted a separate ANOVA with fairness and session (1 and 2) as within-subjects factors to investigate whether participants adjusted their rejection rates from session 1 to session 2. This analysis showed a significant main effect of fairness (F=61.817, P<0.0001), but no main effect of session (F=0.133, P=0.719) and no significant fairness X session interaction (F=1.179, P=0.318).

To examine whether ATD-induced increases in rejection rates were most pronounced in subjects with the greatest biochemical depletion, the change in TRP:ΣLNAA ratio (ATD - placebo) was correlated with the change in rejection rate of unfair offers (ATD - placebo). There was a modest positive correlation between degree of biochemical depletion and degree of increase in rejecting unfair offers across subjects, but this relationship was not statistically significant (r=0.260, P=0.268).

PANAS scores were analyzed immediately before drink ingestion, and immediately before the UG. A repeated-measures ANOVA with treatment (ATD, placebo) and time point (baseline, +5.5h) found no significant effect of ATD on PANAS ratings at time of testing on positive affect (F=0.820, P=0.377) or negative affect (F=0.137, P=0.716). Entering PANAS ratings as covariates into the repeated-measures ANOVA did not affect the significant treatment X fairness interaction (F=5.754, P=0.007).

We measured response times for each offer and performed a repeated-measures ANOVA on the data, with treatment and fairness as within-subjects variables. There was a significant main effect of fairness on response time (F=4.549, P=0.017). Pair-wise comparisons revealed that participants responded significantly faster to 50% (fair) offers than to 30% (unfair) offers (P=0.011), and tended to respond faster to 45% (fair) offers than to 20% (most unfair) offers (P=0.065). There was no significant main effect of ATD on response times (F=1.058, P=0.317), and no significant interaction between fairness and ATD (F=0.542, P=0.586).

Consistent with previous research (*1,5)* we did not find any effects of ATD on basic response inhibition, as measured by the Go/No-go task. Repeated-measures ANOVAs indicated no significant effect of treatment on the Go reaction time (F=0.710, P=0.410), proportion of hits (F=0.009, P=0.924), and critically, proportion of commission errors (F=0.273, P=0.607).

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