The Gut-Brain Axis: Impact of a Probiotic Intervention on Neurocognitive Measures of Emotion and Cognitive Control

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The human gut microbiome plays a key role in human well-being. Specifically, differences have been found between the gut microbiome compositions of healthy individuals compared with patient groups suffering from affective psychiatric disorders. Research has indicated that probiotics can have beneficial effects on the gut microbiome and its functioning as well as on the brain. In the present study we investigated the effects of a multispecies probiotic on neurocognitive measures of emotion and cognitive control. We were interested in whether the effects of a probiotic intervention can extend beyond effects on affective measures and associated brain regions, also including cognitive control (of emotion). Analyses were carried out on a first, small subset of participants (n = 11) who were divided into a placebo (n = 5) and a probiotic group (n = 6). In this study all participants were healthy women (mean age 23.3 years) with a body mass index (BMI) ranging from 19.2 – 24.8. The study consisted of two measurement points separated by a four-week probiotic vs. placebo intervention. In this preliminary study we did not find significant effects of the probiotic intervention on the brain. However, on exploratory thresholds, findings indicated an effect of the probiotic intervention on the amygdala during emotion regulation as well as on lateral frontal regions during general cognitive control processes, in line with our hypotheses. From these preliminary results we can conclude that probiotic effects tend to extend beyond modulating affective processes, also tending to affect prefrontal cortex and associated cognitive control processes.

Keywords: gut microbiome, affective psychiatric disorders, probiotic, amygdala, prefrontal cortex, hypothalamic-pituitary-adrenal (HPA) axis, inflammation

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The human gut microbiome plays a key role in human well-being (Dinan, Stanton, & Cryan, 2013; Rook, Lowry, & Raison, 2013). The intestinal microbiota, collectively referred to as gut-microbiome, is a metabolic ecosystem consisting of microorganisms that outnumber the total amount of human cells in the body by far (Dinan et al., 2013). The gut microbiome is crucial for digestion and is involved in the development of the immune system, helping protect the body against pathogens (e.g., Shreiner, Kao, & Young, 2015; Smith, McCoy, & Macpherson, 2007; Wu & Wu, 2012). Additionally, it has a role in regulating endocrinological processes as well as gastrointestinal nerve activity (Allin, Nielsen, & Pedersen, 2015) and has been shown to influence several aspects of behaviour, stress, mood and cognition (Cryan & Dinan, 2012; Desbonnet, Clarke, Shanahan, Dinan, & Cryan, 2014; Mayer, 2011; Neufeld, Kang, Bienenstock, & Foster, 2011).

Bercik et al. (2011) provided evidence for the involvement of gut microbiota in mice's behaviour by showing that a short-term perturbation of the microbiota with an administration of antimicrobials[1] (ATM) increased exploratory behaviour in mice. Additionally, germ-free mice, ones without a gut microbiome or only a small amount of it therefore characterised by an undeveloped immune system, did not show altered behaviour in response to ATM. These findings indicate that the microbiome is necessary for these behavioural effects to take place rather than ATM working on the brain directly (Bercik et al., 2011). Furthermore, when mice showing timid behaviour received a gut microbiome transplant of mice that were relatively outgoing, a personality shift towards more outgoing behaviour could be observed (Bercik et al., 2011).

The gut microbiome has also been indicated to be crucial for stress regulation, since it is involved in the development of the hypothalamic-pituitary-adrenal (HPA) axis (i.e., an important stress regulation mechanism). Germ-free mice, for instance, displayed an increased HPA reaction to stress indicating a malfunction of the HPA axis and associated hormone secretion (Sudo et al., 2004). Additionally, early life stress has been linked to alterations in gut microbiome composition and was found to be a risk factor for the development of depression later in life (Juruena, 2014; O'Mahony et al., 2009). Excessive amounts of stress signals have generally been linked to various changes in the body, for instance altered hormone levels (Hargreaves, 1990) that can in turn lead to changes in neuroendocrine processes such as the HPA response to stress (e.g., Sudo et al., 2004). Additionally, inflammations can follow from stress signals that can, for example, take place in the gut. These inflammations are able to disrupt the functioning of the intestinal epithelial barrier. This barrier consists of a single layer of mucosa and restricts access to the gut to water, nutrients and electrolytes, preventing toxins and bacteria from entering. Its dysfunction, not only caused by stress but multiple factors including heredity, diet, exercise and drugs, can lead to increased amounts of lipopolysaccharides (LPS), parts of the cell membrane of gram-negative bacteria, entering the blood (Söderholm & Perdue, 2001; Santos et al. 2001; Van Hemert & Ormel, 2014). Big quantities of LPS can in turn provoke increased immune signalling causing new inflammatory reactions (Van Hemert & Ormel, 2014). Food allergies, diabetes, chronic fatigue and chronic intestinal disorders such as irritable bowel syndrome (IBS), associated with impaired epithelial barrier function, can follow from these inflammatory reactions (Dinan et al., 2013; Messaoudi et al., 2011).

The pro-inflammatory cytokines which are released by the immune system as a reaction to increased levels of LPS are also able to enter the central nervous system with the potential of influencing various processes in the brain by interacting with its cytokine network (Capuron & Miller, 2011). Increased levels of pro-inflammatory cytokines may, for instance, have effects on the neuroendocrine system in the context of depression; alterations such as stimulation of the corticotrophin-releasing hormone and thereby HPA activity, which is important for stress regulation (Miller, 1998), can occur. Therefore, a link between the gut microbiome and affective psychiatric disorders has gained recent interest. Naseribafrouei and colleagues (2014) have shown that healthy individuals exhibited a different gut microbiome composition relative to individuals suffering from depression. Additionally, differences in immune functioning as well as related increased levels of inflammation markers in the blood and brain have been linked to different psychiatric disorders (mainly depression) as well as to declines in cognitive functions such as working memory and learning performance (Capuron & Miller, 2011; Gimeno et al., 2009; Rook et al., 2013; Sparkman et al., 2006). The high co-morbidity between affective psychiatric symptoms (e.g., anxiety or depression) and various chronic intestinal disorders such as IBS provides additional evidence for a role of the intestinal microbiota in gut-brain communication (Bercik et al., 2011).

1 Agent that can kill microbiota.

Probiotics[2] are one kind of treatment indicated to have the potential of beneficially influencing gut-functioning and gut microbiome composition in animals and humans and has been found to reduce symptom severity in patients with (chronic) intestinal disorders (e.g., Kajander, Hatakka, Poussa, Färkkilä, & Korpela, 2005; Moayyedi et al., 2010). By means of probiotics, it was possible to investigate the role of the intestinal microbiota in the regulation of anxiety, mood, cognition, pain and behaviour in rodents (Chen, D'Souza, & Hong, 2013; Cryan & Dinan, 2012; Foster & McVey Neufeld, 2013). Probiotics have been shown to lower levels of systemic inflammatory cytokines and up-regulate plasma IL-10 levels, a cytokine suggested to have anti-inflammatory properties, in vivo (in mice) and in vitro. These two findings are of importance considering the link between elevated pro-inflammatory cytokine levels and depression (Ghosh, Van Heel, & Playford, 2004; Kopp et al., 2008; Logan & Katzman, 2005). In a study with human participants a reduction in sad mood by means of a self-reported questionnaire has been shown after a probiotic intervention (Steenbergen, Sellaro, Van Hemert, Bosch, & Colzato, 2015). Additionally, decreased activity in different affective (including amygdala and insula), viscerosensory and somatosensory brain regions in response to an emotional face matching paradigm after intake of a fermented milk product versus no intervention has been indicated in the first neuroimaging (i.e., functional Magnetic Resonance Imaging [fMRI]) study in this field (Tillisch et al., 2013). Overall, existing evidence suggests various positive effects of probiotics on the gut and brain, yet without uncovering the neurocognitive mechanisms underlying these effects. The study by Tillisch and colleagues was a first attempt to investigate probiotic effects on the brain in humans. Nonetheless, due to a number of limitations — very small group sizes (ranging from 10 to 12 subjects) and specific findings showing effects only in comparison with a no-intervention group instead of placebo, results should be interpreted with caution.

To investigate the effects of a multispecies (i.e., consisting of multiple strains of bacteria) probiotic and to unravel its underlying neurocognitive mechanisms (i.e., the gut-brain mechanism) with respect to emotion processing, emotion regulation and cognitive control processes, an extended design was used in the present study. Specifically, we added two extra paradigms in addition to the one used by Tillisch et al. (2013). The design was extended since affective psychiatric disorders are not solely characterised by differences in emotion processing but also by emotion regulation and associated cognitive control processes in which the prefrontal cortex (PFC) plays an important role (Joormann & Gotlib, 2010). In a study by Bishop et al. (2004) for instance, decreased activity in PFC regions has been found in patients suffering from anxiety in a task used to study processing of threat-related distractors. These findings indicate a dysfunction of cognitive control in the context of threat in this patient population. In line with this finding, functional connectivity between PFC and amygdala has been found to increase when healthy participants were presented with unexpected threat-stimuli, which suggests an important role of the PFC to control amygdala in order to maintain goal-directed behaviour in the context of threat (Gold, Morey, & McCarthy, 2015). Additionally, since the effects of a probiotic specifically designed to improve epithelial barrier function were investigated in the current study, it is unlikely that effects are being specific to emotion processing alone since immunological mechanisms (here one of the mechanisms assumed to underlie the effects) can affect the whole system including various brain regions (Van Hemert & Ormel, 2014). For instance, cytokines, which are important in immune defense, have been shown to be able to act on the central nervous system with diverse consequences such as changes in gastric function, induction of fever, increased metabolism as well as changes in behaviour (Rothwell & Hopkins, 1995). Furthermore, as inflammations can lead to a decline in cognitive control functions such as working memory (see Sparkman et al., 2006), probiotics that improve the intestinal barrier and thereby presumably decrease inflammation, might also increase such cognitive control functions by acting on brain regions other than those involved in emotion.

In our design we included the emotional face matching paradigm (see methods for detailed task descriptions) as used by Tillisch et al. (2013) in order to study effects of the probiotic on affective brain regions (e.g., amygdala) involved in emotion processing. Furthermore, an emotional face Stroop paradigm as used by Etkin, Egner, Peraza, Kandel, & Hirsch (2006) has been chosen to investigate intervention-induced effects on emotion regulation. This paradigm has been shown to capture activation of PFC (medial and lateral PFC [mPFC/lPFC], including supplementary motor areas) and affective

2 Probiotics are defined by the World Health Organisation as "live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host.

regions (e.g., amygdala) in particular (Etkin, Egner, & Kalisch, 2011). In addition, investigating intervention-induced effects on cognitive control processes in the absence of emotion, widely associated with dorso-medial (dm) frontal regions (e.g., anterior cingulate cortex [ACC] and [pre-] supplementary motor area [SMA]) and lateral PFC (e.g., inferior frontal gyrus [IFG] and middle frontal gyrus [MFG]) was implemented by means of a classic colour-word Stroop task (Courtney, Petit, Haxby, & Ungerleider, 1998; Stroop, 1935).

In the present study we put forth several predictions: based on the findings by Tillisch et al. (2013) we expected to find (1) intervention-induced *decreases* in the activity of affective brain regions (e.g., amygdala) in response to the emotional face matching paradigm. In addition, we predicted to find (2) intervention-induced increases in PFC activity, especially ventro-medial (vm) and dorso-medial (dm) PFC (including [pre-]SMA) in response to the emotional face Stroop paradigm along with (3) *decreased* activity in affective brain regions (e.g., amygdala). Beyond that we predicted (4) an increase in the connectivity between prefrontal and affective cortices in this task, suggesting intervention-induced enhances in cognitive control over affective cortices (not further described here). In the classic colour-word Stroop task we also expected to find (5) intervention-induced increases in PFC activity, especially dmPFC and lPFC, suggesting enhancement of cognitive control processes in absence of emotion.

This part of the study includes only a small, first subsample (n = 11) of the data (the final sample will include sixty participants) in order to test the validity of the paradigms used in the study and to explore some of the hypotheses stated above.

Methods

Participants

In total, 11 of the planned 60 participants, randomly (double-blind) divided into the probiotic intervention group (n = 6, age M = 21.3 years, SD = 2.3) and the placebo group (n = 5, age M = 25.6 years, SD = 3.8) were analysed in this study. All participants were healthy women (age M = 23.3 years, SD = 3.6), with a body mass index (BMI) ranging from 19.2 – 24.8 (M = 22.0, SD = 2.0), a range considered to be healthy taking age and gender into account, both in the probiotic (BMI M = 21.1, SD = 1.4) and the placebo group (BMI M = 23.1, SD = 2.2). Except for two participants who graduated from university already, all participants were university students. All participants took hormonal contraceptives and were not in the stop week during test sessions to ensure similar hormone levels between both sessions across participants. They were screened for medical conditions (including neurological, psychiatric, gastrointestinal or endocrine disorders) and relevant medical history. Furthermore, participants were screened for MRI compatibility, probiotics and prebiotic use, diet, alcohol and smoking behaviour. In order to ensure good task comprehension and clear understanding of the neuropsychological questionnaires (not described here), all participants exhibited sufficient knowledge of Dutch. The study was conducted following the Declaration of Helsinki with human subjects and the complete procedure was approved by the local Ethics Committee CMO Arnhem-Nijmegen. Written informed consent was obtained from each participant.

Procedure

We employed a double-blind, randomised, placebo-controlled, between-subject design. The study consisted of two sessions separated approximately five weeks in time. During four (28 days) of the five weeks an intervention consisting of daily probiotic or placebo intake was implemented. Both sessions of the experiment were conducted at the Donders Centre for Cognitive Neuroimaging in Nijmegen, the Netherlands. Feces samples were taken, one before the start of the intervention and one after taking the last probiotic/placebo (within a time window of approximately 29 – 40 days). At the beginning of the first test session, the experimental procedure was explained and informed consent was obtained from the subjects. Physical measurements including height, weight, waist circumference and blood pressure were taken. Participants practised the tasks that were performed in the scanner at a later stage and were asked to fill out different questionnaires (not described here). An MRI part of 75 minutes including acquisition of anatomical, functional and resting state images followed during which participants had to perform the tasks they had practised earlier (starting with an emotional face matching paradigm, followed by an emotional Stroop task and ending with a classical colour-word Stroop task). The MRI part was followed by another session outside of the scanner consisting of neuropsychological and dietary questionnaires participants had to fill in as well as a stress test (not reported here). At the end of the first test session,

subjects were provided with the probiotics/placebo and instructions on how to take it as well as with the toolkit for the feces samples. After the intervention, participants came to the centre for the second test session, which resembled the first one.

fMRI tasks

In this study, three tasks were chosen to be performed in the MRI scanner, including the emotional face matching paradigm, an emotional Stroop task and a classic colour-word Stroop paradigm. The experiment was performed using Presentation® software (Version 0.70, www.neurobs.com). Trial sequences of each task were pseudo-randomised in order to guarantee equal numbers of presentations of each stimulus type. A different version of each task was performed in the first and the second test session; the order was counterbalanced.

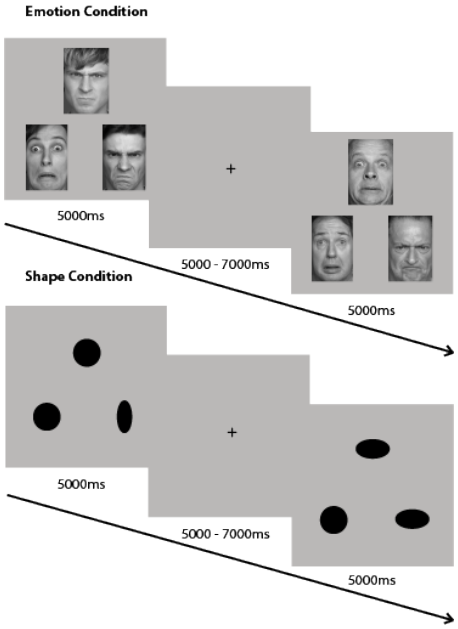

Fig. 1. Emotional face matching paradigm: example stimuli.

Emotional face matching paradigm. This paradigm was chosen to investigate intervention-induced changes in emotional processing or 'reactivity' (Hariri, Bookheimer, & Mazziotta, 2000). A block design was used for this task with a total of 18 blocks consisting of three stimuli each. The task included two different conditions, a control and an emotion condition. In the control condition subjects had to match one of two geometric shapes presented at the bottom to a target shape presented at the top of the screen. The experimental condition involved subjects choosing one of two emotional (angry or fearful) faces presented at the bottom of the screen as best matched the emotional expression of a face seen at the top of the screen (see Fig. 1 for example). The condition was kept constant over a block duration of 17 seconds, but was randomised between blocks. Participants were asked to react as fast and accurately as possible. The total duration of the task amounted to seven minutes.

Emotional face Stroop paradigm. A Dutch version of the emotional face Stroop task (Etkin et al., 2006) was used to assess intervention-induced differences in cognitive control in the face of emotional distractors. During this task, participants were presented with pictures of male faces expressing fear or happiness. On top of the faces, the Dutch words for happy (i.e., *blij*) and fearful (i.e., *bang*) were presented in prominent red letters (see Fig. 2 for example). The emotions described by the words were either congruent with the emotion of the face or incongruent and subjects had to indicate the

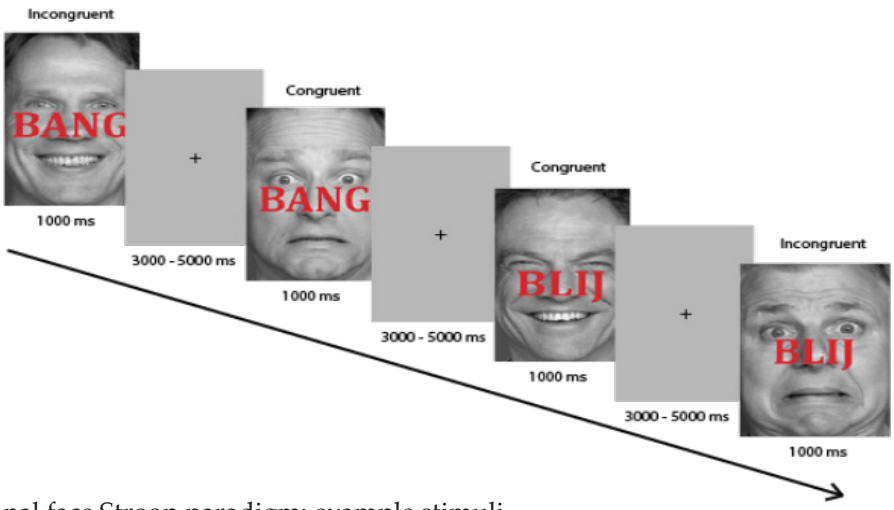

Fig. 2. Emotional face Stroop paradigm: example stimuli.

emotion of the face ignoring the emotion word. In total, stimulus presentations amounted to 148 presentations of happy or fearful male faces. The order of stimulus presentation was pseudo-randomised. The total duration of the task added up to 15 minutes.

Classic colour-word Stroop paradigm. A Dutch version of the classic colour-word Stroop task (Stroop, 1935) was used to assess intervention-induced differences in general cognitive control in absence of emotional stimuli. During this task, participants were presented with four different colour words written either in the same ink colour as the word (e.g., red written in red ink) or in an incongruent colour (e.g., red written in blue ink, see Fig. 3 for example). The task was to indicate the ink colour of the word by pressing a button mapped to that colour, and ignore the word meaning. The task consisted of 80 stimulus presentations in total. As in the other tasks, participants were asked to react as fast and accurately as possible. Colour-button mappings were randomised across subjects, but kept constant between the two sessions of each subject. The total duration of the task amounted to approximately 10 minutes, depending on participants' performances on the practice trials.

MRI data acquisition

MRI data were acquired using a 3T MAGNETOM Prisma system, equipped with a 32-channel head coil. During the three tasks, 3D echo planar imaging (EPI) scans (using a T2*-weighted gradient echo multi-echo Echo Planar Imaging (EPI) sequence [Poser, Versluis, Hoogduin, & Norris, 2006]) were acquired (voxel size = 3.5 × 3.5 × 3 mm isotropic; repetition time [TR] = 2070 ms; echo time [TE] = 9 ms, 19.25 ms, 29.5 ms, 39.75 ms; field of view [FoV] = 224 mm). The slab positioning and rotation (average angle of 14 degrees to AC axis) optimally covered both prefrontal and deep brain regions (i.e., including affective brain regions such as amygdala). During the tasks, thirty dummy volumes were discarded immediately before the main scan to allow for the weight calculations of the four echoes used for image reconstruction. The total scan duration was about 75 minutes. Whole-brain high-resolution T1-weighted anatomical scans were acquired using an MPRAGE sequence (voxel size = 1.0 × 1.0 × 1.0 mm isotropic, TR = 2300 ms, TE = 3.03 ms, 192 slices).

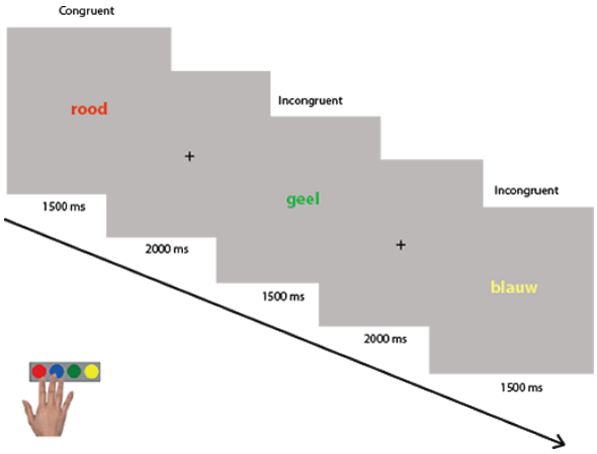

Fig. 3. Classic colour-word Stroop paradigm: example stimuli.

fMRI data preprocessing

Processing of the data was implemented using Statistical Parametric Mapping (SPM8, Wellcome Department of Imaging Neuroscience, London). Volumes for each echo-time were realigned using six rigid body spatial transformations (translations and rotations). Thirty volumes acquired before the tasks were used to combine the four echo images into a single MRI volume using an echo weighting method known as PAID-weighting (Poser et al., 2006). Resulting combined functional (EPI) images were slice-time corrected by realigning the time series for each voxel to the time of acquisition of the reference slice (here slice 17). Subject-specific structural and functional data were subsequently co-registered to a standard structural or functional stereotactic space respectively, to Montreal Neurological Institute (MNI) templates. A unified segmentation approach was then used to segment the structural images, which were subsequently spatially co-registered to the mean of the functional images. The transformation matrix resulting from the segmentation step was used to normalise the structural and functional images to MNI space, resampled at a voxel size of 2 × 2 × 2 mm. In a final step, normalised functional images were spatially smoothed using an 8-mm full-width at half maximum (FWHM) Gaussian kernel.

Statistical analyses

First level fixed effects analyses of fMRI data were performed using an event-related approach for both Stroop paradigms. The statistical model for event-related fixed effects analyses contained two

regressors of interest for the classic colour-word Stroop paradigm representing the different task conditions (correct incongruent and congruent trials) and four regressors of interest for the emotional face Stroop paradigm (incongruent trials followed by congruent trials, congruent trials followed by incongruent trials, congruent trials followed by congruent trials and incongruent trials followed by incongruent trials). Miss and incorrect trials were taken into account in a regressor of no interest for both of these paradigms. First level analyses of the emotional face matching paradigm were carried out using a block-design fMRI approach with block duration of 17 seconds. Onsets of the independent regressors for the event-related Stroop paradigms were modelled as a stick function convolved with the canonical hemodynamic response function (HRF) (Friston et al., 1998). Additionally, twelve regressors of no interest were added in order to account for motion artifacts consisting of twelve rigid-body transformation parameters (i.e., movement regressors consisting of three translations and rotations and their linear derivatives) obtained during realignment. A high-pass filter with a cut-off of 128 seconds was applied to the time-series of the functional images to remove low-frequency drifts. By applying an autoregressive AR(1) model, correction for serial correlations was carried out.

Three GLMs were run as random effect second level analyses based on the different contrast images of the contrasts applied in the first level analyses of each of the three tasks. For the emotional face Stroop, a GLM with the contrast images of adaptation minus non-adaptation ([incongruent – congruent, congruent – incongruent] > [congruent – congruent, incongruent – incongruent]) was run. For the classic colour-word Stroop paradigm, incongruent minus congruent contrast images were used (incongruent > congruent) for the GLM and the GLM for the emotional face matching paradigm was based on the contrast images of emotion minus shape (emotion > shape). Analysis of variance (ANOVA) was performed in a full-factorial design with the above-specified contrast images from first level analyses and two additional factors were added at second level analyses, 'Group' (probiotic, placebo) as a between-subject factor and 'Time' (pre- or post-intervention) as a within-subject factor.

In the presented figures, results are displayed at exploratory thresholds of $p < .001$ (uncorrected) and $p < .005$ (uncorrected). Whole-brain corrected results at p *corr (FWE)* $< .05$ (cluster-level, with intensity threshold $p < .001$) are reported in the tables. MarsBaR was used to extract regionally-averaged beta weights at $p < .001$ (uncorrected) and $p < .005$ (uncorrected) for two brain regions in the Stroop paradigms for illustrative purposes.

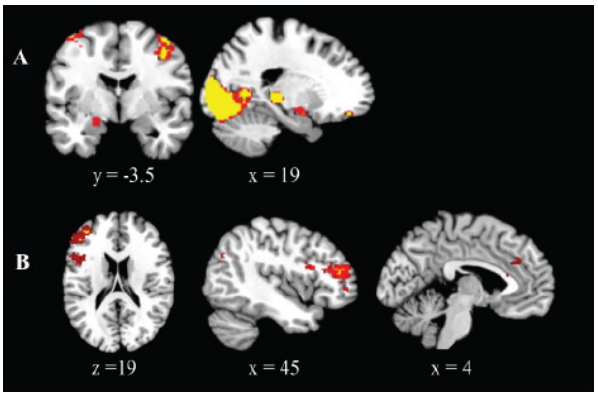

Fig. 4. Emotional face matching: positive task effects and effects of probiotic intervention on brain activity. **A.*** Positive task effects of affective matching (emotion > shape). **B.*** Effects of the probiotic intervention on the brain (Probiotic > Placebo, Pre > Post). Here regions are displayed that showed more activity for the probiotic group relative to the placebo group, in the pre- compared with the post-session of the study.
* Results are displayed at exploratory thresholds in *yellow* at $p < .001$ (uncorrected) and in *red* at $p < .005$ (uncorrected). Images are shown in radiological orientation, left = right (MNI coordinates).

Results

Imaging data

Emotional face matching paradigm. A main task effect of emotion processing, matching affect (emotion > shape) was shown in various brain regions including occipital, temporal and frontal regions as displayed in Table 1. On an exploratory threshold of $p < .005$ (uncorrected) amygdala activation could be observed as well (see Fig. 4A). We did not find a significant effect of the probiotic (Probiotic > Placebo, Pre > Post) on brain regions during emotional face matching at the stringent threshold of p *corr (FEW)* $< .05$ for this paradigm. Even on exploratory thresholds of $p < .001$ (uncorrected) and $p < .005$ (uncorrected) we did not observe significant amygdala deactivation, yet a deactivation of a medial frontal region ($p < .005$, uncorrected) as well as IFG could be observed when the probiotic group was compared with the placebo group (Pre > Post, $p < .001$, uncorrected) (see Fig. 4B).

Table 1.

Main task effect of emotion processing (emotion – shape) and (shape – emotion). Clusters showing greater BOLD* activity for the match emotion condition compared to the match shape condition, (whole brain corrected *pFWE* < .05). MNI stereotactic coordinates of local BOLD maxima.

Region	cluster *p* (FEW-corrected)	cluster equivk	peak T	x, y, z {mm}
Emotion processing				
(emotion > shape)				
Right inferior occipital gyrus	.000	13661	26.00	44, -80, -10
Left inferior occipital gyrus			20.74	-18, -98, -8
Right cuneus			19.42	16, -96, 6
Right hippocampus	.001	489	8.19	22, -30, -2
Left thalamus			7.55	-20, -28, -2
Midbrain			4.41	-4, -32, -4
Right inferior frontal gyrus pars opercularis	.000	746	6.37	38, 14, 26
Right middle frontal gyrus			5.40	44, 32, 18
Right inferior frontal gyrus pars triangularis			5.21	48, 14, 24
Right superior temporal gyrus	.004	363	5.50	50, -40, 14
Right middle temporal gyrus			4.28	50, -48, 6
Left inferior frontal gyrus pars triangularis	.021	248	4.56	-44, 14, 26
Left inferior frontal gyrus pars triangularis			4.01	-44, 20, 18
Right pre-supplementary motor area	.038	212	4.55	4, 16, 52
Left pre -supplementary motor area			3.91	-2, 24, 48
(shape > emotion)				
No significant clusters				

*BOLD = Blood oxygenated level dependent

Emotional face Stroop paradigm. Main task effects of emotional Stroop adaptation revealed significant medial and lateral frontal cortex activation as expected at *p corr (FEW)* < .05 (see Table 2, Fig. 5A). Additionally, on an exploratory threshold of *p* < .005 (uncorrected) amygdala deactivation could be observed. We did not observe any significant effects of the probiotic intervention on brain regions during emotional Stroop adaptation. Yet at an exploratory threshold of *p* < .005 (uncorrected),

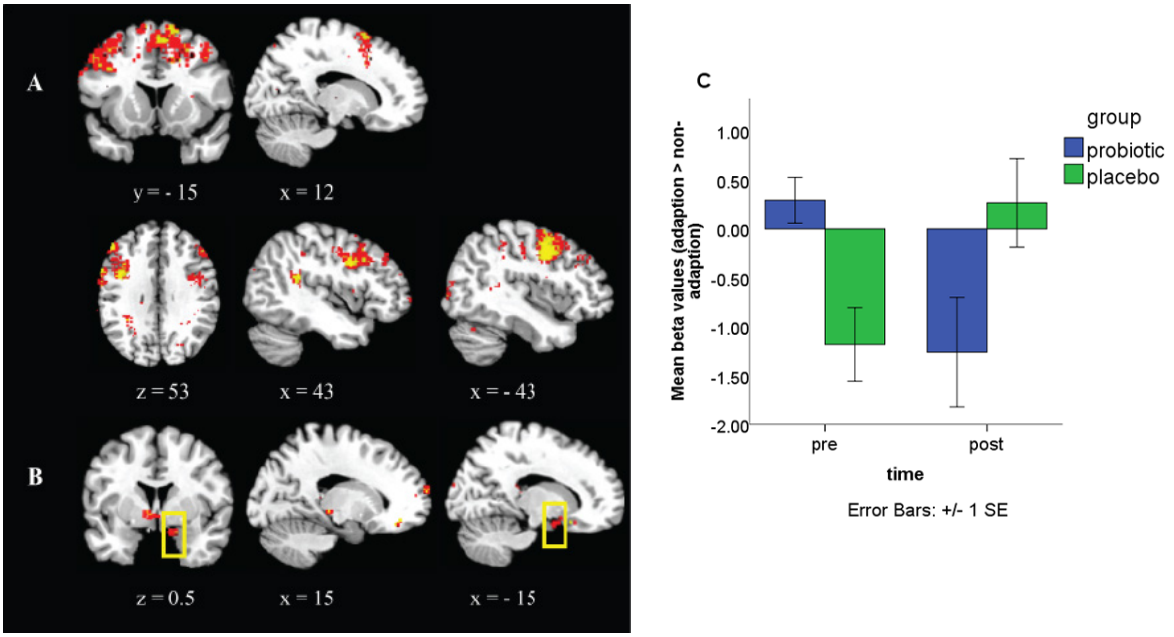

Fig. 5. Emotional face Stroop: positive task effects and effects of probiotic intervention on brain activity. **A.*** Positive task effects of emotional Stroop adaptation (adaptation > non-adaptation). Here, brain regions are displayed showing more activity during adaptation compared with non-adaptation trials. **B.*** Effects of probiotic intervention on the brain during emotional Stroop adaptation (adaptation > non-adaptation) (Probiotic > Placebo, Pre > Post). Here, brain regions are displayed showing more activity for the probiotic relative to the placebo group in the pre- compared with the post-session.
C. Extracted mean beta values (adaptation > non-adaptation) from an ROI created for the amygdala (MNI coordinates: -16 4 -22) based on the functional images received from the Group × Time interaction contrast on (Probiotic > Placebo, Pre > Post) at $p < .001$ (uncorrected).
* Results are displayed at exploratory thresholds in *yellow* at $p < .001$ (uncorrected) and in *red* at $p < .005$ (uncorrected). Images are shown in radiological orientation, left = right (MNI coordinates).

regions in the frontal cortex including vmPFC were shown to be *deactivated* for the probiotic versus placebo group after the intervention, contrary to our hypothesis. Minor amygdala *deactivation* could be observed as well ($p < .001$, uncorrected; more clearly at $p < .005$, uncorrected) when groups were compared after intervention, being in line with our hypothesis (see Fig. 5B). For illustrative purposes we created a region of interest (ROI) for this region of activation (see Fig. 5C) from functional images received from the Group × Time interaction contrast (Probiotics > Placebo, Pre > Post; $p < .001$, uncorrected). Mean beta values (adaptation > non-adaptation) were extracted showing a *decrease* in amygdala activity for the probiotic group (Pre > Post), as expected. The opposite could be observed for the placebo group.

Classic colour-word Stroop paradigm. Main task effects of the Stroop effect revealed significant activations for regions in left lateralised frontal regions (see Table 3 and Fig. 6A). We did not observe significant effects of the probiotic intervention on brain regions during this paradigm, however at an exploratory threshold of $p < .005$ (uncorrected), activation of lPFC (i.e., IFG) for the probiotic group compared with the placebo group (Post > Pre) could be observed in line with our hypothesis. Additionally, we found vmPFC activity for this contrast (see Fig. 6B). For illustrative purposes we created an ROI for the area in the lPFC (see Fig. 6C) based on the functional images received from the Group × Time interaction contrast (Probiotic > Placebo, Post > Pre; $p < .005$, uncorrected). Mean beta values were extracted showing an increase in activity for the probiotic group, as expected (Post > Pre). The placebo group showed effects in the opposite direction.

Discussion

In the present study our aim was to investigate the effects of a multispecies probiotic on brain functioning and its underlying mechanisms. We were particularly interested in whether the probiotic effects would extend beyond emotion processing and associated brain regions, also affecting brain regions involved in emotion regulation and general cognitive control processes.

Table 2.

Main task effects of emotional Stroop adaptation (adaptation – non-adaptation) and (non-adaptation – adaptation). Clusters showing greater BOLD* activity for adaptation trials compared to non-adaptation trials (whole brain corrected $p_{FWE} < .05$). MNI stereotactic coordinates of local BOLD maxima.

Region	cluster *p* (FWE-corrected)	cluster equivk	peak T	x, y, z {mm}
Emotional Stroop adaptation				
(adaption > non-adaptation)				
Left precentral gyrus	.000	441	6.24	-38, -2, 44
Left precentral gyrus			5.96	-46, -2, 38
Left precentral gyrus			5.25	-38, 0, 36
Right precentral gyrus	.001	189	5.30	42, 0, 32
Right inferior frontal gyrus pars opercularis			5.28	38, 16, 32
Right inferior frontal gyrus pars opercularis			4.31	46, 14, 34
Right pre-supplementary motor area	.002	167	4.93	6, 4, 68
Left pre-supplementary motor area			4.47	0, 8, 54
Left pre-supplementary motor area			4.36	-8, 16, 54
(non-adaptation > adaptation)				
No significant clusters				

*BOLD = Blood oxygenated level dependent

Main effects of tasks

Despite the small sample size studied here, we found sufficient task-related brain activations in line with findings of earlier studies, thus we can confidently conclude that the tasks used in our design functioned as expected. This is not very surprising since we used three robust paradigms in order to ensure that we could measure the effects we were interested in. The significant activation of thalamus during the emotional face matching task for instance, a region known to influence amygdala activity, is in line with findings by Hariri et al. (2000). Additionally, activation in bilateral inferior occipital gyri (IOG), a region shown to be involved in face processing, also indicated that this task worked well since it was found when brain activation for matching of emotional faces was compared with matching of shapes (Haxby, Hoffman, & Gobbini, 2000). Significant task-related brain activations in the emotional face Stroop task in medial and later PFC, particularly right IFG and bilateral pre-SMA, as well as *deactivation* of the vmPFC when adaptation trials were compared with non-adaptation trials were also in line with earlier studies using similar paradigms (Roberts & Hall, 2008; Etkin et al., 2006). Finally, the classic colour-word Stroop task activated medial and lateral frontal areas as well, here also including left pre-SMA and left IFG, regions often found to be activated during (Stroop) conflict paradigms (e.g., Roberts & Hall, 2008; Zoccatelli, Beltramello, Alessandrini, Pizzini, & Tassinari, 2010).

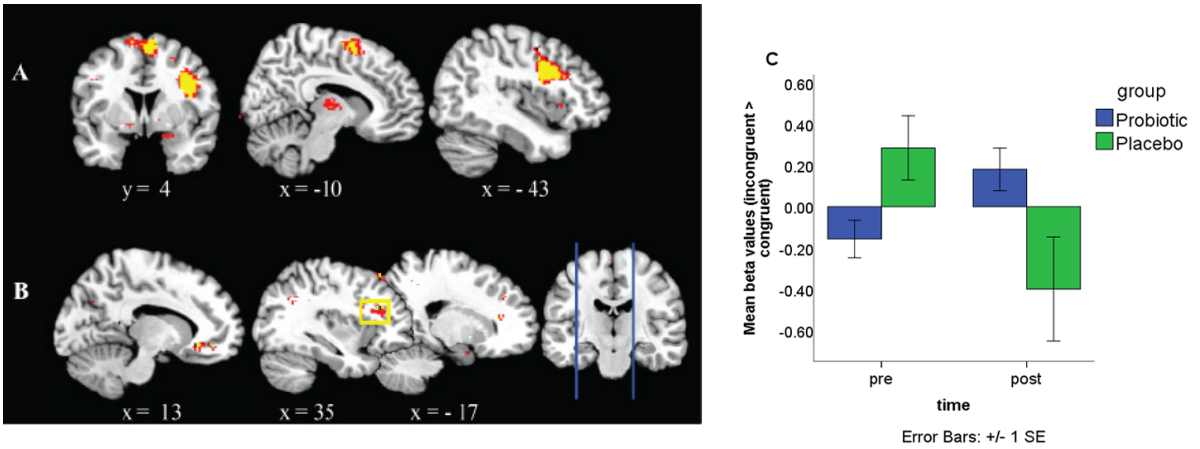

Fig. 6. Classic colour-word Stroop: positive task effects and effects of probiotic intervention on brain activity. **A.*** Positive task effects of Stroop effect (incongruent > congruent). Here, brain regions are displayed showing more activation during incongruent versus congruent trials. **B.*** Effects of probiotic intervention on the brain during Stroop effect (incongruent > congruent) (Probiotic > Placebo, Post > Pre). Here, brain regions are displayed showing more activation for the probiotic relative to the placebo group in the post- compared with the pre-session.
C. Extracted mean beta values (incongruent > congruent) from an ROI created for a region in the LPFC (MNI coordinates: 34 26 20) based on the functional images received from the Group × Time interaction contrast on (Probiotic > Placebo, Post > Pre) at $p < .005$ (uncorrected).
* Results are displayed at exploratory thresholds in *yellow* at $p < .001$ (uncorrected) and in *red* at $p < .005$ (uncorrected). Images are shown in radiological orientation, left = right (MNI coordinates).

Probiotic intervention effects

The probiotic intervention did not show significant effects on brain regions during the utilised paradigms, which is not surprising considering the very small sample size of $n = 6$ in the probiotic group and $n = 5$ in the placebo group. Yet, with exploratory, uncorrected thresholds indications of probiotic effects on brain activation in all three tasks could be seen of which some were in line with our hypotheses. Analyses showed an intervention-induced decrease in amygdala activity for the probiotic group compared with the control group only for the emotional face Stroop paradigm. Earlier studies have found the amygdala to be crucial for experiencing emotional conflict (Etkin et al., 2006). This preliminary finding could thus indicate that participants in the probiotic group experienced less emotional conflict in this task after the intervention compared with the placebo group. Additionally, this indication of *decreased* amygdala activity fits with what Tillisch et al. (2013) described in their results. However, they found this *deactivation* only in comparison with a non-intervention group than in comparison between the experimental group and the control group. This result might be due to the small group sizes investigated in their study or could indicate effects of the fermented milk product (here delivery vehicle) itself. In case our preliminary finding reaches significance with the final sample in our study, we could provide evidence for effects of probiotics on this affective brain region, unlikely induced by the delivery vehicle of the probiotics since here the probiotic and placebo group were compared.

In addition to decreased activity in the amygdala we expected to find enhanced activations in lPFC and mPFC areas during both Stroop paradigms due to the probiotic intervention. However, intervention-induced enhancements in lateral frontal activation, here left IFG, could be observed only for the classic colour-word Stroop paradigm. The IFG has been shown to be involved in cognitive control processes and to be important for inhibitory control (e.g., Swick, Ashley, & Turken, 2008). This preliminary finding could thus indicate an improvement of inhibitory control in the probiotic group by means of the probiotic intervention. Intervention-induced enhancement of vmPFC activation was also found in this paradigm for the probiotic group compared with the placebo group after the intervention. This finding is not in line with our hypotheses since this region has been shown to be more involved in cognitive control processes involving emotion whereas this paradigm does not include emotion stimuli (e.g., Winecoff et al., 2013). Nevertheless, in addition to many studies indicating its importance with respect to decisions involving

emotions or varying degrees of (un)certainty, it has also been indicated that this region is crucial for general decision making (Fellows & Farah, 2007). Therefore, these preliminary findings could hint towards an improvement of general decision making, for instance fewer impulsive decisions due to the probiotic-intervention. Intervention-induced *deactivations*, rather than the expected enhancement of vmPFC, were found in the emotional face Stroop paradigm as well as the emotional face matching paradigm; for the latter we did not predict any probiotic effects on prefrontal regions. Even though these preliminary results are not in line with our hypotheses, all regions found here were close to or overlapping with the regions activated during main task effects. This overlap indicates an intervention-induced *decrease* of activity of task-related regions during emotion processing and regulation.

Nevertheless, these preliminary brain-related findings, whether unexpected or in line with our hypothesis, need to be interpreted with caution due to the small sample size studied here, which is underlined by the findings of ROI beta value extraction. The mean beta values extracted from the ROIs created from the classic colour-word Stroop (lPFC) and the emotional face Stroop paradigm (amygdala) showed effects in line with our hypothesis for the probiotic group for both brain regions. Differences in activation in these regions could be seen when the baseline activation was compared to the activation at the post-session for the two groups, revealing group differences. However, group differences at baseline could also be observed. It is thus difficult to interpret these preliminary findings. With the final sample, unbiased statistical ROI analyses wherein ROIs are created based on anatomical images, will be performed in order to see by which group and time point the effects are driven.

Underlying gut-brain mechanisms

As we had a small sample size of eleven participants and therefore examined at exploratory, uncorrected thresholds, it is difficult to draw significant conclusions about underlying neurocognitive mechanisms in this study. With the complete sample of participants we hope to find effects of the probiotic on brain regions extending beyond emotion regions, which have already been indicated with this small sample size. In case these findings reach significance in the final sample it would be plausible to suggest a general gut-brain mechanism to underlie the effects such as immunological mechanisms or the metabolic pathway (described below). The immunological mechanisms (as described in the introduction) may lead to rather whole brain than very specific effects since this mechanism is involved in various processes in the body. Additionally, gut-bacteria can produce metabolites (metabolic pathway) that can enter the bloodstream thereby affecting the local enteric nervous system as well as the central nervous system, which also suggests whole brain effects. Interactions of neurochemicals between the central nervous system and gut microbiota have been shown to be bidirectional (e.g., Lyte, 2014). The produced metabolites can be precursors of neurotransmitters or can affect those travelling through the bloodstream and cross the blood-brain barrier to affect neurotransmitter synthesis in the brain (Collins, Surette, & Bercik, 2012). Some bacterial strains have been indicated to produce, for instance gamma-Aminobutyric acid (GABA) or tryptophan, a precursor of serotonin (O'Mahony, Clarke, Borre, Dinan, & Cryan, 2015). Tryptophan, for example, can affect various brain regions as serotonergic projections can be found throughout the brain, indicating that in case this mechanism is one to play a role here, effects would concern whole brain effects rather than simply affecting specific brain regions (e.g., Charnay & Leger, 2010).

Additionally, gut microbiota can produce hormones and regulate their secretion (Neuman, Debelius, Knight, & Koren, 2015), which is important concerning stress regulation, which has been shown to be malfunctioning in germ-free mice (Sudo et al., 2004). The immunological mechanism is tightly coupled with other mechanisms and systems in the body such as the metabolic mechanism and endocrine system. Through the regulation of hormone levels the gut-bacteria can, for instance affect the immune system (Neuman et al., 2015). In order to find out which one of these mechanisms plays a significant role in the current study, or whether they interact, it is necessary to include blood samples of participants in future studies. These samples can be used to measure the amount of inflammation markers in the blood of subjects providing a more direct measurement of the mechanism via which the probiotic changes in gut microbiome might affect cognitive functions. In case results concerning the PFC disappear with the final sample and effects are specific to affective brain regions, we might have reason to conclude another gut-brain mechanism to underlie the findings. In a study by Cryan and O'Mahony (2011), anxiolytic and antidepressant effects of a bacterium in mice were prevented when animals underwent vagotomy, suggesting a role

of the vagus nerve in gut-brain communication. The vagus nerve transmits signals from the enteric nervous system to the central nervous system. Its efferent pathway is crucial for the regulation of a number of cytokines in response to stress signals in the gut, suggesting a certain degree of overlap between the vagus nerve and the immune system (Sherman, Zaghouani, & Niklas, 2015). The vagus nerve sends signals about sensations occurring in the gut back to the brain providing the nucleus tractus sollitarius with gut-related information. Subsequently the information is transmitted to the parabrachial nucleus, which is connected to various brain regions (including insular, hypothalamus and amygdala) (King, 2007; Mayer, 2011) amongst others involved in emotion processing and regulation and maintenance of bodily homeostasis. Thus, in case the vagus nerve may play a major role in gut-brain communication here, we would expect more specific, direct effects on brain areas involved in emotion processing and regulation (rather than whole brain effects, including the PFC). Nevertheless, it is likely that these different mechanisms work together to a certain degree rather than functioning completely independently.

Limitations, strengths, and future directions

For future studies, it might be interesting to include a vagus nerve intervention. Current research is intended to test effects of blocking the vagus nerve in patients with obesity (e.g., Shikora et al., 2015). In a future study the vagus nerve could be blocked for a specific period of time for one group of participants, thereby adding a direct measurement of vagal nerve contribution to gut-brain communication. However, so far there are only invasive techniques available to block or stimulate the vagus nerve, which is not ethically appropriate for the present study. However, it is also possible to make predictions about vagal activity by means of a person's heart rate variability (HRV), more specifically the respiratory sinus arrhythmia (RSA) – a measurement of heart rate change in response to inhalation and exhalation – which was first proposed to be associated with vagal activity by Hering (1910) (as stated by Berntson et al., 1997). If the vagus nerve plays a role in gut-brain communication, changes in vagal tone should be observable (Alcock, Maley, & Aktipis, 2014).

In this preliminary data set one of the biggest limitations is the small sample size per group, which limits the amount of meaningful analyses that can be applied and decreases the power of related results. The final study will include 60 participants, 30 per group, which will lead to more reliable results and presumably less noise in the data. Additionally, due to time limits in the present study trials in the classic colour-word Stroop paradigm were evenly split into congruent and incongruent ones. However, Stroop interference has been shown to be stronger when overall proportion congruency is higher, that is, when the paradigm consists of proportionately more congruent than incongruent trials, which was seen in several studies (e.g., Kane & Engle, 2003; Logan & Zbrodoff, 1979). In future studies, additional congruent trials could thus be added in order to increase the Stroop interference and related brain activations. Furthermore, we aim at performing additional analyses with the complete sample such as brain-behaviour correlation analyses, in order to receive a more detailed picture of the data. In order to investigate whether the probiotic intervention affected affective brain regions directly or rather indirectly via the PFC, connectivity analyses between mPFC regions and the amygdala will be carried out for this paradigm with the complete sample of 60 participants.

A future strength of this study concerns the collected feces samples from participants. With the final sample we will apply analyses to the feces, enabling us to look for different bacteria strains in the sample. By means of a database it is then possible to find out which metabolites these bacteria can produce.

Conclusion

Taken together, the present study investigated the effects of a multispecies probiotic in a first, small subsample of participants. We can conclude that our tasks functioned as expected, although they did not show complete congruency with findings of earlier studies. Additionally, we could observe that indications of probiotic effects extended beyond affective brain regions as was expected. These effects included *decreased* vmPFC activity during emotion regulation, and more importantly, lPFC activity enhancement during a 'pure' non-emotional cognitive control task.

Clinical relevance

This study is of high clinical relevance since it aimed at finding effects of a probiotic on brain regions such as the amygdala involved in a variety

of affective psychiatric disorders (e.g., Peluso et al., 2009). Our preliminary results indicate a reduction of amygdala activity due to the probiotics in one of the tasks, which is of specific relevance for depression as depression disorder is often associated with hyperactivation of the amygdala (Peluso et al., 2009). If this hyperactivation could be reduced by means of probiotics it might lead to a reduction in symptom severity. Thereby it might represent a potential new treatment for affective psychiatric disorders since gut microbiota have been suggested to play a crucial role in these kinds of disorders. Patients with depression disorder have been shown to have increased levels of antibodies in the blood that are secreted as immune response against LPS, suggesting a dysfunction of the epithelial barrier (Maes, Kubera, & Leunis, 2008). Restoring the epithelial barrier function might thus lead to fewer circulating inflammation markers in patients with depression disorder and might have beneficial effects on their symptom severity. Additionally, gut microbiota are involved in the production of metabolites of, for instance, precursors of serotonin. Due to evidence suggesting serotonin deficiency as a possible causal factor in a number of affective disorders, bacteria producing this precursor might be beneficial for this group of disorders (Dinan et al., 2013; Lakhan & Vieira, 2008). Future studies in these patient populations could be set up in order to test the effectiveness of a probiotic treatment for these specific disorders.

References

Alcock, J., Maley, C. C., & Aktipis, C. A. (2014). Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. *BioEssays, 36*(10), 940–949.

Allin, K. H., Nielsen, T., & Pedersen, O. (2015). Mechanisms in endocrinology: Gut microbiota in patients with type 2 diabetes mellitus. *European Journal of Endocrinology, 172*(4), R167–R177.

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., … & Collins, S. M. (2011). The Intestinal Microbiota Affect Central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice. *Gastroenterology, 141*(2), 599–609.

Berntson, G. G., Thomas Bigger, J., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., … & Van Der Molen, M. W. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology, 34*(6), 623–648.

Bishop, S., Duncan, J., Brett, M., & Lawrence, A. D. (2004). Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli. *Nature Neuroscience, 7*(2), 184–188.

Capuron, L., & Miller, A. H. (2011). Immune system to brain signaling: Neuropsychopharmacological implications. *Pharmacology & Therapeutics, 130*(2), 226–238.

Charnay, Y., & Leger, L. (2010). Brain serotonergic circuitries. *Dialogues in Clinical Neuroscience, 12*(4), 471–487.

Chen, X., D'Souza, R., & Hong, S.T. (2013). The role of gut microbiota in the gut-brain axis: current challenges and perspectives. *Protein & Cell,* 4(6), 403–414.

Collins, S. M., Surette, M., & Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. *Nature Reviews Microbiology, 10*(11), 735–742.

Courtney, S. M., Petit, L., Haxby, J. V., & Ungerleider, L. G. (1998). The role of prefrontal cortex in working memory: examining the contents of consciousness. *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 353*(1377), 1819–1828.

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience, 13*(10), 701–712.

Cryan, J. F., & O'Mahony, S. M. (2011). The microbiome-gut-brain axis: from bowel to behavior. *Neurogastroenterology & Motility, 23*(3), 187–192.

Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2014). Microbiota is essential for social development in the mouse. *Molecular Psychiatry, 19*(2), 146–148.

Dinan, T. G., Stanton, C., & Cryan, J. F. (2013). Psychobiotics: A Novel Class of Psychotropic. *Biological Psychiatry, 74*(10), 720–726.

Etkin, A., Egner, T., & Kalisch, R. (2011). Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends in Cognitive Sciences, 15*(2), 85–93.

Etkin, A., Egner, T., Peraza, D. M., Kandel, E. R., & Hirsch, J. (2006). Resolving Emotional Conflict: A Role for the Rostral Anterior Cingulate Cortex in Modulating Activity in the Amygdala. *Neuron, 51*(6), 871–882.

Fellows, L. K., & Farah, M. J. (2007). The role of ventromedial prefrontal cortex in decision making: judgment under uncertainty or judgment per se? *Cerebral Cortex, 17*(11), 2669–2674.

Foster, J. A., & McVey Neufeld, K. A. (2013). Gut–brain axis: how the microbiome influences anxiety and depression. *Trends in Neurosciences, 36*(5), 305–312.

Friston, K. J., Fletcher, P., Josephs, O., Holmes, A., Rugg, M. D., & Turner, R. (1998). Event-Related fMRI: Characterizing Differential Responses. *NeuroImage, 7*(1), 30–40.

Ghosh, S., Van Heel, D., & Playford, R. J. (2004). Probiotics in inflammatory bowel disease: is it all gut flora modulation?. *Gut, 53*(5), 620–622.

Gimeno, D., Kivimäki, M., Brunner, E. J., Elovainio, M., De Vogli, R., Steptoe, A., … & Ferrie, J. E. (2009). Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychological*

Medicine, 39(03), 413–423.

Gold, A. L., Morey, R. A., & McCarthy, G. (2015). Amygdala–Prefrontal Cortex Functional Connectivity During Threat-Induced Anxiety and Goal Distraction. *Biological Psychiatry, 77*(4), 394–403.

Hargreaves, K. M. (1990). Neuroendocrine markers of stress. *Anesthesia Progress, 37*(2–3), 99– 105.

Hariri, A. R., Bookheimer, S. Y., & Mazziotta, J. C. (2000). Modulating emotional responses: effects of a neocortical network on the limbic system. *Neuroreport, 11*(1), 43–48.

Haxby, J.V., Hoffman, E.A., & Gobbini, M.I. (2000). The distributed human neural system for face perception. *Trends in Cognitive Sciences, 4*(6), 223–233.

Joormann, J., & Gotlib, I. H. (2010). Emotion Regulation in Depression: Relation to Cognitive Inhibition. *Cognition and Emotion, 24*(2), 281–298.

Juruena, M. F. (2014). Early-life stress and HPA axis trigger recurrent adulthood depression. *Epilepsy & Behavior, 38*, 148–159.

Kajander, K., Hatakka, K., Poussa, T., Färkkilä, M., & Korpela, R. (2005). A probiotic mixture alleviates symptoms in irritable bowel syndrome patients: a controlled 6-month intervention. *Alimentary Pharmacology & Therapeutics, 22*(5), 387–394.

Kane, M. J., & Engle, R. W. (2003). Working-memory capacity and the control of attention: the contributions of goal neglect, response competition, and task set to Stroop interference. *Journal of Experimental Psychology. General, 132*(1), 47–70.

King, M. S. (2007). Anatomy of the Rostral Nucleus of the Solitary Tract. In R. M. Bradley (Ed.), *The Role of the Nucleus of the Solitary Tract in Gustatory Processing*. Boca Raton (FL): CRC Press/Taylor & Francis.

Kopp, M. V., Goldstein, M., Dietschek, A., Sofke, J., Heinzmann, A., & Urbanek, R. (2008). Lactobacillus GG has in vitro effects on enhanced interleukin-10 and interferon-gamma release of mononuclear cells but no in vivo effects in supplemented mothers and their neonates. *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology, 38*(4), 602–610.

Lakhan, S. E., & Vieira, K. F. (2008). Nutritional therapies for mental disorders. *Nutrition Journal, 7*, 2.

Logan, A. C., & Katzman, M. (2005). Major depressive disorder: probiotics may be an adjuvant therapy. *Medical Hypotheses, 64*(3), 533–538.

Logan, G. D., & Zbrodoff, N. J. (1979). When it helps to be misled: Facilitative effects of increasing the frequency of conflicting stimuli in a Stroop-like task. *Memory & Cognition, 7*(3), 166–174.

Lyte, M. (2014). Microbial endocrinology: Host-microbiota neuroendocrine interactions influencing brain and behavior. *Gut Microbes, 5*(3), 381–389.

Maes, M., Kubera, M., & Leunis, J.C. (2008). The gut-brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuroendocrinology Letters, 29*(1), 117–124.

Mayer, E. A. (2011). Gut feelings: the emerging biology of gut–brain communication. *Nature Reviews Neuroscience, 12*(8), 453–466.

Messaoudi, M., Lalonde, R., Violle, N., Javelot, H., Desor, D., Nejdi, A., … & Cazaubiel, J.M. (2011). Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. *The British Journal of Nutrition, 105*(05), 755–764.

Miller, A. H. (1998). Neuroendocrine and immune system interactions in stress and depression. *Psychiatric Clinics of North America, 21*(2), 443–463.

Moayyedi, P., Ford, A. C., Talley, N. J., Cremonini, F., Foxx-Orenstein, A. E., Brandt, L. J., & Quigley, E. M. M. (2010). The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut, 59*(3), 325–332.

Naseribafrouei, A., Hestad, K., Avershina, E., Sekelja, M., Linløkken, A., Wilson, R., & Rudi, K. (2014). Correlation between the human fecal microbiota and depression. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society, 26*(8), 1155–1162.

Neufeld, K. M., Kang, N., Bienenstock, J., & Foster, J. A. (2011). Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterology & Motility, 23*(3), 255–e119.

Neuman, H., Debelius, J. W., Knight, R., & Koren, O. (2015). Microbial endocrinology: the interplay between the microbiota and the endocrine system. *FEMS Microbiology Reviews, 39*(4), 509–521.

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut microbiome axis. *Behavioural Brain Research, 277*, 32–48.

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.M., Quigley, E. M. M., … & Dinan, T. G. (2009). Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. *Biological Psychiatry, 65*(3), 263–267.

Peluso, M. A. M., Glahn, D. C., Matsuo, K., Monkul, E. S., Najt, P., Zamarripa, F., … & Soares, J. C. (2009). Amygdala hyperactivation in untreated depressed individuals. *Psychiatry Research, 173*(2), 158–161.

Poser, B. A., Versluis, M. J., Hoogduin, J. M., & Norris, D. G. (2006). BOLD contrast sensitivity enhancement and artifact reduction with multiecho EPI: Parallel-acquired inhomogeneity-desensitized fMRI. *Magnetic Resonance in Medicine, 55*(6), 1227–1235.

Roberts, K. L., & Hall, D. A. (2008). Examining a supramodal network for conflict processing: a systematic review and novel functional magnetic resonance imaging data for related visual and auditory Stroop tasks. *Journal of Cognitive Neuroscience, 20*(6), 1063–1078.

Rook, G. A. W., Lowry, C. A., & Raison, C. L. (2013).

Microbial "Old Friends", immunoregulation and stress resilience. *Evolution, Medicine, and Public Health, 2013*(1), 46–64.

Rothwell, N. J., & Hopkins, S. J. (1995). Cytokines and the nervous system II: Actions and mechanisms of action. *Trends in Neurosciences, 18*(3), 130–136.

Santos, J., Yang, P. C., Söderholm, J. D., Benjamin, M., & Perdue, M. H. (2001). Role of mast cells in chronic stress induced colonic epithelial barrier dysfunction in the rat. *Gut, 48*(5), 630-636.

Sherman, M. P., Zaghouani, H., & Niklas, V. (2015). Gut microbiota, the immune system, and diet influence the neonatal gut-brain axis. *Pediatric Research, 77*(1–2), 127–135.

Shikora, S. A., Toouli, J., Herrera, M. F., Kulseng, B., Brancatisano, R., Kow, L., … & Billingto, C. J. (2015). Intermittent Vagal Nerve Block for Improvements in Obesity, Cardiovascular Risk Factors, and Glycemic Control in Patients with Type 2 Diabetes Mellitus: 2-Year Results of the VBLOC DM2 Study. *Obesity Surgery, 26*(5), 1021–1028.

Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015). The gut microbiome in health and in disease. *Current Opinion in Gastroenterology, 31*(1), 69–75.

Smith, K., McCoy, K. D., & Macpherson, A. J. (2007). Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Seminars in Immunology, 19*(2), 59–69.

Söderholm, J. D., & Perdue, M. H. (2001). II. Stress and intestinal barrier function. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 280*(1), G7-G13.

Sparkman, N. L., Buchanan, J. B., Heyen, J. R. R., Chen, J., Beverly, J. L., & Johnson, R. W. (2006). Interleukin-6 Facilitates Lipopolysaccharide-Induced Disruption in Working Memory and Expression of Other Proinflammatory Cytokines in Hippocampal Neuronal Cell Layers. *The Journal of Neuroscience, 26*(42), 10709–10716.

Steenbergen, L., Sellaro, R., Van Hemert, S., Bosch, J. A., & Colzato, L. S. (2015). A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain, Behavior, and Immunity, 48,* 258–264.

Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of Experimental Psychology, 18*(6), 643–662.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.N., … & Koga, Y. (2004). Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *The Journal of Physiology, 558*(1), 263–275.

Swick, D., Ashley, V., & Turken, A. U. (2008). Left inferior frontal gyrus is critical for response inhibition. *BMC Neuroscience, 9*(1), 102.

Tillisch, K., Labus, J., Kilpatrick, L., Jiang, Z., Stains, J., Ebrat, B., … & Mayer, E. A. (2013). Consumption of Fermented Milk Product With Probiotic Modulates Brain Activity. *Gastroenterology, 144*(7), 1394–1401.

Van Hemert, S., & Ormel, G. (2014). Influence of the Multispecies Probiotic Ecologic®Barrier on Parameters of Intestinal Barrier Function. *Food and Nutrition Sciences, 5*(18), 1739–1745.

Winecoff, A., Clithero, J. A., Carter, R. M., Bergman, S. R., Wang, L., & Huettel, S. A. (2013). Ventromedial Prefrontal Cortex Encodes Emotional Value. *Journal of Neuroscience, 33*(27), 11032–11039.

Wu, H.J., & Wu, E. (2012). The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes, 3*(1), 4–14.

Zoccatelli, G., Beltramello, A., Alessandrini, F., Pizzini, F. B., & Tassinari, G. (2010). Word and position interference in Stroop tasks: a behavioral and fMRI study. *Experimental Brain Research, 207*(1–2), 139–147.