Materials and Methods:

Participants:

Participant recruitment:

Participants were recruited from the University of Sheffield's volunteer database, community groups, and the University's student population using posters, emails and advertisements, as well as websites such as the UK Adult ADHD Network. All recruitment material included a link to the study information sheet and consent form for interested individuals, whereby they completed an online pre-screening survey. Eligible participants were then invited to book a 2.5-hour slot at the University of Sheffield to participate in the trial. Participants were compensated for their time with £30 Amazon vouchers or course credits for undergraduate psychology students.

No prior research is currently available measuring the impact of 5-hydroxytryptophan on distractibility, meaning that we were unable to use pre-existing data to inform power analysis and sample size. A priori power analysis for repeated measures ANOVA were completed using G-power (49), and calculated a participant number of 112 for a medium effect size (two-tailed, alpha=0.05, beta = 0.08, effect size= 0.3). Recruitment stopped when we arrived at 112 participants with usable data. An interim analysis was produced at 105 participants in preparation for the presentation of the data at a conference.

Inclusion criteria:

Participants between the ages of 18 and 65 were selected; the lower bound ensured only adults were assessed, and the upper bound to include a range of adults within working age in the UK, whilst maintaining a low chance of participants experiencing mild cognitive impairment (50). Control participants were selected based on a score of 1 or less on the Adult ADHD self-report screener (ASRS v1.1, items 1-6) (51), whereas participants in the high ADHD group were selected based on a score of 4 or greater, the score considered to be highly consistent with ADHD. To widen participation criteria to nonclinical presentations of ADHD, participants were not required to have a clinical diagnosis of ADHD to participate in the high group.

Exclusion criteria:

Participants were excluded following pre-screening if they were taking medication for the treatment of ADHD to allow us to assess the effect of 5-hydroxytryptophan without the interference of psychostimulants. Individuals who used other serotonin-altering medications, such as selective serotonin reuptake inhibitors, were also excluded. Smokers or vapers were excluded due to the considerable psychoactive effects of nicotine. Other exclusion criteria included pregnant and breastfeeding individuals, and individuals with lactose intolerance or a dairy-free diet, due to the placebo tablets containing lactose. Individuals with dyslexia were also excluded from participating to

minimise extraneous factors which may impact reaction time and accuracy on letter-presenting tasks.

Trial design:

The study consisted of a randomised, double-blind, parallel group trial in a 2 (group: high ADHD traits vs low ADHD traits) x 2 (intervention: placebo or 5-hydroxytryptophan) by 2 (time: pre and post-administration) design. Participants were stratified into two groups of high and low levels of ADHD traits using the ASRS, and assignment to either the acute, high-dose 5-hydroxytryptophan administration group or placebo group was randomised in a 1:1 allocation ratio. The trial primary outcome was a change in distractibility, measured by recording of mean reaction time, the standard deviation of reaction time, accuracy and the percentage of false positives in two distractor tasks. Analysis centred on observing differences between these values pre- and 90 minutes post administration. Public involvement extended only to participation in the trial.

The study protocol and analysis plan were preregistered on the Open Science Framework (OSF) on 20/05/2024 (https://doi.org/10.17605/OSF.IO/H6KET). No changes were made to the preregistered protocol, but some exploratory analyses were conducted on the data to better understand the sample (detailed in the exploratory analyses section). De-identified participant data and experiment code can be accessed at the University of Sheffield Online Research Data Repository (10.15131/shef.data.29183702). Reporting aligns with CONSORT 2025 guidelines (52).

Ethics and harms:

The study was approved by the University Research Ethics at the University of Sheffield on the 4th of March 2024 (RN 058170). All participants had access to the information sheet and online consent form. Consent was taken twice; first for the collection of pre-screen data, and again before participation in the trial. Written consent was collected electronically. Participants were recruited between May 2024 and February 2025. No follow-up was conducted after the in-person session.

Harms were detailed and assessed in a risk assessment prior to initiation of the trial. 5-hydroxytryptophan has been widely established as safe for human consumption, with some common side effects. As such, side effects were assessed non-systematically by collating reports from participants throughout the trial.

Materials and Measures:

Online pre-screen and ASRS v1.1:

Potential participants were required to complete an online screening survey to assess whether the individual met the inclusion criteria. In the interest of brevity, participants were asked for minimal demographic information of age and sex assigned at birth. Participants were then asked screening questions based on the inclusion criteria. If

answers indicated exclusion, participants were screened out of the survey. Participants were also asked to indicate use of psychostimulants or serotonergic-intervening medications and if they had previously received a diagnosis of ADHD, autism, dyslexia or a mental health condition.

ADHD-like traits were assessed in the pre-screen using the 6-question ASRS v1.1 screener, a well-validated clinical tool used in screening for ADHD (51). Participants answered questions regarding their behaviour over the preceding 6 months on a Likert rating scale with the answers never, rarely, sometimes, often or very often. Questions 1-3 score 1 point for answers of sometimes, often or very often; any other answer is given 0 points. The remaining questions score 1 point for often or very often and 0 points for other answers. A score of 4 or more is considered a score highly consistent with an ADHD diagnosis. Therefore, the high ADHD traits group was defined as participants with a score of 4 or more. Reports of ASRS scores in the USA have previously found a mean score of 2.0. Therefore, participants with a score of 1 or less were determined as the low ADHD traits group (53).

All pre-screener respondents also completed the extended ASRS (Kessler et al., 2005), a further 12 questions, which, when coupled with the screener, give 9 questions on hyperactive-impulsive traits and 9 questions on inattentive traits. Questions 9,12,16, and 18 were scored in the same manner as questions 1-3, with all other questions scored with 1 point for answers of often or very often and 0 points for answers of never, rarely or sometimes. Answers were used to characterise the sample and better understand the presentation of ADHD subtypes within the high ASRS group. ADHD subtype was defined based on ASRS score and its correlation to the DSM-5, whereby individuals need a score of 5 or more in inattentive and hyperactive domains for a combined diagnosis, and a score greater than 5 in either the inattentive or hyperactive domains for an inattentive or hyperactive diagnosis, respectively (American Psychiatric Association, 2013).

The lead researcher reviewed pre-screen responses within 5 days of completion, and respondents were contacted via email. Eligible participants were then invited to book a slot for laboratory testing.

5-hydroxytryptophan and placebo preparations:

Participants in the intervention group were given a 200mg dose of 5-hydroxytryptophan via oral administration, based on a recent study looking at acute 5-hydroxytryptophan administration and social cognition (54). 5-hydroxytryptophan was obtained from Nature's Best supplements in a tablet containing 3982mg of Griffonia seed extract, providing 100mg of 5-hydroxytryptophan, along with calcium carbonate, anti-caking agents (silicon dioxide, stearic acid and magnesium stearate), and tablet coating (hydroxypropyl methylcellulose, glycerol). Participants were given 2 tablets with water.

Placebo tablets were sucrose-lactose tablets obtained from Ainsworth's homoeopathic remedies. Again, participants were given two tablets with water.

Task- relevant distractor: Flanker Task

Participants completed an adapted version of the Eriksen flanker test (sets of chevrons were presented rather than the letter sets presented in the original task) (55), considered a reliable test of response to task-relevant distractors. There is evidence that ADHD individuals experience increased reaction time and reduced accuracy on such tests compared to a typically developed population, and that ADHD symptom remission is linked to improved performance on the flanker test (56,57).

The flanker task was built and delivered using Psychopy 2023 2.3 (58). Participants were presented with a series of 5 chevrons and asked to report the direction of the middle chevron using a key press (Z for left, M for right). Chevrons were displayed in either congruent (>>>>>, <<<<<) or incongruent (<<><<, >><>>) displays. The chevrons were presented for 200 ms, and following participant response, a fixation cross was presented for 1000 ms prior to another trial. Participants completed a 20-trial practice block prior to the test phase. The testing phase consisted of 10, 40-trial blocks, each presenting each combination of chevrons 10 times in a random order, resulting in 400 trials with 100 presentations of each condition. Participants were given a minimum 30s break between each block, with the task taking around 15 mins to complete. Participants' performance was assessed with several different measures, using mean reaction time, standard deviation of reaction time, and accuracy as a percentage for congruent and incongruent trials.

Task- irrelevant distractor: N-back task:

Participants also completed a task-irrelevant distractor task. Studies looking at task-irrelevant distraction have observed inflated scores in reaction time in individuals with ADHD or increased ADHD symptoms (59,60). Similar changes in response occur in oddball paradigm tasks, where novel auditory stimuli are presented (61,62). As a result, we incorporated novel auditory stimuli as task-irrelevant distractors into a version of the N-back test. This decision to use an N-back test was based on evidence that individuals with ADHD have poorer performance on the N-back test compared to controls and that ADHD medications ameliorate the observed deficits. (63).

Participants were asked to focus on a fixation cross for 600 ms and then were presented with a letter (H, K, N, W and Z, based on the letters from the Eriksen flanker for their similarity) ((B. Eriksen & Eriksen, 1974). Letters were presented for 400 ms, and a further 600 ms elapsed before the next trial began, giving a 1000-ms window for reaction times. Participants were asked to recall if the letter they were presented with matched the letter presented two trials ago, and to answer in the affirmative by pressing the spacebar (see figure 1). On 25% of trials, a 200 ms, 85 dB noise was presented simultaneously with a

trial letter. Sounds were assigned using stratified randomisation, assigning sound to cues, target and neutral letters in a 1:1:3 ratio, meaning that a sound played on 25% of each condition. The study consisted of a 25-trial practice block, one block without sound, and 4 blocks with auditory stimuli. Presentation of the auditory and silent blocks was counterbalanced between participants in a 1:1 ratio. Each block lasted approximately 2 min 40 s, with a minimum 30 s break between blocks. The task took around 16 min to complete. Participants' performance was measured using reaction time, standard deviation of reaction time, and accuracy as a percentage of targets hit and as a percentage of false positive responses.

Fig 1: Stimuli used in the flanker and N-back tasks: a) the 4 stimuli, congruent and incongruent, displayed in the flanker task. b) Schematic of stimulus presentation in the n-back task. W forms a cue and a target response, and auditory stimuli are present on non-cue, non-target letters.

Randomisation:

Multiple components were randomised throughout the recruitment and testing phases of the trial. Upon completion of the pre-screen survey, participants were assigned a participant number that would be used to identify their data for the rest of the study. Using a random number generator, randomisation was stratified by group (high ADHD traits and low ADHD traits) with a 1:1 allocation to intervention and placebo conditions, recorded as A and B. Task order and block presentation in the n-back task were counterbalanced following the same procedure. Randomisation was completed and implemented by the lead investigator. All randomisation was produced using the web applications available at http://random.org.

To blind investigators enrolling, assigning and delivering the protocol, an assistant extraneous to the trial placed doses of placebo and 5-hydroxytryptophan tablets in A or B envelopes. The contents of A and B were then written down and sealed in an envelope to be opened at the completion of the trial. Participants were also blind as to what intervention they received. Interventions were both tablets in the same quantity, with 5-hydroxytryptophan tablets being slightly larger and darker in colour than the placebo tablets; thus, they were concealed from the investigator with the use of envelopes.

Procedure:

Following engagement with recruitment, individuals were required to complete the pre-screening survey, providing information on demographics and ADHD symptoms. Responses were reviewed, and eligible participants were contacted by email with a link to book a 2.5-h slot at the University of Sheffield for the trial. Participants were contacted 3 days prior to the session with further details and a reminder. Participants were asked at

this time to complete a food diary for the 48 h preceding the test, logging food, alcohol and medication consumption. Participants were also asked to refrain from consuming alcohol, nicotine or caffeine on the day of their appointment and to refrain from eating for two hours before their appointment time.

Upon arrival, participants were given an information sheet and asked to complete a consent form and were reminded that they may withdraw consent at any point during the study. Participants were seated in a quiet room with a monitor and a keyboard, with an investigator present. Tasks were presented and explained to participants by the investigator and presented in their previously assigned, randomised order. Baseline tasks took around 35 min to complete.

Following completion of the initial tasks, participants were given an envelope containing either 5-hydroxytryptophan or placebo and a glass of water and were observed swallowing the tablets. Participants were then shown to a separate room where they were allowed to participate in a self-chosen leisure activity whilst they waited. The waiting period was 90 ± 5 minutes, based on previous pharmacokinetic reports of 5-hydroxytryptophan levels in serum and cerebrospinal fluid (64).

Once the 90 min wait period was completed, participants returned to the quiet room and completed the two tasks again in the exact manner as in the baseline test phase. Upon completion, participants were debriefed and provided with researcher contact details in the event they wished to follow up. The procedure took approximately 2 h and 40 min to complete per participant.

Data preparation and Statistical analysis:

Individual data was prepared by removal of reaction times outside of the range 200-3000 ms for the flanker task and reaction times under 200 ms for the N-back task based on mean population values of simple human reaction time, and boundaries suggested by the NIH toolkit for flanker analysis (65,66). Values that fell outside of ±3 standard deviations of the individual's mean were also removed, following recommendations for reducing outliers in reaction time data (67). All participants maintained at least 95% of data for each task. No missing data occurred in the trial. Mean and standard deviation of reaction time values were calculated, as well as accuracy as a percentage of correct hits. The percentage of false positives was also calculated for the N-back task.

All analyses were completed using IBM SPSS Statistics (Version 29) and, unless otherwise specified, included all participants and were performed with a two-tailed significance value of 5%. Data was checked for normality using histograms, measures of skew and Kurtosis and the Kolmogorov-Smirnov normality test. Significant outliers were noted in all measures of the flanker task, the standard deviations of reaction time in the N-back task, and the false positives in the N-back task. As such, these values were winsored prior to analysis. Due to the abnormality of the distribution, Box-Cox

transformation was used to normalise all measures of the flanker task and the standard deviation of reaction time on silent trials and of false positives in the N-back task. This normalisation still resulted in a non-normal distribution of congruent accuracy in the flanker task, but given the robustness of ANOVAs, parametric testing was still used in the analysis(68). Descriptives in the form of means, standard deviations (SD) and percentages were used to characterise the data.

Data analysis sought to answer three key questions: did the N-back and Flanker tasks manipulate distractibility, did the two tasks provide discrimination between individuals with high and low ADHD traits, and finally, did 5-hydroxytryptophan supplementation affect performance on the tasks. This was assessed by looking at differences in reaction time, individual variance of reaction time (standard deviation), and task accuracy. The N-back additionally looked at the rate of false positive hits as a metric of distractibility.

Test efficacy was assessed using paired t-tests between control (silent [N-back] or congruent [flanker] trials) and distractor (auditory stimulus [N-back] or incongruent [flanker] trials) condition measures at the pre-administration timepoint. To compare task performance between ASRS groups, a MANOVA was used for the variables of the flanker task and the variables of the N-back task. A 2x2 split-plot ANOVA was used to assess differences between the ASRS group and distractor/no distractor conditions, and independent t-tests to look at differences between groups on each measure. Finally, Intervention outcomes were first assessed with a 2x2 ANOVA (intervention x timepoint) to identify effects of the 5-hydroxytryptophan regardless of ASRS group, and then further assessed using a 2 x (Group: High ADHD traits, low ADHD traits) 2 x (Intervention: 5-hydroxytryptophan, placebo) 2 x (time: pre and 90 min post) ANOVA. An ANCOVA with age as a covariate was also performed.