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# Do common antibiotic treatments influence emotional processing?

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## ABSTRACT

Antibiotics are among the most commonly prescribed medications worldwide, yet research in recent years has revealed the detrimental effect they can have on the human microbiome, with implications for health. The community of microorganisms inhabiting the gut has been shown to regulate physiological and neural processes. Since studies in both humans and animal models have revealed that the gut microbiome can affect the brain, influencing emotion and cognition, here we investigate whether antibiotic treatment is associated with changes in emotional processing and mood with a between-subject design in 105 young healthy adult volunteers, using both psychological tests and questionnaires. As both the immune system and vagal signalling can mediate the microbiome–gut–brain axis, we also assess whether there is any evidence of such changes in participant physiology. We find that individuals who have taken antibiotics in the past three months show a stronger emotional bias towards sadness and at a physiological level they have a higher heart rate (though this does not mediate the relationship with negative bias). While we cannot rule out a possible role of prior infection, our findings are in any case highly relevant in light of research revealing that antibiotics are linked to increased susceptibility to depression and anxiety. Our results also have implications for listing antibiotic use as an exclusion criterion in studies on emotional processing and psychophysiology.

## 1. Introduction

Antibiotics are among the most commonly prescribed medicines worldwide and in the UK the average person is estimated to take 70 antibiotic courses during their lifetime [1]. While antibiotics are important for treating bacterial infections, they are too often prescribed unnecessarily, resulting in overuse and misuse of antibiotics [2,3]. Not only is this contributing to the ever-growing issue of antibiotic resistance but it is also a concern with respect to the detrimental effect that antibiotics can have on the gut microbiome (the community of microorganisms living in the gut) since the majority of antibiotics are not very specific and kill beneficial bacteria as well as the target pathogenic bacteria. Recent research in the gut microbiome field has revealed the negative impact that antibiotics can have on both physical and mental health [4–6].

It is an interesting observation that antibiotic treatment in humans is associated with an increased risk of psychological disorders such as depression and anxiety compared to treatment with other antimicrobials like antifungals and antivirals [5,7]. In addition, longitudinal research has revealed that antibiotic exposure during early life is linked to a higher likelihood in childhood of mood and anxiety disorders, as well as

behavioural difficulties [8,9]. While such studies cannot directly inform on mechanism, it has been hypothesised that the impact that antibiotics have on the gut microbiome may increase the risk of suffering from psychological symptoms via the microbiome–gut–brain axis. Studies in rodents have shown that antibiotic exposure can alter neurochemical signalling and cause cognitive deficits, impairments in social behaviour and depressive-like behaviours [10–19]. Indeed, research the past decade has revealed that the microbial environment within the gut interacts with the host's central nervous system, influencing emotion, cognition and behaviour [20–22]. There are various ways in which disruption to the gut microbiome caused by antibiotics may affect brain function, including through alterations in neuronal gene expression [10, 23] and neurotransmitter levels [24], changes in vagal signalling and through its impact on the immune system, including potentially influencing neurogenesis [25].

In animals, the gut microbiome has been found to alter emotional behaviour and its underlying neurochemical pathways, while there is a growing number of human studies linking the microbiome to emotion and personality [10,11,26]. For example, neuroimaging studies have revealed that consumption of probiotics in healthy volunteers can alter neural signatures of emotion [27,28]. Similarly, prebiotic supplements

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(selectively fermented indigestible carbohydrates such as fructo- and galacto-oligosaccharides which promote the growth and/or activity of certain gut bacteria) have also been found to affect emotional processing, decreasing attentional bias towards negative information [29].

In view of the increasing evidence that the gut microbiome can affect human emotion and the fact that antibiotic treatment significantly alters the gut microbial community for several months [26,30], here we investigate for the first time whether recent antibiotic exposure influences emotional processing using a range of psychological tests. While studies in the field of human psychology and behaviour frequently list numerous exclusion criteria in an attempt to mitigate against extraneous effects, recent antibiotic treatment is not normally taken into account. In contrast, antibiotic exposure (typically in the past three or six months) is used as an exclusion criterion in nearly all research studies on the microbiome–gut–brain axis in humans given the evidence that perturbing the microbial community can impact psychological processes. Various mechanisms mediate the interaction between the gut microbiome and the brain, including communication via neural signalling, most notably the vagus nerve, and the immune and endocrine systems [20,31,32]. In this study we therefore also aim to assess potential physiological changes in both the immune system by measuring salivary immunoglobulin A (IgA) levels [33] and in heart rate and its variability (the beat-to-beat variation in heartbeat intervals) as this can be used to assess vagal tone [34].

## 2. Materials and methods

### 2.1. Participants

Two groups of participants were recruited: the control group that had not used antibiotics in the past year and the second group that had taken antibiotics in the past three months. The intention was to include approximately 120 individuals (60 in each group) in order to be able to detect differences between group means, as estimated using a power analysis (parameters: power = 0.8, Cohen's  $d$  = 0.5,  $\alpha$  = 0.05). Due to the pandemic curtailing recruitment and testing, a total of 109 participants (60 in the control group and 49 in the antibiotic group) gave consent and participated in the current study. Participants were required to meet the following criteria: aged between 16 and 35 years old; proficiency in the English language; no recent changes in diet; no use of probiotic supplements in the past three months; no self-reported excessive (>25 units per week) alcohol use; no use of soft or hard drugs in the past month; not pregnant; no gastrointestinal disease; no cardiovascular or respiratory disease; no use of any psychoactive medication; no mental or physical disability that could hinder participation; no neurological or psychiatric disorders or history of such disorders; and no claustrophobia.

### 2.2. Procedure and design

The current study followed a single session, between-subject design (control versus antibiotics). All participants received written and verbal information about the study procedure and signed informed consent before assessment. Upon arrival to the behavioural laboratory, participants filled out a form to confirm inclusion criteria and collect demographic information. Height and weight were measured (using an OMRON Karada Scan) to calculate body mass index (BMI). Participants then answered questions regarding antibiotic use, including how long ago they had taken the antibiotics, the duration of the treatment, the reason for taking the antibiotics and the type of antibiotic.

Participants sat in a soundproof room, were asked to turn off all mobile and Bluetooth devices, and to apply a Polar chest belt used to collect heart rate data. They were then asked to remain seated and answer, in the following order, a number of questionnaires using Qualtrics online survey software: the Bristol Stool Scale [35]; the Positive and Negative Affect Scale (PANAS [36]); the Difficulty in Emotion

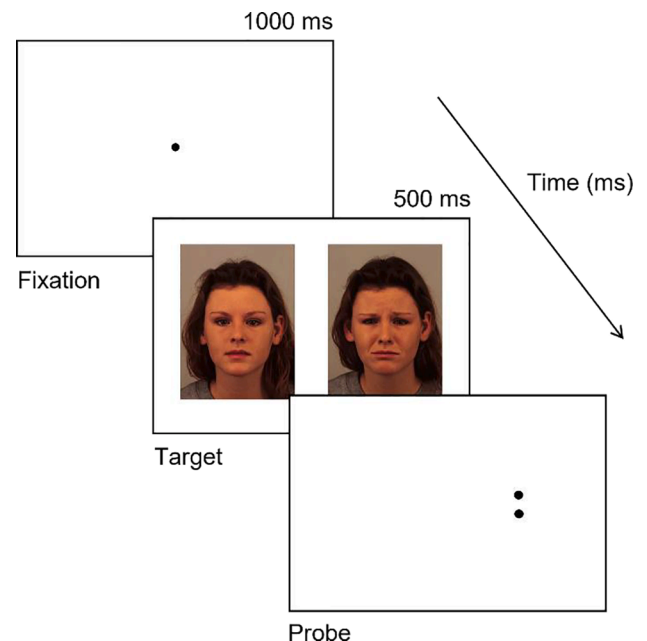
Regulation Scale (DERS-18 [37]); the Empathy Quotient [38]; the Leiden Index of Depression Sensitivity – Revised (LEIDS-R [39]); and the Bermond–Vorst Alexithymia Questionnaire (BVAQ [40]). For more details on each questionnaire, please see Supplementary material. Subsequently, participants were instructed to place both feet on the ground and relax for five minutes while resting-state heart activity was recorded. Next, participants removed the Polar chest belt and performed the first task from the emotional test battery [41] called the emotional categorization task (ECAT). This was followed by the scrambled sentences task (SST) and the emotional dot-probe task. Participants then performed the remaining tasks from the emotional test battery, namely the emotional recall task (EREC) and the emotional recognition memory task (EMEM). Upon completion of these tasks, participants were asked to provide a saliva sample by collecting 2 ml of their saliva within a time period of five minutes.

These procedures followed the ethical standards of the 1975 Declaration of Helsinki [42] and subsequent amendments, and were approved by the local ethics committee (CEP19-0225130, Psychology Research Ethics Committee, Institute of Psychology, Leiden University).

### 2.3. Psychological tasks

E-Prime 2.0 software (Psychology Software Tools, Inc., Pittsburgh, PA) was used to programme the tasks, present stimuli and collect participants' responses.

The emotional dot-probe task (Fig. 1) was used to assess selective attention for emotions (emotional bias). Participants are shown two faces presented concurrently for 500 ms; a face expressing an emotion and a face with a neutral expression. Immediately after the pictures disappear, the probe (which is depicted as two dots) re-appears at one of the locations and participants are asked to click the probe as quickly as possible. The experimental task consisted of 10 practice trials and 120 test trials. Test trials comprised stills of peak emotional expressions



**Fig. 1.** Schematic of the emotional dot-probe task. The task presents two faces simultaneously (one with an emotional expression and the other a neutral expression), followed by a probe in place of one of the faces. The reaction time to click the probe is taken as a measure of attention. In congruent trials, the probe appears at the position where the emotional face was previously shown and for incongruent trials the probe appears at the position where the neutral face was previously shown. Reaction times from the trials can be used to assess the degree to which participants are biased towards emotional expressions (sadness, fear, anger, happiness and surprise) versus neutral ones.

of eleven male and nine female actors from the Amsterdam Dynamic Facial Expressions Set [43] depicting six expressions (sadness, fear, anger, happiness, surprise and neutral). Trials were counterbalanced regarding location of the emotional picture (left or right) and location of the probe (congruent with the emotional picture or incongruent with the emotional picture). The outcome variable of this task is an emotional bias score calculated for each emotion using the following formula:  $RT_{neutralprobe} - RT_{emotionalprobe}$ . Although no response deadline is defined, responses faster than 200 ms or slower than 2000 ms were filtered out as is common practice [44,45].

The scrambled sentences task (SST) was originally developed to identify increased vulnerability for a depressive disorder [46]. The task includes one practice trial and 20 test trials in which participants are shown six scrambled words. Participants are instructed to create a sentence by choosing five out of six words, with each trial containing two target words. For example, the words “looks”, “the”, “future”, “bright”, “very” and “dark” can become “the future looks very bright” or “the future looks very dark”. For the duration of the task, participants were asked to remember a six-digit number since cognitive load can help minimise deliberate response bias. Based on the number of positive versus negative sentences constructed, this task allows assessment of an individual’s positive or negative cognitive bias [47].

The ECAT consists of 40 trials; during each trial participants are shown an adjective selected from Anderson [48] that is either positive or negative. Participants are instructed to indicate whether they would like to be described using this adjective by clicking ‘agreeable’ or ‘disagreeable’. The EREC is an unannounced recall task during which participants are asked to write down as many words as they are able to remember from the previously finished ECAT. This task is designed to measure an emotional recall bias which is reflected in the proportion of positive words that participants recall. The outcome measures are the number of correctly remembered positive words, the number of correctly remembered negative words, the number of incorrectly remembered positive words and the number of incorrectly remembered negative words.

The EMEM is designed to measure emotional recognition bias. During this task participants are shown 80 words (one per trial) and instructed to indicate whether this word had appeared in the previously completed ECAT. Forty of these words had been shown during the ECAT whereas the other 40 words are distractors. This task therefore measures the number of positive and negative hits (i.e. correct recognition of positive and negative words from the ECAT), the number of positive and negative correct rejections (i.e. rejection of words that were not shown before during ECAT), the number of positive and negative false alarms (i.e. words not shown during ECAT but participant reports to recognise them) and the number of positive and negative misses (i.e. words shown during ECAT but participant does not recognise them).

#### 2.4. Physiological measures

Heart rate and interbeat intervals were recorded over a timespan of approximately five minutes using a wireless H7 Polar heart rate sensor (Polar Electro, Finland, for validity see reference [49]) in conjunction with the Elite HRV application (Elite HRV version 4.7.0). Raw data were extracted from the app as individual text files and processed using Kubios [50] to obtain measures of heart rate (mean, minimum and maximum) and variability [51]. The standard heart rate variability measures assessed were RMSSD (root mean square of successive interbeat interval differences), SDNN (standard deviation of interbeat intervals) and pNN50 (percentage of successive interbeat intervals that differ by more than 50 ms).

Secretory immunoglobulin A (IgA) levels were extracted from a 2 ml saliva sample provided by participants. Samples were analysed using the secretory IgA enzyme-linked immunosorbent assay (ELISA) to determine the IgA concentration, a widely used and proven method for analysing the amount of secretory IgA.

#### 2.5. Statistical analyses

All statistical analyses were conducted using R 3.2.3 software [52] and reported according to the journal guidelines using ‘new statistics’ rather than null-hypothesis significance testing [53]. The majority of analyses determined the mean difference between groups and the 95% confidence interval of that difference. Where there was a clear difference between the antibiotic group and control group, multiple regression was conducted within the antibiotic group to determine whether the recency of treatment, duration of treatment or type of antibiotic influenced the variable of interest (ensuring the assumptions of normality and homogeneity of variance were met). To assess the relationship between the psychological and physiological variables measured in the study, an intercorrelation analysis was performed using pairwise Kendall’s Tau-b correlation.

### 3. Results

#### 3.1. Participant characteristics

Three participants who had been recruited as part of the antibiotic group were not able to be included since the full information they provided during participation revealed that they had been treated with alternative medication to antibiotics and a further participant was also excluded since they had taken their antibiotic course over three months prior to the testing date. This resulted in 45 participants in the antibiotic group and 60 in the control group considered for the analyses (Table 1). There were no apparent differences in basic characteristics of the participants recruited for the two groups. For the antibiotic group, the average time since the antibiotic course was 31.26 days (sd ± 25.12 days) and the average duration was 10.11 days (sd ± 12.95 days). For a list of antibiotics taken, please see Table S1.

#### 3.2. Psychological tasks

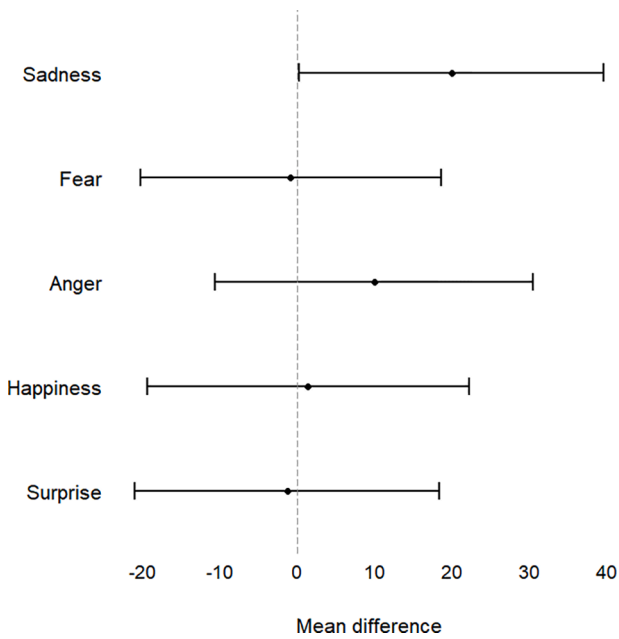
One participant from the antibiotic group did not complete the dot-probe task. Out of the five emotions, the largest and most notable difference between the means was for the emotional bias towards sadness where the antibiotic group showed a greater bias by 19.91 ms, 95% CI [0.26, 39.56] (Fig. 2). For the summary statistics of participants’ reaction times in the dot-probe task, please see Table S2.

Since the antibiotic group displayed a greater bias towards faces expressing sadness than the control group, multiple regression was conducted with the predictor variables as days since treatment, duration of treatment and antibiotic class (broad versus narrow spectrum). The estimated regression coefficients ( $df = 34$ ) were -0.52, 95% CI [-1.17, 0.14], -0.80, 95% CI [-1.97, 0.37] and 24.94, 95% CI [-7.40, 57.27] respectively (for the latter coefficient the reference level is narrow-spectrum antibiotics and so a positive value indicates increased bias with broad-spectrum antibiotics).

There was no evidence in the antibiotic group of a negative or positive bias in the SST, nor the majority of variables measured in the emotional test battery (Fig. 3). In the EREC, the antibiotic group showed a higher percentage of negative false alarms by 9.21%, 95% CI [-3.87, 22.29], though the confidence intervals for the mean difference overlapped with 0. For the summary statistics of participants’ responses to

**Table 1**  
Participant characteristics. Mean values are given with standard deviations in parentheses.

	Antibiotic group	Control group
Sample size	45 [8M, 37F]	60 [14M, 46F]
Age	22.1 (3.1)	22.3 (2.0)
BMI	24.2 (5.0)	24.7 (4.5)
English proficiency	8.6 (1.4)	8.7 (1.0)



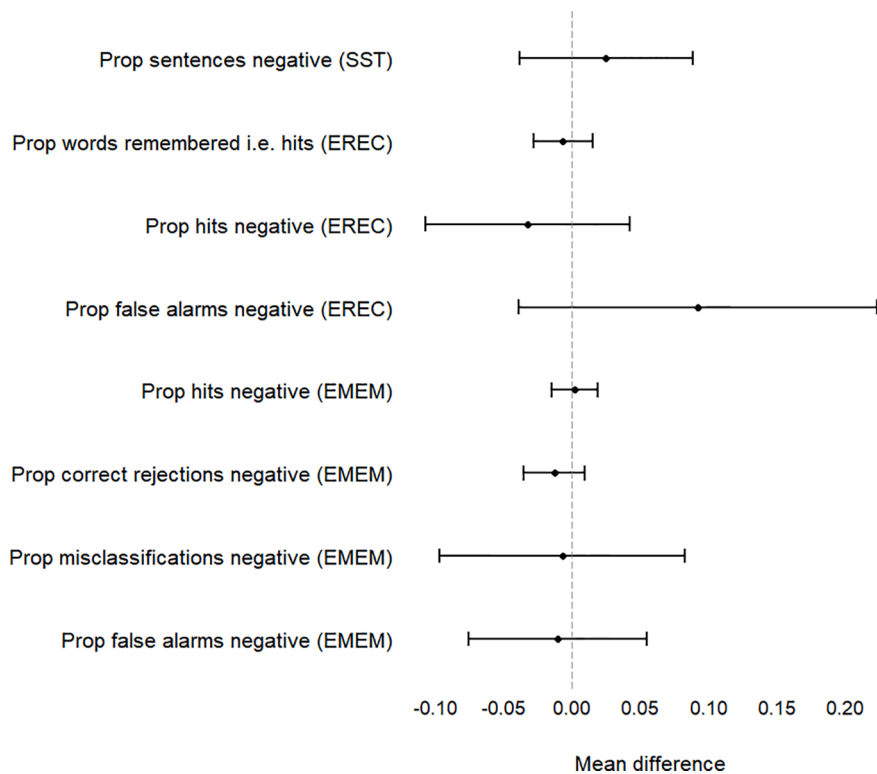
**Fig. 2.** Mean difference in emotional bias (ms) between antibiotic and control group (with 95% confidence intervals) for the dot-probe task. Difference represents the bias for the control group subtracted from the bias for the antibiotic group, such that a positive mean difference indicates a higher value in the antibiotic group.

this task battery, please see Table S3.

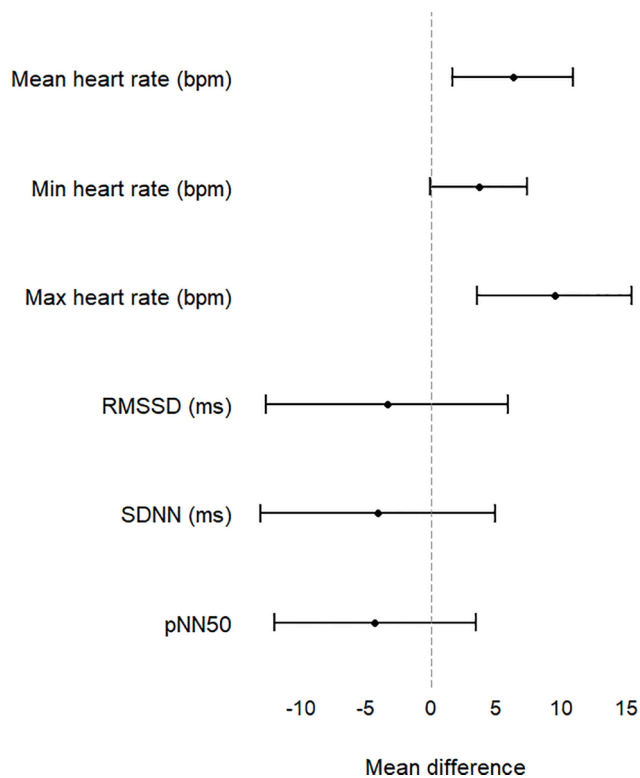
### 3.3. Physiological measures

Heart rate measurements for one participant were not obtained due to connection issues with the heart rate monitor and data for a further 23 participants (10 in the control group, 13 in the antibiotic group) could not be included in the analyses due to unreliable heart rate data obtained (see Table S4 for exclusion reasons). The antibiotic group displayed a higher heart rate than the control group, most notably the mean heart rate which was 6.27 bpm higher, 95% CI [1.62, 10.92] and the maximum heart rate which was 9.45 bpm higher, 95% CI [3.52, 15.37] (Fig. 4). In contrast, there was little evidence of differences between the two groups for the measures of heart rate variability, namely RMSSD, SDNN and pNN50. In terms of gastrointestinal transit, there was no evidence of differences in the Bristol Stool Scale (95% CI [-0.32, 0.49]) or defecation frequency (95% CI [-0.47, 0.13]) between the two groups.

Since the antibiotic group showed a higher mean and maximum heart rate than the control group, multiple regression was conducted with the predictor variables as days since treatment, duration of treatment and antibiotic class (broad versus narrow spectrum), but there was no evidence any of these antibiotic characteristics were associated with heart rate. The estimated regression coefficients ( $df = 25$ ) predicting mean heart rate were -0.03, 95% CI [-0.21, 0.14], 0.10, 95% CI [-0.17, 0.37] and -3.06, 95% CI [-11.85, 5.73] respectively (for the latter coefficient the reference level is narrow-spectrum antibiotics) and predicting maximum heart rate were -0.04, 95% CI [-0.31, 0.22], 0.03, 95% CI [-0.39, 0.45] and -0.48, 95% CI [-14.02, 13.06] respectively. A further regression analysis was conducted to check that the higher heart rate in the antibiotic group did not underlie their increased emotional bias. The regression coefficients ( $df = 77$ ) predicting bias towards sadness were 23.63, 95% CI [0.63, 46.63] for treatment (antibiotic group versus the control group as the reference level) and -0.12, 95% CI [-1.18, 0.95] for mean heart rate.



**Fig. 3.** Mean difference between antibiotic and control group (with 95% confidence intervals) for the scrambled sentences task and the emotional test battery. Difference is the value for the control group subtracted from the value for the antibiotic group, such that a positive mean difference indicates a higher value in the antibiotic group.



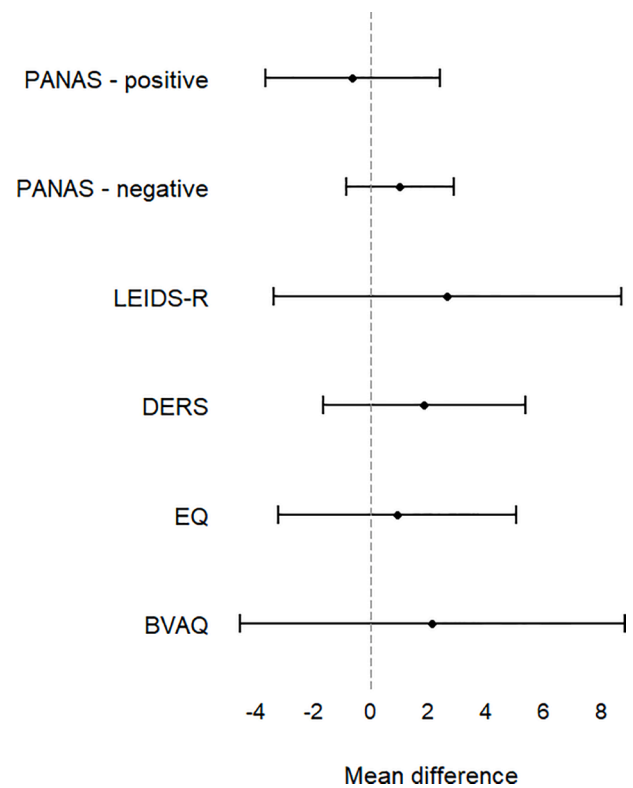
**Fig. 4.** Mean difference in heart rate variables between antibiotic and control group (with 95% confidence intervals). Difference is the value for the control group subtracted from the value for the antibiotic group, such that a positive mean difference indicates a higher value in the antibiotic group.

Due to disruption caused by the pandemic (see Supplementary material), a limited dataset was obtained for salivary IgA levels which prevented comparison between the two groups. A within-group multiple regression was conducted for 27 antibiotic samples with IgA concentration as the independent variable and the predictor variables as days since treatment, duration of treatment and antibiotic class (broad versus narrow spectrum). The estimated regression coefficients ( $df = 20$ ) were 2.05, 95% CI [-0.62, 4.72], 2.11, 95% CI [-2.69, 6.91] and 13.81, 95% CI [-137.59, 165.21] respectively (for the latter coefficient the reference level is narrow-spectrum antibiotics). For the summary statistics of participants' physiological measures, please see Table S6.

### 3.4. Trait measures

There was no evidence of a difference between the mean questionnaire scores (assessing mood, depression sensitivity, emotion regulation, empathy and alexithymia) of the antibiotic group and control group (Fig. 5). For the summary statistics of participants' questionnaire responses, please see Table S7.

Intercorrelation analysis (Fig. S1) revealed expected correlations between some of the questionnaire scores and there were also expected correlations between the heart rate variables. Correlations detected in this exploratory analysis include the positive associations between the proportion of negative sentences in the SST and scores for negative mood ( $\tau = 0.34$ , 95% CI [0.23, 0.46]), LEIDS-R ( $\tau = 0.22$ , 95% CI [0.10, 0.34]), DERS ( $\tau = 0.35$ , 95% CI [0.23, 0.46]) and BVAQ ( $\tau = 0.17$ , 95% CI [0.04, 0.31]). Participants with a more positive mood showed less bias towards sad faces ( $\tau = -0.17$ , 95% CI [-0.31, -0.03]) and fearful faces ( $\tau = -0.14$ , 95% CI [-0.27, -0.02]) in the dot-probe task. Individuals tended to remember fewer words during the EREC if they reported less proficiency in English ( $\tau = 0.18$ , 95% CI [0.06, 0.31]) or a more negative mood ( $\tau = -0.19$ , 95% CI [-0.33, -0.06]). The intercorrelation analysis



**Fig. 5.** Mean difference in questionnaire scores between antibiotic and control group (with 95% confidence intervals). Difference is the value for the control group subtracted from the value for the antibiotic group, such that a positive mean difference indicates a higher value in the antibiotic group.

also revealed a positive association between LEIDS-R score and BMI ( $\tau = 0.17$ , 95% CI [0.05, 0.30]).

## 4. Discussion

An important finding from this research is the greater emotional bias shown by the antibiotic group towards sad faces in the dot-probe task. As opposed to antibiotics which are considered detrimental to microbiome health, intake of prebiotic supplements has been shown to decrease bias towards negative information in a dot-probe task in healthy participants [29]. In addition, probiotic studies in healthy volunteers have found that probiotic consumption can reduce cognitive reactivity to sad mood [54] and improve symptoms of depression and anxiety [55]. Furthermore, brain imaging has revealed that probiotics can decrease the activity of brain regions involved in emotional processing when viewing negative facial expressions [27].

While studies in the field of the microbiome–gut–brain axis have shown that the microbiome plays a role in emotion, the strongest link has been found with depressive symptoms [56,57]. Since antibiotics disrupt the composition and reduce the diversity of the gut microbiome, this may in turn influence emotional processing and bring about an increased bias towards sadness via the microbiome–gut–brain link. In fact, as opposed to the increased bias we report here in antibiotic-treated participants, stimulating the vagus nerve (one of the mechanisms via which the gut microbiome can interact with the brain) has been shown to reduce emotional bias towards sadness in the dot-probe task [82].

Our finding is particularly interesting in light of research showing that even just a single course of antibiotics is associated with a greater risk of depression and anxiety, with the risk increasing with subsequent antibiotic exposures [5]. While previous research has linked antibiotic treatment to clinical outcomes, the novelty of our research lies in assessing the underlying cognitive mechanism to detect changes in

emotional processing. Thus perhaps this increased bias towards sad stimuli that we observe in antibiotic-treated participants may provide one mechanism via which antibiotics may heighten the risk of certain psychological conditions, particularly since a stronger emotional bias towards sad stimuli is associated with depression [58–60].

Notably, previous research reporting associations between antibiotic exposure and mental health outcomes [5] additionally looked at people who had taken antifungals or antivirals and found that there was a mild increase in the risk of depression and anxiety with a single course of treatment (though less than the increased risk with antibiotics), and this risk did not increase with further exposures. A separate study also found that the increased risk of developing schizophrenia or affective disorders including depression was primarily driven by infections treated with antibiotics rather than antivirals, antifungals or antiparasitic agents [7]. While definitive conclusions cannot be drawn from studies like these, the comparison between antibiotics and other antimicrobials does provide a way to try and disentangle any effect of infection from the direct effect of antibiotics themselves.

Although we cannot rule out that previous infection may contribute to the increased negative bias in the antibiotic group, it is relevant to note that the average time between taking the antibiotic course for the infection and participating in the study was over a month. The participants were young, otherwise healthy, students who had been treated for relatively minor ailments (with infections of the urinary tract, throat and skin making up the majority) and when participants were recruited for the study they reported that they had recovered from their prior infection. However, it is well known that the gut microbiome typically takes much longer to recover from a course of antibiotics, resulting in the microbial community being significantly disturbed for up to several months [30]. Furthermore, it has been repeatedly demonstrated in animals that antibiotics alone, without any infection, can result in depressive-like symptoms and cognitive and behavioural impairments [10–19]. Our regression analysis provided some weak evidence that individuals who have taken antibiotics more recently or were prescribed broad-spectrum antibiotics tended to show greater bias towards sadness. However, the sample size of this within-group regression limits its power such that we cannot draw conclusions. It would therefore be interesting for further research to investigate different characteristics of antibiotic courses in relation to their psychological effects. In addition, it should be noted that while the main intended activity of antibiotics is their antimicrobial effect, they can also possess other biological properties. Although antibiotics negatively affect the gut microbiome, a few antibiotics, most notably minocycline, have also been identified for their anti-inflammatory effects and potential therapeutic use in psychiatric and neurological disorders [61,62].

It is perhaps not surprising that a bias towards sadness was detected in the dot-probe task for the antibiotic group but there were no notable differences between the two groups in the questionnaire scores, including those assessing mood, depression sensitivity and emotion regulation. Indeed, the premise of the dot-probe task is that cognitive effects precede subjective reporting, and so it is a much more sensitive tool to detect changes in emotional processing [63]. While there were no clear differences in questionnaire scores, it is interesting to note that for five of the six trait measures the mean score for the antibiotic group was in the expected direction compared to the control group if antibiotics are negatively associated with emotional state. Perhaps therefore the standard questionnaires used in this study do not provide enough variation to be able to assess small differences in a non-clinical population. In addition, the lack of substantial differences between groups for measures obtained from the EREC and EMEM suggest that the antibiotic group did not show a detectable memory bias.

In terms of any physiological differences, while the IgA data were not suitable for comparison between the two groups, the multiple regression within the antibiotic group does indicate a possible trend towards higher levels of IgA the longer the time since the antibiotic course was taken. However, since only IgA data from one batch could be included in the regression (see Supplementary material), more data would be needed to

confirm this effect. If so, this would indicate that the antibiotic treatment (or possibly the infection itself) may deplete IgA levels. It is unlikely the infection results in depletion of IgA since IgA is our body's primary defence against infection, serving to inhibit bacteria and viruses from attaching to cells and neutralising their toxins. However, research has shown that antibiotic treatment can reduce IgA levels via its depletion of the microbiota [64]. Indeed, microbial signals activate immune pathways in the body that promote IgA production and also microbial metabolites, particularly short-chain fatty acids, play an important role in regulating IgA levels [65].

While heart rate measurements revealed an elevated heart rate in the antibiotic group, there were no differences observed in the variables assessing heart rate variability (RMSSD, SDNN, pNNS50) between the two groups. Although heart rate variability is often used as an indirect measure of vagal activity, a recent study in rodents where vagal tone can be measured directly, unlike in humans, did not find any correlation with heart rate variability [66]. In fact, numerous studies have now led researchers to question the extent to which measures of heart rate variability can be used to accurately determine vagal tone [67,68]. The elevated heart rate in the antibiotic group could be caused by a number of factors. Resting heart rate is estimated to be 80% attributable to the parasympathetic nervous system (which consists primarily of the vagus nerve) and 20% to the sympathetic nervous system [69]. Since the vagus nerve acts to lower heart rate, one possible explanation is a reduction in vagal activity in the antibiotic group since disruption of the gut microbiota may result in reduced stimulation of the vagus nerve [70] and animal studies have shown that a dysbiotic microbiota can lead to a decrease in vagal innervation [71]. While we did not find a difference in heart rate variability, this does not necessarily mean there were no differences in vagal activity [66–68]. A further possible explanation for the higher heart rate in the antibiotic group may be because they had an increased stress response compared to the control group during the testing session. The microbiota is known to be a key regulator of the stress response [72] and thus its disruption due to antibiotic treatment may have resulted in the participants exhibiting a stronger stress response and therefore a higher heart rate while testing. Another explanation for both the differences in heart rate and in emotional bias may be the prior infection itself. A heightened inflammatory state, as is typical of infection, may result in a higher heart rate and may also increase susceptibility to negative mood [73] given the evidence that the immune system interacts with emotions [74] and that inflammation can influence emotional processing [75]. However, the regression analysis revealed that this increased heart rate did not mediate the association between antibiotic treatment and emotional bias.

Since antibiotics are so frequently used, and in fact are the most commonly prescribed drug to children [83], it is important that we have a comprehensive understanding of both their short-term and potential long-term effects and the underlying mechanisms. Human population studies have linked antibiotic use during early life to increased risk of various conditions including inflammatory bowel disease [76] and obesity [77,78], as well as mental health disorders [8,9]. Indeed, the microbiome is also considered to play an important role in inflammatory bowel disease [79] and obesity [80]. Since these conditions are often associated with psychological comorbidities, this highlights the value of considering the microbiome in systemic health and disease [81].

In terms of the generalizability of our findings, while the study was conducted on a young student population, there is no reason to expect our findings would not be applicable across age groups. With a larger sample it would be interesting to determine whether the effect sizes differ between the sexes. Since the recruitment requirements for the antibiotic group did not restrict participants to a certain type of antibiotic or duration of treatment, this does make it more likely that our findings are generalizable to common antibiotic treatments, though of course particular antibiotics may have a greater or lesser effect. However, as previously noted, since this is not an intervention study it cannot be ruled out that the observed effect is not, at least in part, a result of the

infection itself. An intervention study is more ethically challenging in terms of justifying treatment with a course of antibiotics when the participant has no infection, though it may be possible to recruit participants who have been prescribed antibiotics prophylactically, although this is not common medical practice.

## 5. Conclusion

Our findings reveal that antibiotic treatment is linked to increased bias towards negative information, as well as a higher heart rate, though this is not a mediating factor in the relationship with emotional bias. Since antibiotics are so commonly prescribed, this biobehavioural association between antibiotic treatment for infection and emotional processing is an interesting observation that warrants further investigation, particularly given previous research linking antibiotic exposure to depression and anxiety. In light of our results, human psychological and behavioural research should consider including recent antibiotic use as a possible exclusion criterion, particularly for studies assessing emotional processing and those taking psychophysiological measures such as heart rate. In conclusion, while the field of psychology primarily seeks to explain emotional and cognitive differences between people from the perspective of brain function, there is now growing evidence of the importance of the gut microbiome's interactions with the nervous system and its link to mental health. Thus greater consideration should be given to the ways in which this microbial environment, and perturbations to it, may affect human psychology and behaviour.

## Author contributions

The idea for the study was first discussed by L. Steenbergen who managed ethical approval of the study. Together with students, L.S. piloted and managed detailed configuration of the tasks and procedure. L.S. supervised data collection, managed and processed the raw data, and K.V.A. Johnson performed statistical analyses. K.V.A.J. drafted the manuscript and both authors revised the draft and approved the final version.

## Open practices statement

All stimuli, tasks, data and analysis code needed to replicate the reported results are available through Leiden University's archive on DataverseNL.

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## Competing interests

The authors declare that they have no competing interests.

## Data availability

Stimuli, tasks, data & code, (Original data)

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.physbeh.2022.113900](https://doi.org/10.1016/j.physbeh.2022.113900).

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