No evidence of emotional biases in the processing of emotionally relevant stimuli as a result of antibiotic use in healthy university students

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Abstract

Recent research has established a link between the gut microbiota and depression. However, it remains unclear how changes in the gut microbiota relate to changes in depressive symptoms. There is some evidence suggesting that alterations in the gut microbiome may affect the processing of emotional information by inducing a negativity bias or by causing a more general sensitivity towards emotional stimuli. The present study aimed to investigate whether the use of antibiotics, which induces microbial dysbiosis, affected the processing of both negative and positive stimuli by comparing the performance of participants that recently used antibiotics to controls on a series of tasks that tap into different aspects of emotional processing, i.e. categorization, recall, and recognition of emotional stimuli. No difference between groups concerning any of the aspects was found. The absence of a group difference may indicate that the use of antibiotics may not affect emotional processing in a healthy sample of young adults. Alternatively, it is possible that the relation between antibiotic use and emotional processing is mediated and or moderated by immunologic factors.

Introduction

According to the WHO (2017), more than 300 million people worldwide suffer from depression, making depression one of the leading causes of non-fatal disease burden. It has been estimated that the economic impact of depressive disorders in Europe alone are over €136 billion per year (WHO, 2016). Given these enormous costs it is vital to identify all factors that reduce the risk of developing a depressive disorder in order to develop new preventative interventions that can reduce the impact of depression on the individual and societal level. Interestingly, recent studies demonstrated that the gut microbiota may be a potential target for such interventions, however, it remains unclear how the gut microbiota affects depressive symptoms (Sarkar et al., 2018; Sherwin, Dinan, & Cryan, 2018). Therefore, the aim of this study is to investigate possible cognitive mechanisms through which the gut microbiota may affect depressive symptoms.

The human gut is populated by a great number of different bacteria (Eckburg et al., 2005). Most of these microbes are harmless or share a symbiotic relation with their host (Lozeupone, Strombaugh, Gordon, Jansson, & Knight, 2012). Studies that manipulated the gut microbiota using probiotics, which have been defined as live cultures of beneficial bacteria that may normalize the gut microbiota (Hill et al., 2014), have shown that symptoms of depressions decreased after the administration of probiotics in both rodents (Abildgaard, Elfving, Hokland, Wegener, & Lund, 2017; Bravo, et al., 2011; Desbonnet, Garrett, Clarke, Bienenstock, & Dinan, 2008; Desbonnet et al., 2010; Gareau et al., 2007; Guo et al., 2019;Murray et al., 2019) and humans (Huang, Wang, & Hu, 2016; Liu, Walsh, & Sheehan, 2019; Nikolova, Zaidi, Young, Cleare, & Stone, 2019). Antibiotics on the other hand have been associated with an increased risk of depression (Lurie, Yang, Haynes, Mamtani, & Boursi, 2015; Sternbach & State, 1997) in otherwise healthy individuals. The physiological mechanisms of these effects are hypothesized to be driven by a change in inflammatory activity in the gut, which has been associated with depressive symptoms (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Maes et al., 2009; Maes, Kubera, & Leunis, 2008; Maes, Kubera, Leunis, & Berk, 2012). Probiotics are understood to reduce gut permeability (Hill et al., 2014), which makes it more

difficult for pathogens to cross the intestinal barrier which would otherwise cause an inflammatory response (Doran, Banerjee, Disson, & Lecuit, 2013). On the other hand, antibiotics have been observed to be related to an indirect increase in inflammatory activity (Van Ampting et al., 2010) which may have resulted from microbial dysbiosis caused by antibiotics use (Nicholson et al., 2012; Preidis & Versalovic, 2009; Zarrinpar et al., 2018). This microbial dysbiosis may decimate the microbes that protect the intestinal barrier by either outcompeting invasive strains (Wine, Gareau, Johnson-Henry, & Sherman, 2009) or by reducing gut permeability indirectly by producing short fatty chain acids such as butyrate (Macfarlane & Macfarlane, 2012) which decreases gut permeability (Lewis et al., 2009; Peng, He, Chen, Holzman, & Lin, 2007; Peng, Li, Green, Holzman, & Lin, 2009; Plöger et al., 2012; Suzuki, Yoshida, & Hara, 2008).

While the physiological mechanisms that link the gut microbiota to depression are somewhat understood, the psychological effects of manipulation of the gut microbiota appear to be more elusive (Sarkar, 2018). A potential pathway through which the gut microbiome may modulate mood is through affecting emotional processing, as many cognitive theories of depression propose that depression is preceded and maintained by a bias towards the processing of self-related negative information (Beck, 2008; Lau, Segal, & Williams, 2004). The link between negativity biases in emotional processing and depression has been established in studies using antidepressant drugs which decrease this negativity bias by facilitating the processing of positive emotional information (Harmer, 2008). Interestingly, antidepressants drugs also appear to have antimicrobial properties (Carlessi, Borba, Zugno, Quevedo, & Reus, 2019; Cussotto, Clarke, Dinan, & Cryan, 2019; Macedo et al., 2017; McGovern, Hamlin, & Winter, 2019). It may therefore be possible that antidepressants induce changes in the gut microbiota which in turn may affect emotional processing. However, as previously mentioned, microbial dysbiosis is likely to be detrimental to the gut microbiota as it kills the microbes that would otherwise prevent the crossing of pathogens through the intestinal barrier which then cause inflammatory activity. This apparent paradox may be solved by the observation that individuals displaying depressive symptoms often suffer microbial dysbiosis (Calarge, Devaraj, &

Shulman, 2019; Jiang et al., 2015; Kelly et al., 2016; Liu et al., 2016; Naseribafrouei et al., 2014; Painold et al.,2019; Vinberg et al., 2019: Zheng et al., 2016). Moreover, depressive symptoms are associated with elevated blood serum Immunoglobulin A and Immunoglobulin M levels in response to toxic bacterial products of normally harmless bacteria that passed the gut barrier (Maes, Kubera, & Leunis, 2008; Maes, Kubera, Leunis, & Berk, 2012). Therefore, antidepressants may reduce inflammatory activity in the gut of depressed individuals by reducing the number of bacteria in the gut, which results in a reduced number of microbes that can access the blood stream from the gut. Additional evidence for this hypothesis comes from studies that administered antibiotics to depressed individuals, which decreased the self-reported symptoms of depression in humans (Dean et al., 2014; Dean et al., 2017) and reduced depressive behavior in mice displaying depressed behavior (Mello, 2013). Moreover, given that increased levels of inflammation are associated with an increase in negative biases in emotional processing (Bollen et al., 2017) it may be that the effect of antidepressants is (partly) caused by a reduction of prereferral inflammation which in turn reduces the negativity bias in emotional processing depressed individuals. Interestingly, probiotics that reduce inflammatory activity by reducing gut permeability (Ait-Belgnaoui et al., 2012; Zareie et al., 2006), have been shown to affect emotional processing as indicated by reduced neural activity towards emotional faces in brain regions involved in emotional processing, such as the periaqueductal grey, somatosensory cortex (Tillisch et al., 2013). Moreover, probiotics have also been demonstrated to reduce cognitive reactivity (Steenbergen, Sellaro, Van Hemert, Bosch & Colzato, 2015), which may limit the processing of negative emotional information. Taken together, these studies suggest that the gut microbiota may indeed affect emotional processing.

Despite the fact that there is preliminary evidence suggesting that changes in the gut microbiota may affect emotional processing, it remains unclear whether the gut microbiome affects emotional processing in a general manner or whether it affects positive emotional processing and negative emotional processing in different ways (Sarkar et al., 2018). While most studies have shown that probiotics tend to alleviate depressive symptoms (Huang, Wang, & Hu, 2016; Liu, Walsh, &

Sheehan, 2019; Nikolova, Zaidi, Young, Cleare, & Stone, 2019) the previously mentioned studies by Steenbergen et al. (2015) and Tillisch et al. (2013) argue for a more general effect of the gut microbiota on emotional processing. Disentangling these effects is important as a general effect would mean that interventions targeting the gut microbiota are not without drawbacks as probiotic treatments could potentially reduce the processing of positive emotional information as well. In addition to these uncertainties, it remains unclear which sub-processes of emotional processing are affected by the gut microbiome. Therefore, aim of the present study is to investigate whether antibiotics which are known to cause gut microbial dysbiosis (Nicholson et al., 2012; Preidis & Versalovic, 2009; Zarrinpar et al., 2018) affect the processing of emotional stimuli. More specifically, the present study aims to investigate whether antibiotic use affects emotional categorisation, emotional memory and emotional attention as negativity biases in these cognitive processes are predictive of the development of depressive symptoms even before any changes in mood are observed (Harmer, 2008).

Alterations in the ability to classify emotional information have been observed in both individuals displaying depressive symptoms (Bouhuys, Geerts, & Gordijn, 1999; Gur et al., 1992; Harmer et al., 2009; Ruhe et al., 2019; Surguladze et al., 2004) and non-depressed individuals at risk of developing a depressive disorder (Chan, Goodwin, & Harmer, 2007; Chan, Harmer, Goodwin, & Norbury, 2008; LeMoult, Kircanski, Prasad, & Gotlib, 2017). As these populations were generally faster in classifying negative, stimuli compared to controls and were more likely to classify neutral stimuli as negative. Moreover, the administration of antidepressant drugs appears to reverse this negativity bias in depressed patients (Harmer et al., 2009). Given the antimicrobial properties that antidepressant possess (Carlessi et al., 2019; Cussotto et al., 2019; Macedo et al., 2017; McGovern et al., 2019) the reduction of the negativity bias may reflect a reduction of inflammation caused by a reduction of level of microbes in the gut resulting in a reduction of the number of bacteria that are able to cross the intestinal barrier which appears to be increasingly permeable in depressed individuals (Maes et al., 2008; Maes et al., 2012). Given that the administration of antibiotics may

increase intestinal permeability (Van Ampting et al., 2010) it is possible that antibiotics may induce a negativity bias in the categorization of emotionally laden stimuli on an emotional categorization task as a result of increased inflammatory activity as increased inflammatory activity has been linked to negativity biases in emotional processing (Bollen et al., 2017). However, if manipulations of the gut microbiome result in a more general effect on emotional processing as described by (Sarkar et al., 2018) the use of antibiotics may facilitate the classification of both positive and negative information on an emotional categorization task.

Changes in emotional memory have also been observed in both individuals suffering from depressive symptoms (Joormann & D'Avanzato, 2010) and in non-depressed individuals at increased risk of developing a depressive disorder (Chan et al., 2007) as these individuals tend to remember more negative stimuli. Interestingly, the effect of antidepressants seems to reduce this negativity bias by allowing increased recall of emotional information (Harmer et al., 2009; Harmer et al., 2011) and by resulting in more falsely remembered positive stimuli (Harmer, Heinzen, O'Sullivan, Ayres, & Cowen, 2008). Again, given the increased gut permeability in depressed individuals this reduction of the negativity bias may reflect a reduction in inflammatory activity as a result of the antimicrobial properties of these drugs (Carlessi et al., 2019; Cussotto et al., 2019; Macedo et al., 2017; McGovern et al., 2019) reducing the number of bacteria that are able to cross the intestinal barrier which appears to be increasingly permeable in depressed individuals (Maes et al., 2008; Maes et al., 2012). Given that antibiotic use is known to cause increased gut permeability (Van Ampting et al., 2010) it may be the case that antibiotic use in healthy individuals may increase inflammatory activity, which is in turn associated with a bias towards negative stimuli (Bollen et al., 2017), which may result in a negativity bias on an emotional recall task. This negativity bias may be reflected as an increase in correctly recalled negative stimuli and as an increase in falsely remembered negative stimuli on an emotional recall task. Alternatively, if manipulations of the gut microbiota result in a general effect on emotional processing as proposed by (Sarkar et al., 2018) it may be the case that the use of antibiotics results in an increase in correctly recalled positive and

negative stimuli but also in an increase in falsely recalled positive and negative stimuli on an emotional recall task.

Whereas recollection is more strongly related to explicit memory, recognition on the other hand appears to be more affected by implicit memory (Yonelinas, 2002). Negativity biases in recognition have also been associated with depression albeit to a lesser extent compared to biases in the recall of emotional information (Joorman & D'Avanzato, 2010). Studies have shown that, under normal circumstances, healthy participants appear to have a protective positive recognition bias for emotional information which may shield from the induction of negative mood as a result of the recognition of negative information (Deldin, Keller, Gergen, & Miller, 2001; Ellis, Beevers, & Wells, 2011). This protective bias appears to be absent in individuals suffering from depression as depressed individuals appear to recognize more negative stimuli (Jermann, Van der Linden, Laurencon, & Schmitt, 2009) and experience more falsely remembered negative stimuli (Howe, & Malone, 2011; Moritz, Glascher, & Brassen, 2005; Wittekind et al., 2014). Given that depression, which is strongly related to biases in emotional processing (Harmer, 2008; Rude, Valdez, Odom, & Ebrahimi, 2003; Rude, Wenzlaff, Gibbs, Vane, & Whitney, 2002), is associated with increased inflammatory activity as a result of increased gut permeability (Dantzer et al., 2008; Maes et al., 2009) it is possible that antibiotics which are known to increase gut permeability (Van Ampting et al., 2010) may induce a negativity bias in recognition as a result of increased inflammation which has been associated with a negative bias in emotional processing (Bollen et al., 2017). This recognition bias may manifest on an emotional recognition task as an increase in correctly recalled negative items and or as an increase in falsely recalled negative items. However, if manipulation of the gut microbiota results in a more general sensitivity towards emotional stimuli as proposed by Sarkar et al. (2018) an increase in the (false) recognition of both positive and negative stimuli at the expense of correct rejections stimuli would be expected on an emotional recognition task.

Lastly, biases in emotional attention regarding negative stimuli have also been observed in depressed individuals and have been hypothesized to increase the risk of developing a depressive disorder (Bourke, Douglas, & Porter, 2010; Joorman & D'Avanzato, 2010). Emotional attention can be dissected into two separate components namely: the engagement of emotional stimuli and the disengagement of emotional stimuli (Posner & Petersen, 1990). The engagement of emotional information, also known as vigilance, describes the orientation of attention towards an emotional stimulus (Koster, Crombez, Verschuere, & de Houwer, 2004). In contrast, disengagement of emotional stimuli refers to the releasing attention from an emotional stimulus when attention needs to be relocated to a new target (Koster, et al., 2004). Increased negative affect and rumination, which are hallmarks of depression (Beck , 2008), appear to affect the engagement to negative stimuli and the disengagement from negative stimuli (Duque & Vazquez, 2015; Grafton, Watkins & MacLeod, 2012; Southworth, Grafton, MacLeod & Watkins, 2017) but also the reduced attentional engagement of positive stimuli (Winer & Salem, 2016). Based on the observation that depression, which is preceded and maintained by biases in emotional processing (Harmer, 2008), is associated with increased inflammation (Dantzer et al., 2008; Maes et al., 2009) it may be the case that antibiotics which are known to increase gut permeability (Van Ampting et al., 2010) may result in a negativity bias in vigilance and or increased difficulties disengaging from negative stimuli such as fearful, angry and sad faces on an emotional attention task as a result of increased inflammatory activity which has been associated with a negativity bias in emotional processing (Bollen et al., 2017). However, if manipulations of the gut microbiome result in a generalized effect on emotional processing as described by Sarkar et al. (2018) it is expected that antibiotic use may result in an attentional bias in vigilance and/or increased difficulties disengaging from both positive (happy and surprised faces) stimuli and negative stimuli (sad, fearful and angry faces) on an emotional attention task.

Method

Design

To investigate whether antibiotic use was associated with an increased bias towards negative and/or positive stimuli in emotional processing the performance of participants that recently used antibiotics on several computerized tasks were compared with the performance of a control group. These tasks were designed to measure the classification, recall, recognition and attention of emotional stimuli. The present study employed a between-subject design as the performance on these tasks was compared between the antibiotic and control group. The rationale behind this design was that antibiotic use is assumed to cause microbial dysbiosis in the gut (Nicholson et al., 2012; Preidis & Versalovic, 2009; Zarrinpar et al., 2018) which allows for the investigation of an association between presumed antibiotic-induced gut microbial dysbiosis and emotional processing without needlessly exposing participants to the potential detrimental effects of experimental microbial dysbiosis. However, given that no analysis of the gut microbiome was performed this study was unable to establish a direct link between the gut microbiome composition and emotional processing.

Participants

A total of 106 participants aged 18 to 35 were recruited from the student population of Leiden University of whom 46 had used antibiotics during the past three months prior to participation. Participants were compensated with either €7.50 or with two participation credits. Screening occurred using a self-report questionnaire based on the Mini International Neuro-psychiatric Interview (Sheehan et al., 1998). Similar to the study by Steenbergen et al. (2015) participants that had a personal or family history of neurologic or psychiatric disorder were excluded from participation. Moreover, given that heart variability was measured participants suffering from conditions related to their cardiovascular and respiratory systems (Berntson et al., 1997) were also excluded from participation. Furthermore, individuals suffering from conditions related to the digestive system were excluded as well given that bowel diseases such as the irritable bowel

syndrome are associated with an altered gut microbiota (Jeffery et al., 2012). Additionally, participants that recently implemented a dramatic change in diet (e.g. fasting) were also excluded from participation given that diet influences microbiota composition (De Filippo et al., 2010) and gastrointestinal transit time (Kashyap et al., 2013), which was also measured during this research project. Lastly, to minimize confounding influences participants that used (illegal) drugs seven days prior to participation (with the exception of hormonal contraceptives) or probiotic supplements three months prior to participation were excluded.

Procedure

Given that the present study was part of a larger research project, not all data collected by the procedures below were analysed in the present study. Prior to participation the informed consent was obtained and participants were informed of the procedure. Next, participants were asked to fill out a background screening questionnaire and asked to report the details regarding their antibiotics use. Following the background screening the weight, height and waist/hip ratio was obtained. Next, participants were asked to put on a heart rate monitor to measure their heart rate variability later on during the experiment. After putting on the heart rate monitor participants were asked to complete the following computerized questionnaires: the Bristol Stool Scale, the Positive and Negative Affect Schedule, the Difficulties in Emotion Regulation Scale, the Empathy Quotient, the Leiden Index of Depression Sensitivity and the Bermond–Vorst alexithymia questionnaire. Following the completion of the questionnaires the heart rate variability was measured. Prior to the recording of the heart rate variability participants were instructed to place both feet on the ground, not to talk during the recording and to sit still during the recording. Next, participants performed the Emotional Categorization Task. Completion of the Emotional Categorization Task was followed by the Scrambled Sentences task. After completion of the Scrambled Sentences task participants performed the Faces Dot Probe Task. The final two computerized task consisted of the Emotional Recall Task and the Emotional Recognition Memory Task. Instructions for the aforementioned tasks were presented both orally by the experimenter and in written form on the screen. Lastly,

participants received instructions for the saliva sample. Participants were asked to collect 2ml of their saliva within a time period of 15 minutes. They received explicit instructions not to spit or to manually create extra saliva. At the end of the session participants were compensated for their time with either money or credits and debriefed. The procedure was approved by the ethics committee of Leiden University and in line with the ethical standards of the Declaration of Helsinki regarding human experimentation (WMA, 2009).

Materials

Given that the present study was part of a larger research project not all materials that are discussed below were relevant to the present study. Materials that were irrelevant to the present studies are only be discussed briefly.

Questionnaires

Several self-report questionnaires were used for screening purposes and to measure psychological constructs as well as intestinal transit time. For the present study only the Mini International Neuro-psychiatric Interview was used to check whether the participants matched the inclusion criteria.

Mini International Neuro-psychiatric Interview (Sheenhan et al., 1998). For screening purposes an adapted version of the Mini International Neuro-psychiatric Interview was used. This self-report questionnaire consists of several items that assessed whether participants met the inclusion criteria.

The Bristol Stool Scale (Lewis & Heaton, 1997). The Bristol Stool Scale is a self-report questionnaire designed to measure the intestinal transit time. This questionnaire required participants to report the number of times they defecate each day along with the form and consistency of their stool on a 7-point scale.

The Positive and Negative Affect Schedule (PANAS) (Watson, Elgoff, & Tellegen, 1998). The PANAS is designed to measure different aspects of self-reported positive and negative affect. The PANAS consists of 20 items that are rated on a five-point Likert scale by the participant. All items were presented in both English and Dutch.

The Empathy Quotient (EQ) (Lawrence, Shaw, Baker, & Baron-Cohen, 2004). The EQ is a self-report questionnaire aimed at measuring empathy. This questionnaire consists of 60 items that are rated on a four-point Likert scale. All items were presented in both English and Dutch.

The Difficulties in Emotion Regulation Scale (DERS; Gratz & Roemer, 2004). The DERS is a self-report questionnaire designed to assess the extent to which individuals are able to regulate their own emotions. The DERS consists of 36 items that are all rated on five-point Likert scale. All items were presented in both English and Dutch.

The Leiden Index of Depression Sensitivity (LEIDS-R; Van der Does, 2002). The LEIDS-R consists of 26 items which are answered on a five-point Likert scale by the participant and is aimed at measuring dysfunctional thought patterns. All items were presented in both English and Dutch.

Bermond–Vorst Alexithymia Questionnaire (BVAQ; Vorst; 2001). The BVAQ is a self-report questionnaire aimed at measuring alexithymia. This questionnaire consists of 40 items which are rated on a five-point Likert scale. All items were presented in both English and Dutch.

Physiological measures

Several physiological measures were taken as part of the larger overall research product. None of the physiological measures described below were analyzed in the present study.

Anthropometric measurements. Height and waist-hip ratio were obtained using a simple tape-measure whereas weight was measured using a Karada scan scale.

Heart-rate variability. Heart-rate varability recordings were obtained using an h7 polar heart rate sensor and the HRV Elite application (Elite HRV version 4.7.0).

Behavioral tasks

Several behaviour tasks were employed to measure different components of emotional processing. All task described below were analysed in the present study except for the scrambled sentences task.

Scrambled Sentences Task. The scrambled sentences task has been developed by Wenzalff (1991) to identify depressogenic cognitions which are hypothesized to increase the odds of developing a depressive disorder (Beck, 2008; Lau et al., 2004). During each trial (21 in total of which one was a practice trial) participants were shown six scrambled words and were instructed to create a sentence out of five of these six words. Each trial contained two target words of which only one could be used in the creation of the sentence for example: *future bright my will dismal be* could become *my future will be bright* or *my future will be dismal*. To prevent participants from actively repressing negative cognitions participants were presented with six digits and instructed to keep these digits active in their working memory until the end of the tasks.

Emotional Test Battery. In addition to the Scrambled Sentences Task several tasks from the Emotional Test Battery were used to measure emotional processing. This test battery has been designed as an early detection tool to detect the early effects of pharmaceutical drugs on emotional processing in depression and has been validated in both healthy populations (Harmer et al., 2003; Harmer, Heinzen, O'Sullivan, Ayres, & Cowen, 2008; Harmer, Shelley, Cowen, & Goodwin, 2004; Horder, Cowen, Di Simplicio, Browning, & Harmer, 2009) and in populations consisting of individuals suffering from depression (Browning et al., 2015; Harmer et al., 2009; Harmer et al., 2011; Post et al., 2014). During the present study the Emotional Categorization Task, The Faces Dot Probe Task, The Emotional Recall Task and The Emotional Recognition Memory Task of the Emotional Test Battery were used and are described in greater detail below.

Emotional Categorization Task. During each trial participants were shown 40 descriptors selected from Anderson (1968) that are either positive such as *cheerful*, *honest* and *optimistic* or

negative, for example, *domineering*, *untidy* and *hostile*. Participants were instructed to indicate whether they would like or dislike to be associated with each descriptor by pressing the word like or dislike using the left mouse button. Participants were given five practice trials prior to the start of the actual task. Th Emotional Categorization task allowed the measurement of the time participants require to decide whether a descriptor is positive or negative for both positive and negative descriptors. This task therefore allowed comparison of reaction times to positive and negative words between the antibiotic and control group. Furthermore, the tasks also allowed to compare the number of correctly classified items between both groups.

Faces Dot Probe Task. During each trial participants were presented with a black dot in the middle of the screen to which they had to respond as fast as possible using the left mouse button. After the presentation of the first dot part participants were shown two faces, one appearing on the right side and the other on the left side of the screen for 500 milliseconds. During each trial one of the faces displayed a neutral expression whereas the other face displayed either a positive emotion (joy or surprise) or a negative emotion (anger or fear). Following the presentation of the faces the black dot would reappear on either the right side or the left side of the screen after which participants were required to click the black dot as fast as possible. The location of the face could be either emotion-congruent (emotional face matches location of the second dot) with the location of the next location of the black dot or emotion-incongruent (emotional face does not match the location of the second dot). The faces were selected from the pictures of facial affect by Ekman and Friesen (1976). Prior to the start of the task participants were given 20 training trials with pictures of nature (e.g. flowers) instead of faces. The Faces Dot Probe task is designed to assess emotional attention by measuring reaction time when presented with emotional faces on both congruent and incongruent trials. Increased emotional attentional engagement results in faster reaction times when the emotional face matches the location of the target compared to trials with two neutral stimuli. Increased difficulty in attentional disengagement, on the other hand, results in increased

reaction times on trials where the target in incongruent with the emotional stimuli compared to neutral trials.

Emotional Recall Task. The Emotional Recall Task is an unannounced recall task during which participants were asked to write down as many words as they were able to remember from the Emotional Categorization Task within two minutes. This task is designed to measure an emotional recall bias which is reflected in the amount of positive versus negative words participants are able to write down. The outcome measures were the number of correctly remembered positive words, the number of correctly remembered negative words, the number of incorrectly remembered positive, the number of incorrectly remembered negative items and the total of the remembered items. These outcome variables allowed for the calculation of the percentage of correctly remembered words and the percentage of falsely remembered words for both the positive and negative items.

Emotional Recognition Memory Task. The Emotional Recognition Memory Task is the final task of the Oxford Emotional Test Battery and is designed to measure emotional recognition bias. During this task participants were shown 80 words (one per trial) and were instructed to indicate whether these words had appeared in the Emotional Categorization Task. This task measures the number of correctly recognized positive and negative words and the number of incorrectly recognized positive and negative words, which allows for a decision criterion to be calculated based on signal detection theory.

Statistical analysis

Data analysis was primarily performed using SPSS (version 26), however, Bayesian analyses were performed using JASP (version 0.12.2). Responses that took longer than 10 seconds on tasks that were reaction time sensitive were removed from the data as these cases may have represented lapses in memory or non-compliance with the instruction. Boxplots were used to screen for

remaining outliers. Reaction times that deviated more than three standard deviations from the mean were analyzed twice (with and without the outliers). No outliers were observed unless otherwise reported. The assumption of normality was assed using the Shapiro-Wilk test. However, given the strictness of this test and the relative robustness of the t-test against violations of normality the data was also visually assed using Q-Q plots. If the data approximated a normal distribution based on inspection of the Q-Q plots the t-test was performed usual. If a serious violation of normality was observed based on both the Shapiro-Wilk test and the Q-Q plots the Mann-Whitney test was performed instead. The assumption of homogeneity of variances was assessed by computing Levene's test for homogeneity of variances. If heterogeneity of variances was observed Welch's t-test was computed instead of Student's t-test. Assumptions of every statistical model were met unless otherwise reported. An alpha level of .05 (two-tailed) was adopted for all tests. Bayesian factors were calculated using a two-sided test based on a Cauchy scale of .707.

Emotional Categorization Task

To test whether the antibiotics group differed in terms of reaction time on both positive and negative items reaction times for correctly classified items were compared between groups using independent sample t-tests. An item was considered correctly classified if the classification of the participant matched the classification by Anderson (1968), see appendix 1. To test whether a speed-accuracy was present the number of correctly classified items were also compared between groups using an independent sample t-test.

Emotional Recall Task

In order to assess whether recall of emotional stimuli of negative and positive items differed between individuals that recently used antibiotics and controls by comparing the percentage of correctly remembered positive and negative words between groups. Moreover, the percentage of falsely remembered positive and negative items were also compared. The reason percentages were chosen instead of numbers of remembered words was because the present study focuses on

emotional bias within an individual's memory and not the functioning of the memory itself. The percentage of correctly positive and negative items was obtained using formula 1. The percentage of falsely remembered membered positive and negative items was obtained using formula 2.

$$Percentage\ correctly\ remembered\ negative\ words = \left(\frac{Number\ of\ remembered\ negative\ words}{Total\ number\ of\ remembered\ words}\right) * 100 \quad (1)$$

$$Percentage\ falsely\ remembered\ negative\ words = \left(\frac{Number\ of\ falsely\ remembered\ negative\ words}{Total\ number\ of\ falsely\ remembered\ word}\right) * 100 \quad (2)$$

Emotional Recognition Memory Task

To test whether individuals in the antibiotics group differed in terms of emotional recall bias the decision criteria based on signal detection theory (Grier, 1971) for both positive and negative items were compared using an independent sample t-test. A shift in decision criteria from zero towards the negative indicates a stricter decision criterion which reflects an increase in correct rejections of stimuli that did not appear in the emotional classification task at the expense of correct hits. Alternatively, a shift in decision criteria from zero towards the positive indicates a more relaxed decision criterion which indicates an increase in correctly remembered stimuli but also in an increase of falsely remembered stimuli. The average reaction times and average number of correct items were also compared to assess whether there was a speed-accuracy trade-off between the two groups. The decision criterion was calculated by first transforming the number of hits and false alarms into z-scores for both positive and negative items which allowed for the calculation of the decision criteria using formula 3.

$$Decision\ criterion = -\left(\frac{Z(hit)+Z(false\ alarm)}{2}\right) \quad (3)$$

Faces Dot Probe Task

To investigate whether individuals who recently used antibiotics differed in terms of vigilance towards emotional faces and disengagement from emotional faces from the control group vigilances and disengagement scores were calculated and compared for each emotion. Vigilance scores were computed for each individual emotion using formula 4 as described by Rooijen et al.

(2017). Disengagement scores were computed for each individual emotion using formula 5 as described by Rooijen et al. (2017). Both vigilance and disengagement scores where then compared between both groups for each individual emotion using independent sample t-tests.

$$Vigilance = (\frac{(RT\ congruent\ neutral\ + RT\ incongruent\ neutral)}{2}) - RT\ emotional\ congruent \quad (4)$$

Note. RT refers to reaction time per trial

$$Disengagement = RT\ incongruent\ emotional - \left(\frac{(RT\ congruent\ neutral + RT\ incongruent\ neutral)}{2}\right) \quad (5)$$

Note. RT refers to reaction time per trial

Results

Participant characteristics

Two participants in the antibiotic groups were excluded from analysis as they did not take medication that could be classified as antibiotic. A total of 106 participants were included (unless otherwise specified) in the analysis of which 46 were in the antibiotics group and 60 in the control group. Age ranged from 18 to 29 years old for both group, average age is shown in table 1. There was no statically significant difference in terms of age between groups $t(72.48) = -.53$, $p = .60$, equal variances not assumed. As shown in table 1 most participants were female, sex distribution did not differ statically significantly between the antibiotics and control group $\chi^2(1, N = 106) = 0.56$, $p = .46$. Average English comprehension for both groups is shown in table 1 and did not statistically significantly differ between both groups $t(77.77) = -.43$, $p = .67$, equal variances not assumed, $F(1, 104) = 5.78$, $p = .02$.

Table 1

Group	***N***	**Average age**	**Average English comprehension**
Antibiotics	46 (38 females)	22.02	8.6
Control	60 (46 females)	22.3	8.7

Note. No differences between groups were observed in distribution of sex, age, and English comprehension.

Emotional Categorization Task

To test whether the control antibiotics group differed from the control group in terms of reaction times on correctly categorised positive an independent sample t-test was computed. The antibiotic group (*M* = 1353.13 milliseconds, *SD* = 353.71) did not differ statistically significantly from the control group (*M* = 1328.69 milliseconds, *SD* = 256.93 in terms of reaction times on correctly classified positive items based on an independent sample t-test $t(104) = .41$, $p = .68$, $d = .08$, see figure 1. These results indicate that both groups did not differ in terms of performance on the emotional categorisation task. The Bayesian factor of this finding was $BF_{01} = 4.48$, indicating that these results were 4.48 times more likely to have occurred under the assumption that there is no relation between antibiotic use and a positivity bias in the categorization of emotional information than under the hypothesis that antibiotic use is associated with a bias in categorization of positive stimuli. Based on the criteria developed by Jeffries (1998) these results can be interpreted as substantial support for the hypothesis that there is no relation between antibiotic use and emotional processing in the present sample.

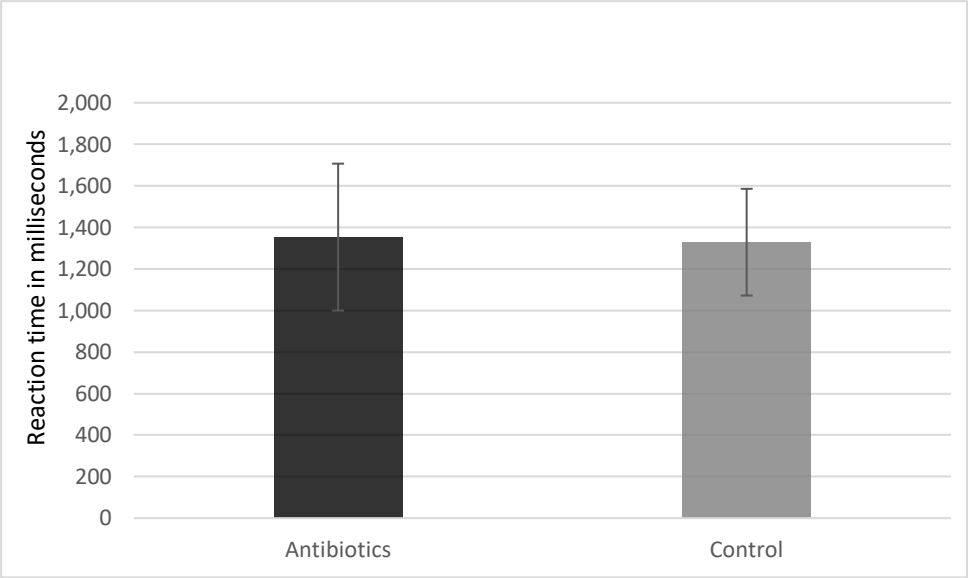

Figure 1. Reaction times of correctly classified positive words on the emotional categorisation task. No statistically significant difference was observed between groups. *N* Antibiotics = 46, *N* Control = 60.

To assess whether a speed-accuracy trade-off was present for the positive items the number of correctly answered positive items were compared between groups. Participants that recently used antibiotics (*M* = 17.17, *SD* = 1.77) did not differ statically significantly from controls (*M* = 17.20 s, *SD* = 1.47) in terms of the number of correctly classified positive items based on an independent sample t-test $t(104) = -.08$, $p = .93$, $d = .02$.

To test whether individuals in the antibiotic group differed in reaction times on correctly classified trials with negative items an independent samples t-test was computed. The assumption of homogeneity of equal variances appeared to be violated based on Levene's test for equality of variances $F(1, 102) = 4.83$, $p = .03$, therefore, Welch's t-test was computed. The antibiotic group (*M* = 1542.05 milliseconds, *SD* = 435.21) did not differ statistically significantly from the control group (*M* = 1439.80 milliseconds , *SD* = 305.90) in terms of reaction times on correctly classified negative items based on an independent sample t-test $t(77.11) = 1.36$, $p = .18$, $d = .27$. Thus, providing no evidence that the two groups differed from each other, in terms of reaction time on correctly classified

negative items, see figure 2. The Bayesian factor of this finding was BF_{01} = 1.97, indicating that these results are 1.97 times more likely to have occurred under the assumption that there is no relation between antibiotic use and a negativity bias in the categorization of emotional information than under the hypothesis that antibiotic use is associated with a bias in categorization of negative stimuli. Based on the criteria developed by Jeffries (1998) these results can be interpreted as anecdotal support for the hypothesis that there is no relation between antibiotic use and the categorisation of emotional information in the present sample.

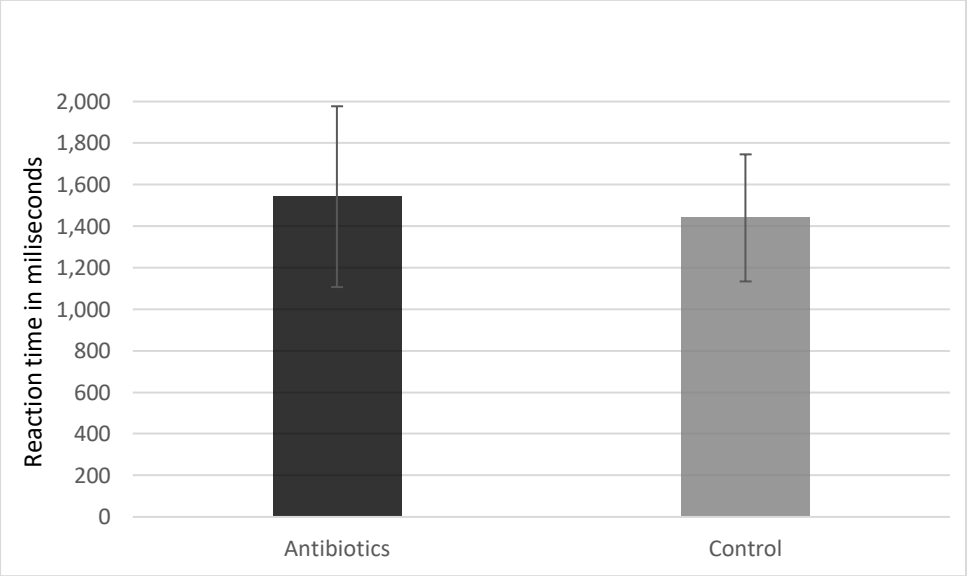

Figure 2. Reaction times of correctly classified negative words on the emotional categorisation task. *N* Antibiotics = 46, *N* Control = 60.

To investigate whether this difference in reaction time was due to a speed-accuracy trade-off an independent sample t-test was computed for the number of correctly classified negative items. Individuals in the antibiotics group (*M* = 17.94 s, *SD* = 1.42), did not statistically significantly differ from individuals in the control group (*M* = 18.23 s, *SD* = 1.28), based on an independent sample t-test *t*(106) = -1.06, *p* = .29, *d* = .20, indicating that there was no evidence of a speed-accuracy trade-off for the negative items.

Emotional Recall Task

To test whether participants that recently used antibiotics suffered from an emotional memory bias, the percentage of negatively remembered items of the total number of remembered items was compared between groups using an independent sample t-test. The independent samples t-test revealed that the individuals in the antibiotics group (*M* = 34.33s, *SD* = 19.37) did not differ in the percentage of correctly remembered negative words from the control group (*M* = 36.96 s, *SD* = 18.73), $t(104) = .85$, $p = .40$, $d = .14$ in a statically meaningful way. Given that the percentage of remembered negative items was based on the total number of items it could be concluded that there was also no difference in percentage of remembered positive items between groups, see table 3 for an overview. To determine the strength of the null effect the Bayesian factor was calculated, $BF_{01} = 3.87$. This result indicates that the present results were 3.87 times more likely to occur under the assumption that antibiotics do not affect the correct recall of negative items than under the alternative hypothesis which assumed that antibiotic use would result in an increase in the number of correctly recalled negative stimuli. Based on the criteria developed by Jeffries (1998) these results can be interpreted as substantial support for the hypothesis that there was no relation between antibiotic use and the recall of emotional stimuli in the present sample.

To test whether the antibiotics group differed in terms of falsely recalled negative words an independent sample t-test was performed. Data of one participant in the control group were missing due to dropping out. Two other participants in the antibiotics group and one in the control group did not report any falsely remembered words, therefore, percentages for these participants could not be calculated. Therefore, a total of 103 participants were included in the analysis of which 44 used antibiotics and 59 did not. The independent t-test revealed that the antibiotics group (*M* = 35.76, *SD* = 30.96) did not statistically significantly differ from the control group (*M* = 31.93 s, *SD* = 26.26), $t(101) = .68$, $p = .50$, $d = .14$, indicating that the percentage of falsely remembered negative items did not differ between the two groups providing no evidence for a difference in negativity bias between groups. Given that the percentage of remembered negative items was based on the total number of

items it could be concluded that there was also no difference in percentage of falsely remembered positive items between groups, see table 2 for an overview, indicating that there was no difference between the groups in terms of a positivity recall bias. The Bayesian factor of the null hypothesis was, BF_{01} = 3.88, indicating that the present results were 3.88 times more likely to occur under the assumption that antibiotics do not affect the correct recall of negative items than under the alternative hypothesis which assumed that antibiotic use would result in an increase in the number of falsely remembered negative stimuli. Based on the criteria developed by Jeffries (1998) these results can be interpreted as substantial support for the hypothesis that there was no relation between antibiotic use and the recall of emotional stimuli in the present sample.

Table 2

Percentage correct and percentage of falsely remembered items on the emotional recall task

Variable	**Mean Antibiotics (SD)**	**Mean control (SD)**
Correctly recalled negative words	34.33%(19.37)	36.96%(18.73)
Correct recalled positive words	65.67%(19.37)	63.04%(18.73)
Falsely recalled negative words	35.76%(30.96)	31.93%(26.26)
Falsely recalled positives words	64.24%(30.96)	68.07%(26.26)

Note. For correctly recalled words: Control *N* = 60, Antibiotics *N* = 46*,* For falsely recalled words: Control *N* = 59, Antibiotics *N* = 44. No statically significant differences between groups were observed.

Emotional Recognition Memory Task

To test whether the decision criteria differed for positive items between the antibiotics and control group an independent sample t-test was computed. Data of one participant of the antibiotics group was missing due to dropping out. Therefore, the antibiotics group contained 45 participants whereas the control group still contained 60 participants. The t-test revealed that the antibiotics group (M = .08 s, SD = .75) did not differ from the control group (M = -.01 s, SD = .77) in terms of decision criteria for positive items $t(103) = .58$, $p = .56$, $d = .12$. Therefore, it could be concluded that the criteria bias for identifying positive words did not differ between groups in a statically meaningful way. The Bayesian factor of the null hypothesis was, $BF_{01} = 4.13$, indicating that the present result were 4.13 times more likely to occur under the assumption that antibiotics do not induce a bias in the recognition of positive emotional information than under the alternative hypothesis which assumed that antibiotic use would result in an increased bias in the recognition of positive emotional information. Based on the criteria developed by Jeffries (1998) these results can be interpreted as substantial support for the hypothesis that there was no relation between antibiotic use and the recognition of positive emotional stimuli in the present sample.

The same procedure was performed for the negative items. Data of a single participant was missing as this participant dropped out during the study. Therefore, the antibiotics group contained 45 participants whereas the control group still contained 60 participants. The t-test revealed that the antibiotics group (M = .07 s, SD = .74) did not differ from the control group (M = -.04 s, SD = .83) in terms of decision criteria for negative items $t(103) = .73$, $p = .47$, $d = .14$. Therefore, it could be concluded that the criteria bias for identifying negative words did not differ between groups in a statically significantly manner. The Bayesian factor of the null hypothesis was, $BF_{01} = 3.08$, indicating that the present results were 3.08 times more likely to occur under the assumption that antibiotics do not induce a bias in the recognition of negative emotional information than under the alternative hypothesis which assumed that antibiotic use would result in an increased bias in the recognition of negative emotional information. Based on the criteria developed by Jeffries (1998) these results can

be interpreted as substantial support for the hypothesis that there was no relation between antibiotic use and the recognition of negative emotional stimuli in the present sample.

Faces Dot Probe Task

To test whether individuals in the antibiotics group differed from the control group in terms of emotional vigilance towards emotional faces, t-tests were computed for each individual emotion based on the reaction times. Due to the observation that the vigilance scores ranged from negative values to positive values each vigilance score was transformed into a positive value. For each vigilance score the lowest score was changed into 1 by using the formula $x + 1$ whereas x was the number required to transform the lowest score into 0. This formula was then applied to all other vigilance scores for each emotion individually. The transformed vigilances scores were then compared using independent sample t-tests as shown in table 3. Based on results as listed in table 3 it could be concluded that there was no evidence for a statically significant difference between groups in terms of vigilance towards any of the emotional faces. Confidence in the null expressed in Bayesian factors hypotheses is also shown in table 3.

Table 3

Vigilance scores based on reaction times in milliseconds of face dot probe task

Emotion	***M*(*SD*) antibiotics**	***M*(*SD*) control**	***M*(*SD*) antibiotics transformed**	***M*(*SD*) control transformed**	***t*(df)**	***p***	***d***	**BF_{01}**
Anger	-.53(48.78)	1.96(42.46)	166.49(48.78)	168.98(42.46)	-.28(104)	.78	.06	4.66
Fear	0.32(40.58)	-.49(41.84	121.47(40.58)	120.66(41.84)	.10(104)	.92	.02	4.81
Sadness	-2.75(44.94)	-10.24(40.69)	151.35(44.94)	143.85(40.69)	.90(104)	.37	.18	3.37
Joy	-7.81(47.14)	-1.27(47.11)	148.17(47.14)	154.71(47.11)	-.71(104)	.48	.14	3.86
Surprise	-.57(39.18)	-1.91(43.63)	119.31(38.96)	117.97(43.63)	.16(106)	.87	.03	4.77

Note. Antibiotics *N* = 46, Control *N* = 60, transformed scores were used to compare means between groups.

To test whether individuals in the antibiotics group differed from the control group in terms of emotional disengagement from emotional faces independent sample t-tests were computed for each individual emotion. Due to the observation that the disengagement scores ranged from negative values to positive values each disengagement score was transformed into a positive value. For each disengagement score the lowest score was changed into 1 by using the formula *x +1* whereas *x* was the number required to transform the lowest score into 0. This formula was then applied to all other disengagement scores for each emotion individually. The transformed disengagement scores were then compared using independent sample t-tests as shown in table 4. Based on results as listed in table 4 it could be concluded that there was no difference between groups in terms of attentional disengagement from any of the emotional faces. Confidence in the null hypothesis expressed in Bayesian factors hypotheses is also shown in table 4.

Table 4

Disengagement scores based on reaction times in milliseconds of face dot probe task

Emotion	***M*(*SD*) antibiotics**	***M*(*SD*) control**	***M*(*SD*) antibiotics transformed**	***M*(*SD*) control transformed**	***t*(df)**	***p***	***d***	**BF_{01}**
Anger	16.27(42.16)	4.81(35.25)	113.18(42.16)	101.72(35.25)	1.51(104)	.14	.30	1.76
Fear	-.53(35.41)	2.86(44.09)	78.59(35.41)	81.98(44.09)	-.43(104)	.67	.08	4.45
Sadness	16.95(47.67)	5.51(48.87)	106.09(47.67)	94.65(39.23)	1.36(104)	.18	.27	2.13
Joy	15.39(46.04)	8.08(35.06)	126.01(46.04)	118.70(35.06)	.93(104)	.36	.18	3.29
Surprise	11.52(37.78)	14.05(36.82)	84.83(37.78)	87.36(36.82)	-.35(104)	.73	.07	4.58

Note. Antibiotics *N* = 46, Control *N* = 60, transformed scores were used to compare means between groups and calculate Bayesian factors.

Discussion

The aim of the present study was to investigate whether the recent use of antibiotics affected the processing of emotionally relevant information by comparing the performance of individuals that recently used antibiotics to a control group on several tasks tapping into different aspects of emotional processing. Based on the literature two general hypotheses were formulated. Firstly, it was hypothesized that the use of antibiotics may have biased participants towards the processing of negative information based on the notion that antibiotic use has been associated with increased risks of developing a depressive disorder (Lurie et al., 2015; Sternbach & State, 1997) and the fact that negativity biases are thought to play an important role in the development and maintenance of depressive disorders (Beck, 2008; Harmer, 2008). The second hypothesis was based on the suggestion by Sakar et al. (2018) who postulated that manipulation of the gut microbiota may have a general effect on emotional processing. It was therefore hypothesised that antibiotic use may have increased the processing of both positive and negative information.

No statistically meaningful difference was found between the antibiotics group and the control group regarding the categorization of emotional information, recall of emotional stimuli, recall of emotional information and the allocation of attention towards or from emotional stimuli for both positive and negative items. Given these findings, the present study fails to provide evidence for a relation between the use of antibiotics and alterations in emotional processing in healthy university students between 18 and 35 years old. The support for the null hypotheses based on Bayesian factors ranged from anecdotal to substantial based on the criteria developed by Jeffries (1998). Based on these results it is difficult to conclude whether both groups were truly similar in terms of emotional processing leaving the results of the present study inconclusive.

Given that negativity biases are thought to play an important role in the development and maintenance of depressive disorders (Beck, 2008; Harmer, 2008) the results of the present study appear to be at odds with the findings that the use of antibiotics is associated with an increased risk of developing a depressive disorder (Lurie et al., 2015; Sternbach & State, 1997). Moreover, the present results also appear to conflict with the suggestion that probiotics, which in contrast to antibiotics have a beneficial effect on the gut microbiota (Hill et al., 2014), may have a general effect on the processing of emotional information (Sakar et al., 2018). While it is possible that antibiotics did not affect emotional processing in our sample it is equally possible that the lack of an observable effect may have resulted from a lack of statistical power, limits of the design of the current study and underlying mediating or moderating effects of variables that were not accounted for in the present study.

One major limiting factor of the present study was the assumption of antibiotic-induced microbial dysbiosis. While the effects of antibiotics on the gut microbiota are well documented (Nicholson et al., 2012; Preidis & Versalovic, 2009; Zarrinpar et al., 2018), the composition of the gut microbiota was not measured during the current study. Therefore, it is possible that the absence of an observable effect can be attributed to a lack of antibiotic-induced microbial dysbiosis in the

antibiotic group. Secondly, antibiotic use is also associated with a reduction in symptoms of depression (Dean et al., 2014; Dean et al., 2017) in individuals suffering from depression. Given that we collected no data regarding depressive symptoms prior to the antibiotic use it is possible that the antibiotics actually reduced depressive symptoms they may have experienced before taking the antibiotics. Therefore, the antibiotic use may have normalized the processing of emotional information as depressive symptoms are predicted by alterations in emotional processing (Beck, 2008; Harmer, 2008). Another major limiting factor of the present study was the fact that the subjective experience of the stimuli used during all computerized tasked designed to tap into different aspects of emotional processing was not assessed. Therefore, it is possible that the participants did not perceive the selected stimuli as intended (e.g. not perceiving a negative descriptor as an undesirable trait during the emotional categorization task). This potential lack of emotional relevance of the stimuli may have been especially detrimental during the emotional attention task as it may have prevented the activation of emotional appraisal processes which are hypothesized to be required in order to induce attentional biases towards emotional stimuli in most theories of emotional attention (Yiend, 2010). Lastly, given that the types of antibiotics used by the participants appeared to be heterogeneous it is possible that some types of antibiotics had a more profound impact on the gut microbiota than other types. Taken together, these factors along with the limited number of participants in the antibiotics group could have obscured the effect of antibiotic use on emotional processing making it impossible to detect using the present study design.

Related to the previously mentioned limitation regarding microbial dysbiosis the current study also failed to measure immunological factors. Given that the effects of the gut microbiome on cognition are understood to be indirect and complex (Sarkar et al., 2018) it is likely that the potential association between the gut microbiome and emotional processing is moderated and or mediated by other variables. Given that depressive symptoms and negativity biases are associated with increased inflammatory activity (Bollen et al., 2017; Maes et al., 2008; Maes, et al., 2012) it is

possible that the relation between antibiotic-induced microbial dysbiosis, which may result in increased gut permeability (Van Ampting et al., 2010) is mediated by proinflammatory cytokines. This notion would fit the observation that peripheral inflammatory activity activates the hypothalamic pituitary adrenal (HPA) axis through afferent fibers of the vagus nerve (Bonaz, Sinniger, & Pellisier, 2016). Overactivity of the HPA axis is in turn a consistent finding in depressive disorders (Pariante & Lightman, 2008). Moreover, cortisol secretion resulting from HPA axis activity affects the encoding and retrieval of emotional information but also the allocation of attention towards or from emotional stimuli (Erickson, Drevets, & Schulkin, 2003). It is, therefore, possible that alterations of the gut microbiota exert their effect through the modulation of inflammatory activity which in turn affects the HPA axis which may affect emotional processing through cortisol secretion. However, it must be noted that it remains unclear whether the full depression inducing effect of antibiotics is (fully) mediated by proinflammatory cytokines as Lurie et al. (2016) argued based on their previous findings (Lurie et al., 2015) that antibiotics may increase anxiety and depressive symptoms through non-cytokine pathways.

Another immunologic variable which may affect the relation between the gut microbiota and emotional processing is Immunoglobulin A (IgA). IgA can be found in both the serum and in mucosal secretions (Delacroix, Dive, Rambaud & Vaerman, 1982). Serum IgA is understood to serve a regulatory role in immune activity by serving as an anti-inflammatory agent under normal circumstances but as an upregulator of immune system activity during inflection (Leong & Ding, 2014). Depression has been associated with increased serum IgA levels as a result of the leaking of toxic bacterial products through the intestinal barrier (Maes et al., 2008; Maes et al.,2012). Secretory IgA, on the other hand, plays an important role in mucosal immunity by protecting the intestinal epithelium from pathogens (Mantis, Rol, & Corthésy, 2011). Secretory IgA is also understood to coat commensal gut microbes in order to prevent an inflammatory response towards these symbiotic bacteria (Mantis et al., 2011). Gut microbes are also able to induce Secretory IgA release by either binding to receptors located on the intestinal epithelium which results in

upregulation of immunoglobulin receptors which allows for increased secretory IgA release into the gut (Kaetzel, 2014). Additional evidence for this finding is derived from probiotic studies that found that administration of probiotics secretory IgA resulted in an increase in secretory IgA (Carasi et al., 2017; Lai, Chiu, Kong, Chang, & Chen, 2019; Lefevre et al., 2015) and antibiotic studies which showed the opposite pattern (Ruiz et al., 2015; Zhang et al., 2018). Additionally, secretory IgA is also involved in shaping the gut microbiome composition of mice by selectively targeting bacteria in the gut that may cause inflammation and diseases (Kubinak & Round, 2016). Similar results were found in humans by Catanzaro et al. (2019) who demonstrated that individuals diagnosed with selective IgA deficiency, which is characterized by reduced secretory IgA levels, displayed reduced microbial diversity compared to controls. Secretory IgA may therefore moderate the relation between the gut microbiota and emotional processing as secretory IgA may maintain the homeostasis in the gut microbiota and therefore reducing inflammatory activity which in turn may affect emotional processing. Optimal levels of secretory IgA may attenuate the hypothesized detrimental effect of antibiotic-induced microbial dysbiosis on emotional processing whereas suboptimal secretory IgA levels may exacerbate this effect. It is therefore possible that secretory IgA concentrations moderate the strength of the relation between the expected negativity bias in the classification, recall, recognition and attention of emotional stimuli and antibiotic-induced microbial dysbiosis.

To conclude, based on the present study it appears that the use of antibiotics may not affect emotional processing on itself in healthy university students. Immunologic factors are likely to mediate and or moderate the hypothesized relation between alterations of the gut microbiota and changes in emotional processing. Future research should focus on identifying the immunologic factors, such as proinflammatory cytokines and secretory IgA, which may link the composition of the microbiota and emotional processing. However, given that it has been argued that antibiotics may exhort their depressogenic effects through non-cytokine pathways (Lurie et al., 2015; Lurie et al., 2016) future research should also focus on identifying these hypothesized mechanisms. In addition to the previous points, it is important to investigate the effect on the gut microbiome and emotional

processing of different types of antibiotics as this is not only important for follow up studies using a similar design but also for healthcare specialists that prescribe antibiotic treatments. Double-blind studies in which a single type of antibiotic is administered to healthy participants would be most informative. However, this kind of approach may be unfavourable as it has some serious ethical concerns (e.g. causing inflammation and increase the risk of developing a depressive disorder). Creative and innovative study designs are required to tackle such obstacles and deepen our understanding regarding the gut microbiota and emotional processing.

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Appendix 1

Table 1

Emotional categorization task words

Word	Type	Word	Type
Narrow minded	Disagreeable	Respectful	Agreeable
Angry	Disagreeable	Hostile	Disagreeable
Warm	Agreeable	Neurotic	Disagreeable
Unkindly	Disagreeable	Tender	Agreeable
Discourteous	Disagreeable	Optimistic	Agreeable
Heartless	Disagreeable	Untidy	Disagreeable
Dishonest	Disagreeable	Sociable	Agreeable
Dull	Disagreeable	Capable	Agreeable
Tolerant	Agreeable	Egoistical	Disagreeable
Happy	Agreeable	Unsocial	Disagreeable
Good	Agreeable	Underhanded	Disagreeable
Thoughtful	Agreeable	Poised	Agreeable
Touchy	Agreeable	Progressive	Disagreeable
Mean	Disagreeable	Courageous	Agreeable
Gossipy	Disagreeable	Courteous	Agreeable
Unfriendly	Disagreeable	Sportsmanlike	Agreeable
Relaxed	Agreeable	Neglectful	Disagreeable
Unpleasing	Disagreeable	Cheerful	Agreeable
Wasteful	Disagreeable	Kind-hearted	Agreeable
Agreeable	Agreeable		