Eat2beNICE

Effects of Nutrition and Lifestyle on Impulsive, Compulsive, and Externalizing Behaviours

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Table of content

Gut microbiome in children and adolescents with ADHD and its relation to neural activation

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Abstract

Gut Microbiome composition has been shown to be associated with both ADHD symptoms and the functioning of the reward network. The current study aimed to investigate the development of microbiome composition over age, and its interaction with ADHD diagnostic status and reward network responsivity in an fMRI paradigm.

We demonstrate that microbiome abundance in several bacterial genera is altered in ADHD in two cohorts of children and adolescents. We further show that the Rumicococcus genera specifically is associated with reward network fMRI activation, and that this association appears to be stronger in subjects with ADHD than in controls.

Our results indicate that a developmental perspective on the influence of microbiome on ADHD in general and reward network activation in specific is warranted in future studies.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, characterized by symptoms of inattention and/or impulsivity and hyperactivity. One of the key biomarkers for ADHD has been behavioral and neural alterations in reward processing, as reflected by altered behavioral responses and diminished brain activation in the reward network (Scheres et al., 2007; Strohle et al., 2008; Plichta et al., 2014; Hoogman et al., 2011).

Recent studies have shown that dietary interventions for patients with ADHD may lead to a significant reduction in ADHD symptoms, although there is heterogeneity across studies (Nigg et al., 2012; Sonuga-Barke et al., 2013; Pelsser et al., 2011). Conceivably, diet might influence behavior and ADHD symptoms by affecting gut microorganisms (i.e. the gut microbiome) (David et al., 2014). The gut microbiome has an increasingly recognized impact on brain functioning and behavior (Cryan et al., 2012). One proposed mechanism for the effects of gut microbiota on brain and behavior is through their ability to synthesize neurochemicals and their precursors that are analogous in structure to those of the host nervous system (Lyte., 2013). Precursors of monoamines involved in ADHD (i.e. dopamine, noradrenaline, serotonin; see above) are produced by several members of the gut microbiota (Desbonnet et al., 2008; Clayton et al., 2012; Gertsman et al., 2015). These precursors (i.e. phenylalanine, tyrosine, tryptophan) might be absorbed through the intestinal epithelium, enter the portal circulation (Lyte et al., 2013), and cross the blood-brain barrier; in this way, they could potentially influence host monoamine synthesis. Consequently, differences in abundance and/or metabolic activity of monoamine precursor-producing inhabitants of the gastrointestinal tract may affect monoamine-related brain functioning and behavior relevant to ADHD, such as reward processing. Indeed, a lowered abundance of Bifidobacterium in infancy has been associated with increased risk of developing ADHD and Asperger syndrome in childhood in a study focusing on particular microbiota (Partty et al., 2015). A study by our group demonstrated altered microbiome composition in patients with ADHD as compared to controls, and showed that this microbiome function relates to decreased neural responses to reward anticipation (Aarts et al., 2017).

The current report is a follow up of our previous work by Aarts et al., (2017), and aims to expand the size and scope of the previous work by adding a further sample of children, allowing an investigation of the development of microbiome composition over age, and its interaction with ADHD diagnostic status and reward network responsivity.

Methods

Sample

The current study was performed on two separately acquired samples, one being part of the COMPULS (Naaijen et al., 2016) and the other part of the NeuroIMAGE (von Rhein et al., 2015) study. The COMPULS study consisted of 19 cases with ADHD and 25 controls between the ages of 5 and 12 with available microbiome and fMRI data. The NeuroIMAGE study consisted of 41 cases with ADHD and 50 controls between the ages of 14 and 30 years old with available microbiome data.

Of these subjects, a subsample also had task fMRI data available. For this analysis, subjects from both COMPULS and NeuroIMAGE cohorts were merged, resulting in the final sample characterized in table2. A total of 53 cases with ADHD and 64 controls were entered in the fMRI analyses.

	ADHD	Control	Test stat	P-value
Sex, f/m	22/31	28/36	Chi-Sq 0.001	0.955
Age	17.53	17.31	KW 0.028	0.865
IQ	98.72	109.12	F 17.46	<0.0001
Inattention	61.67	45.5	KW 47.50	<0.0001
Hyp/Imp	64.26	47.27	KW 32.69	<0.0001
Motion (FD)	0.12	0.09	KW 0.73	0.39
BMI	21.60	20.71	KW 0.83	0.36

Table 1. demographic factors for the full total sample used in the fMRI analyses.

Microbiome

The human fecal samples were collected at home by the participant and sequenced using the V1-V2 region of the 16S rRNA gene. The sequenced data was analyzed through NG-Tax 16S rRNA pipeline and classified using the SILVA reference database (version 128). For more information about the collection and pre-processing of the microbiome, see Szopinska-Tokov et al. (2020).

A prevalence cut-off of 20% for genera and samples was applied before taxonomic analyses. Three meta-analyses were performed to associate bacterial relative abundance between NeuroIMAGE and COMPULS with 1) participants with and without ADHD, 2) age, only in participants with ADHD, and 3) age, only in participants without ADHD. The package MMUPHin was used with total sum scoring and an arcsine square-root transformation (Ma 2019, Ma et al. 2021). As covariates age, gender and BMI was taken into account.

fMRI tasks

Three distinct fMRI tasks were employed within the NeuroIMAGE and COMPULS studies to operationalize reward sensitivity, and all three were used in the current analysis to estimate a reward anticipation contrast (see Figure 1 below).

NeuroIMAGE Stroop

High vs Low reward

NeuroIMAGE MID

Reward vs Neutral

Compuls Knutson

Reward vs Neutral

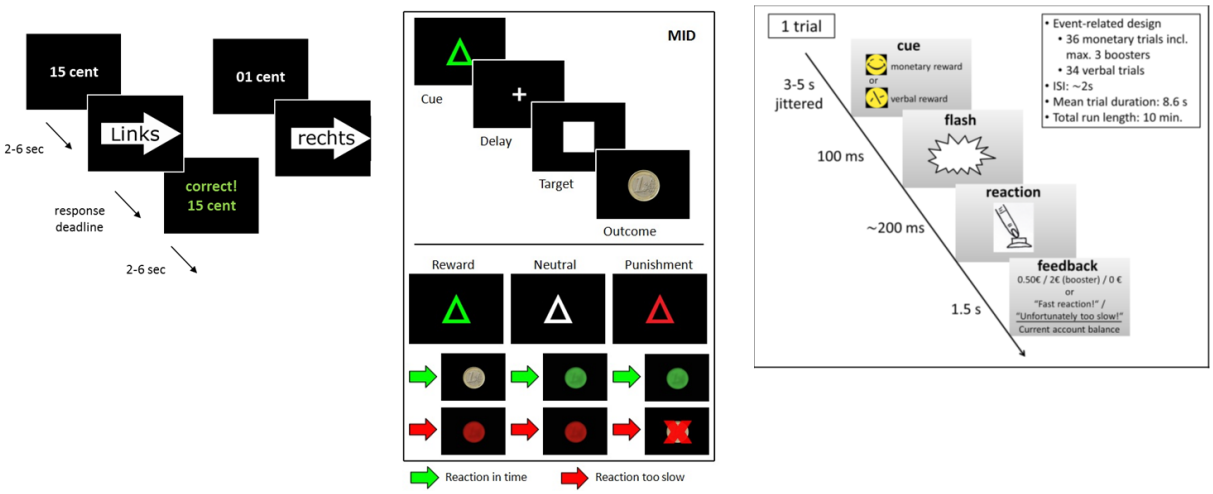

Figure 1. three fMRI tasks used in the current analyses.

fMRI data preprocessing was unified between tasks (following the protocol from Aarts et al., 2017), and the first-level (individual) fMRI analysis was unified between tasks to acquire a comparable reward anticipation contrast (see Figure 2).

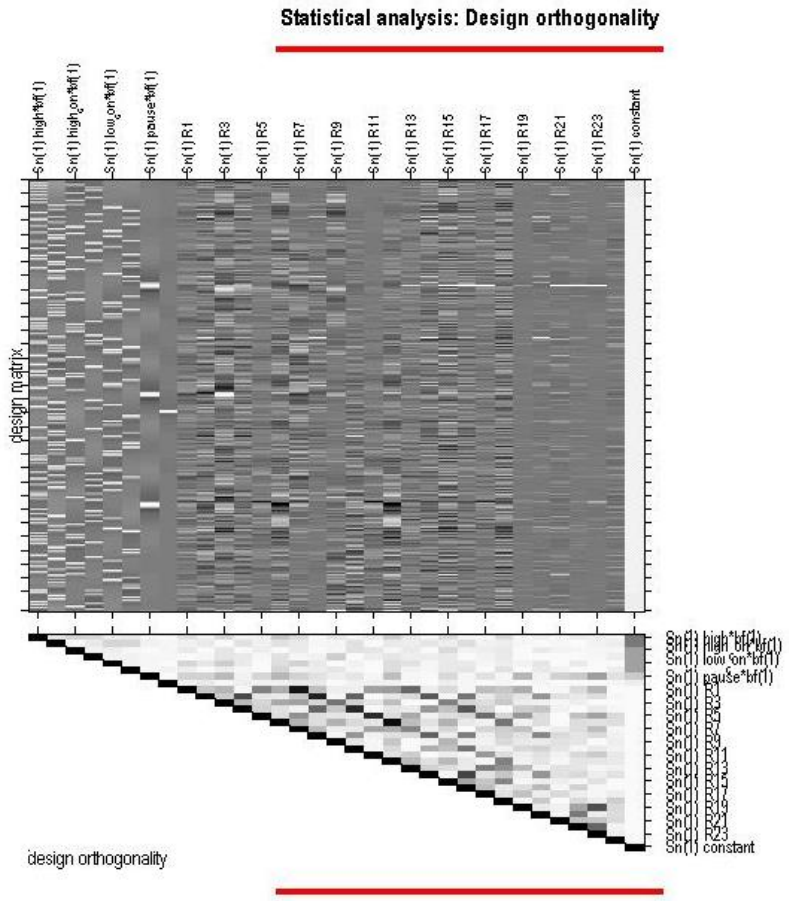

Figure 2 first level design for each fMRI task, including high reward cues and low reward cues/neutral cues as predictors of interest and nuisance regressors dependent on task as well as 24 motion regressors.

Statistics

Subsequently, the beta-values from each ROI were combined over all samples, and the effects of microbiome were investigated using linear mixed effects models (lmer function in the R statistics package), using the formula displayed in Figure 3. Here, bacteria strain was entered as the independent variable. Diagnosis, age, BMI and Sex were covariates, and a random factor was entered to correct for cohort and family effects.

$$Beta's\ func\ ROI \sim Bacteria + diagnosis * + age + BMI + sex + (1|Family) + (1|Cohort)$$

Dependent variable | Indep of interest | covariates | Random factor for family relatedness and cohort

Figure 3. formula for comparison of microbiome effects within each ROI

Results

Microbiome analysis between groups

Between group analysis was performed for all bacterial genera in the three meta-analyses, firstly for cases vs controls, then for the age effect in cases between the two samples and the age effects between controls of the two samples. Significant effects from these three meta-analyses indicate one genera which shows significant abundance differences between cases and controls (ruminococcus: coef=-0.034, p=0.049) (see Figure 4), with several other genera showing a significantly different effect of age between the two samples (see Table 2).

The significant differences observed in the meta-analysis across samples were subsequently entered in the fMRI analysis as predictors of interest.

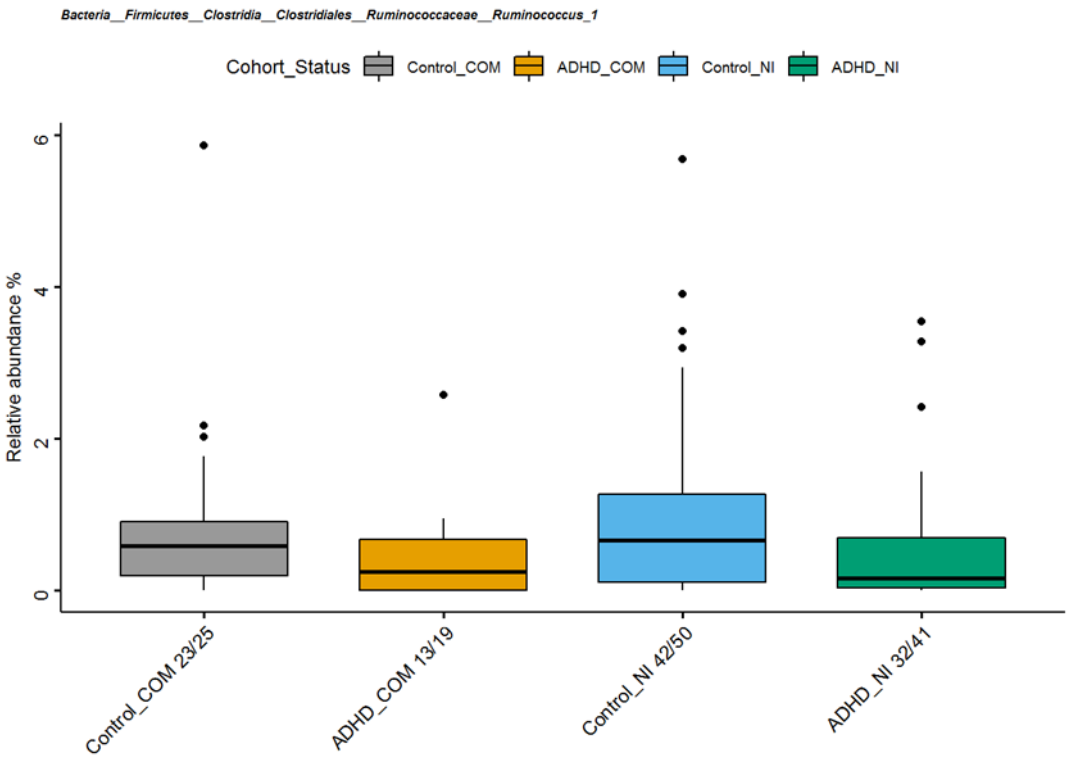

Figure 4. Relative abundance of the Rumicococcus genera bacterium between cases with ADHD and controls in both the COMPULS and NeuroIMAGE samples.

	Cases vs control – disorder_status	Cases vs cases – age	Controls vs controls – age
NeuroIMAGE	Family XIII UCG001 Blautia Lactobacillus Lachnospiraceae - uncultured	Erysipelotrichaceae UCG003 Ruminiclostridium 9 Eubacterium coprostanoligenes group	Eubacterium ventriosum group Ruminiclostridium 9 Paraprevotella Anaerostipes
COMPULS	Dorea Blautia Coprococcus 1	Clostridium sensu scricto 1 Coprococcus 1 Family XIII AD3011 group Christensenellaceae R7 group Coprococcus 2	Turibacter Alistipes Clostridium sensu stricto 1 Eubacterium ventriosum group
Meta-analysis	Ruminococcus 1	Erysipelotrichaceae UCG003 Eubacterium coprostanoligenes group	Ruminiclostridium 9 Paraprevotella Anaerostipes Lachnospiraceae ND3007 group

Table 2. Candidate microbiota with significant differences between groups in each of the two samples as well as in a combined meta analysis.

fMRI task activation

fMRI activation maps were calculated for the reward anticipation contrasts for the NeuroIMAGE sample (MID and Stroop tasks combined) and the COMPULS sample separately. Reward anticipation maps showed a significant activation of the reward network, including bilateral striatum in both samples.

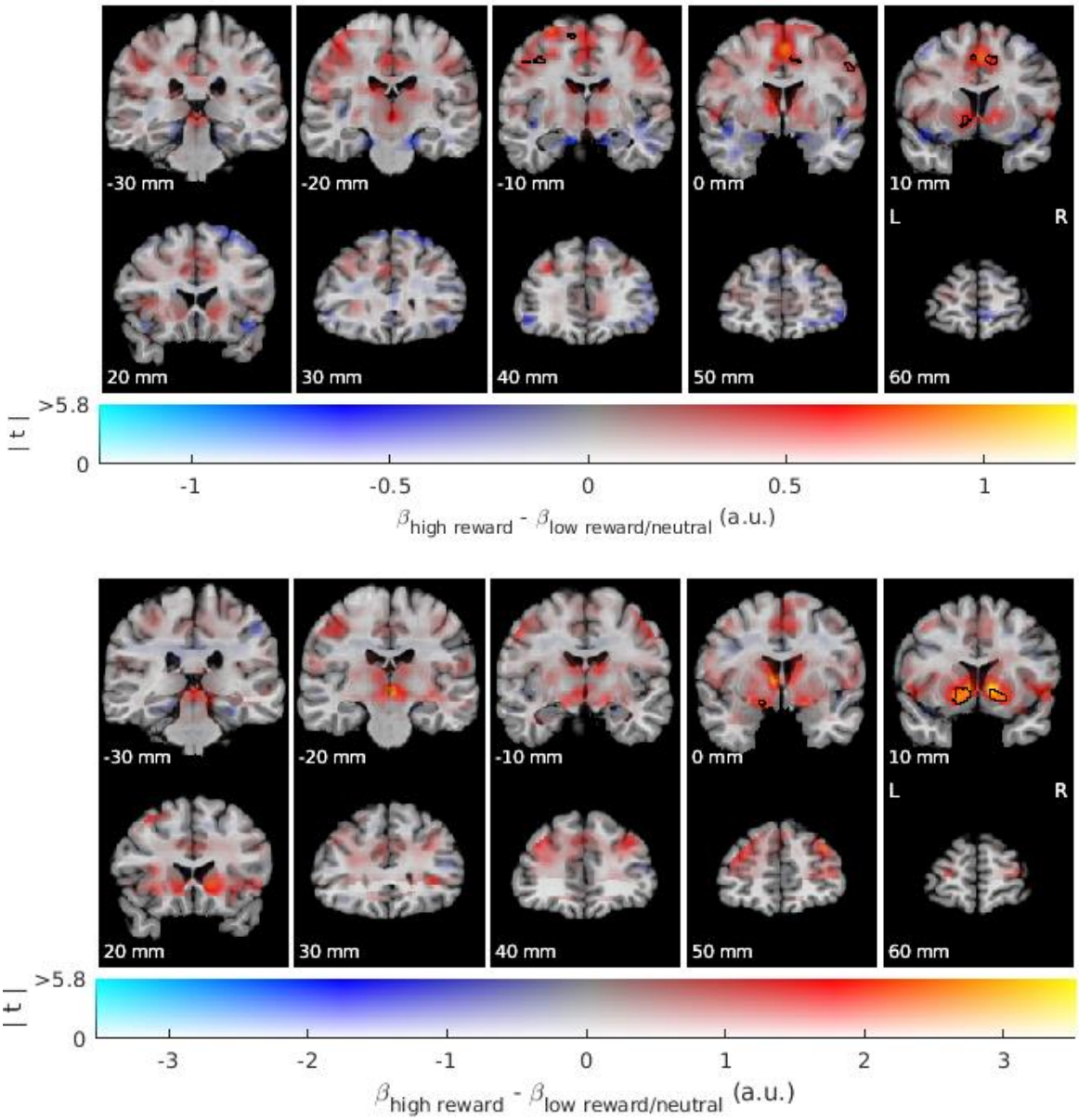

Figure 5. Task activation maps after motion correction for (A) NeuroIMAGE, MID and Stroop taks combined. (B) Compuls MID task during response anticipation.

ROI selection

The regions of interests that were selected to investigate the reward anticipation in each of the samples were based on the task activation patterns overlayed with the anatomical bilateral striatum (see Figure 6). Beta-values were extracted from these ROI's to use as dependent variable in the subsequent fMRI between group analyses.

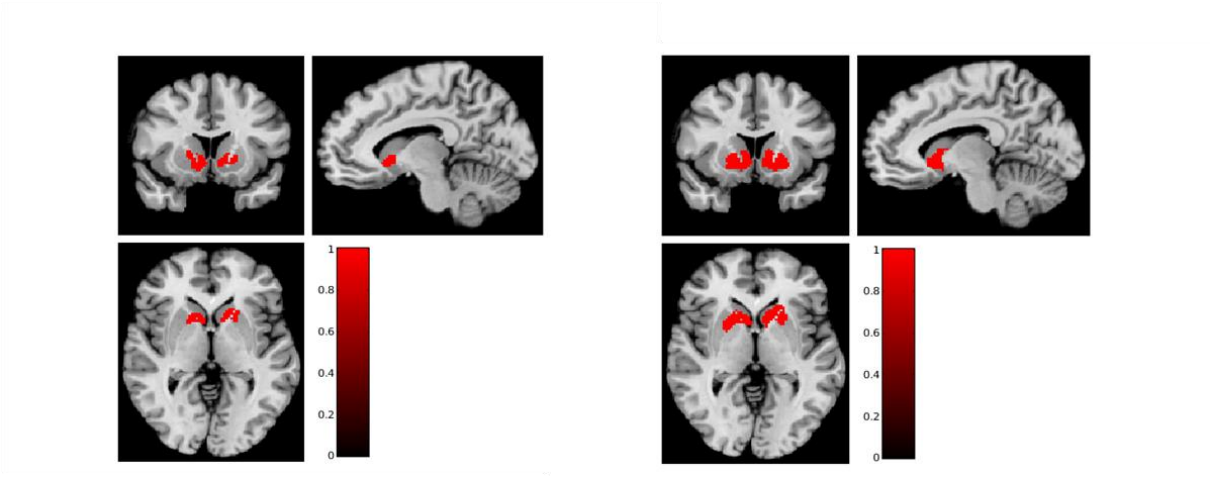

Figure 6. ROI selection for (A) NeuroIMAGE, MID and Stroop taks combined. (B) Compuls MID based on task effect per cohort in high versus low contrast, masked by the anatomical bilateral striatum.

fMRI group and age effects

The primary fMRI analyses fail to show significant associations between striatal brain activation during reward anticipation and any of the bacteria in neither the case-control, case-case or control-control comparisons.

Additional tests show a significant interaction effect of Bacteria*Diagnosis, in the case-control contrast only, for the Ruminicoccus_1 genera (t=-2.218, p=0.04) (see Figure 7). This effect indicates a downward trend in striatal activation with higher Ruminococcus loads for subjects with ADHD, but a stable trend for controls.

A second significant interaction effect was found for Bacteria*Age, in the control-control contrast only, for Anaerostipes genera (t=-2.41, p=0.019) (see Figure 8). This effect indicates that higher neural activation in the older controls as compared to younger controls, which further increases with higher Anaerostipes load.

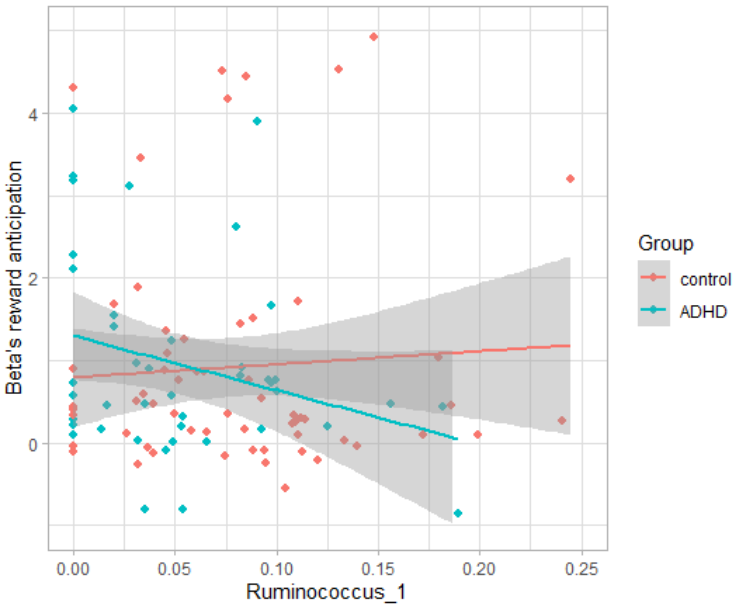

*Figure 7. Bacteria*Diagnosis interaction in striatal activation for Ruminococcus_1*

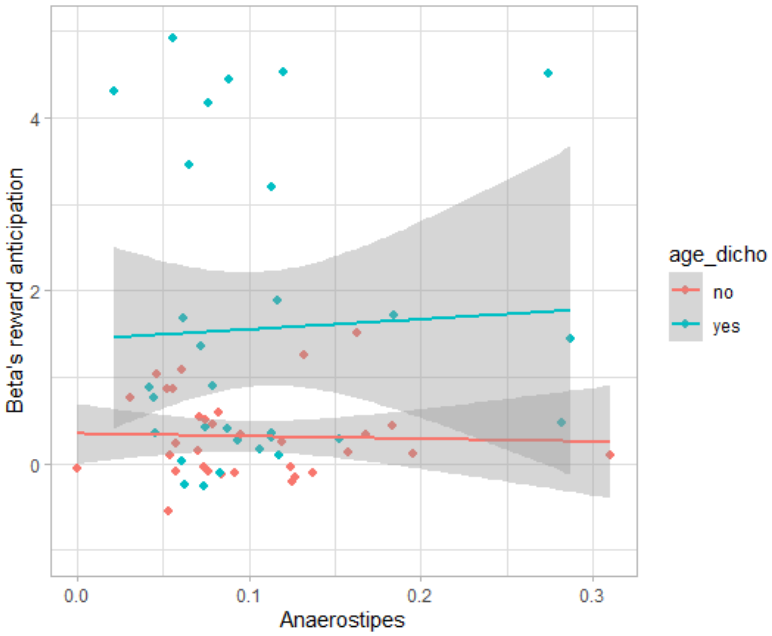

*Figure 8. Bacteria*Age interaction in striatal activation for Anaerostipes*

Discussion

Our results indicate that there are differences in the microbiome between cases with ADHD and controls, specifically in the abundance of the *Rumicococcus* genera bacterium. Subsequent fMRI analyses of the striatal activation during reward anticipation further indicate that there is an interaction between the effect of *Rumicococcus* abundance and diagnosis reward network activation. These results indicate that the abundance of this genera is associated with the reward network activation, and that this association appears to be stronger in subjects with ADHD than in controls.

We also observe several interactions of age*sample in our analyses, indicating differential effects of age in children and adolescents. These results show that the effect of the gut microbiome is not uniform over time, and that the age of a sample may influence the effect of different genera. The observed interaction effect on striatal activation between age and *Anaerostipes* abundance also indicates that these age effects may influence the effects of microbiome on brain and behavior.

Our results indicate that a developmental perspective on the influence of microbiome on ADHD in general and reward network activation in specific is warranted in future studies.

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