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Exploring the links between gut microbiota and excitatory and inhibitory brain processes in alcohol use disorder: A TMS study.

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ABSTRACT

While the impact of the gut microbiota on brain and behavior is increasingly recognized, human studies examining this question are still scarce. The primary objective of the current study was to explore the potential relationships between the gut microbiota composition, motor cortical excitability at rest and during inhibitory control, as well as behavioral inhibition, in healthy volunteers and in patients suffering from alcohol use disorder. Motor cortical excitability was examined using a range of transcranial magnetic stimulation (TMS) measures probed at rest, including the recruitment curve, short and long intracortical inhibition, and intracortical facilitation within the primary motor cortex. Moreover, TMS was applied during a choice reaction time task to assess changes in motor excitability associated with inhibitory control. Finally, behavioral inhibition was investigated using a neuropsychological task (anti-saccade). Overall, our results highlight several interesting correlations between microbial composition and brain measures. Hence, higher bacterial diversity, as well as higher relative abundances of *UGC-002* and *Christensenellaceae R-7 group* were correlated with stronger changes in motor excitability associated with inhibitory control. Also, higher abundance of *Anaerostipes* was associated with higher level of corticospinal excitability. Finally, relative abundances of *Bifidobacterium* and *Faecalibacterium* were positively related to performance in the neuropsychological task, suggesting that they might have a positive impact on behavioral inhibition. Although correlation is not causation, the present study suggests that excitatory and inhibitory brain processes might be related to gut microbiota composition.

1. Introduction

The importance of the gut microbiota, the trillions of microorganisms that live in our intestines, in maintaining tissue homeostasis is well established. The brain, and consequently our behavior, is also under the control of gut microbes. Many animal studies have demonstrated that the communication between the gut and the central nervous system includes immune, neural and metabolic pathways (Cryan and Dinan, 2012). Preclinical studies have also shown that intestinal bacteria influence brain neurotransmission and the balance between inhibitory and excitatory signaling. For instance, chronic treatment with the lactic acid bacteria *Lactobacillus rhamnosus JB-1* induced changes in $GABA_A$ and $GABA_B$ receptors expression in various brain areas, and decreased depression and anxiety-like behaviors in mice (Bravo et al., 2011). Moreover, the use of magnetic resonance spectroscopy (MRS) in mice showed that this bacterial strain is capable of increasing brain neurotransmitters glutamate, glutamine, *N*-acetyl aspartate and GABA (Janik et al., 2016). Treatments with other probiotics such as *Bifidobacterium longum* also induce changes in $GABA_A$ receptor expression in the hippocampus (Liang et al., 2017). In addition, by transplanting the fecal microbiota of psychiatric patients into mice, recent studies have further confirmed the causal impact of the gut microbiota on the main inhibitory (GABA) and excitatory (glutamate) neurotransmitters signaling and behavioral changes (Leclercq et al., 2020; Zheng et al., 2019).

Studies that have examined the interaction between gut microbes, brain and behavior in humans are limited. Brain imaging techniques offer the possibility to study gut-brain interaction *in vivo*. A landmark study combining functional magnetic resonance imaging (fMRI) and

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neuropsychological tasks in healthy women showed that a 4-week ingestion of fermented milk product containing various strains of *Bifidobacterium*, *Streptococcus*, *Lactobacillus* and *Lactococcus* affects activity of brain regions that control central processing of emotion and sensation (Tillisch et al., 2013). Another study showed that gut microbiota profile is associated with brain microstructure and cognitive function in obese and non-obese individuals (Fernandez-Real et al., 2015). Particularly, in that study, the relative abundance of Actinobacteria phylum was related to differences in fractional anisotropy within the amygdala and the thalamus and to cognitive test scores related to speed, attention, and flexibility. The relative abundance of *Prevotella* was also associated with worse cognitive score at the Trail Making Test. Finally, a recent study reported interesting associations of the gut microbiota composition, including the relative abundance of Bacteroidaceae and Lachnospiraceae families, with intrinsic brain activity and cognitive performance in patients suffering from amnestic mild cognitive impairment (Liu et al., 2021).

However, despite the well-established effect of intestinal bacteria on GABA and glutamate signaling, to date, no clinical study has investigated the relation between the microbiome and the balance between neural inhibition and excitation. In humans, these processes can be evaluated at the level of the motor system by the use of single- or paired-pulse transcranial magnetic stimulation (TMS), a non-invasive tool allowing to probe corticospinal excitability and intracortical circuits (Di Lazzaro and Ziemann, 2013; Ziemann, 2017). Hence, when applied over the primary motor cortex (M1), single-pulse TMS elicits motor-evoked potentials (MEPs) in targeted contralateral muscles, and the amplitude of these MEPs reflects the global level of corticospinal excitability at the time of stimulation (Bestmann and Duque, 2016; Derosiere et al., 2020; Derosiere and Duque, 2020). By applying TMS at different intensities, this technique allows to obtain the so-called recruitment curve, depicting the rise in MEP size with increasing TMS intensities. A steeper slope demonstrates higher cortical excitability and, even though the relationship with neurotransmission is not fully understood, it seems to reflect increased glutamatergic activity (Di Lazzaro et al., 2003; Stagg et al., 2011).

Moreover, TMS can be used in paired-pulse protocols to selectively probe intracortical inhibitory and excitatory circuits (Kujirai et al., 1993; Valls-Solé et al., 1992; Ziemann et al., 1996b). Such protocols assess the impact of a first conditioning stimulus on the MEP response elicited by a subsequent test pulse generated through the same stimulating coil. Depending on the intensity of the conditioning stimulus and the interval between the two pulses, specific intracortical circuits can be evaluated. In particular, some protocols, called short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI), result in a suppression of the conditioned MEP. Importantly, SICI and LICI depend, respectively, on GABA-A (Ziemann et al., 1996a) and GABA-B (McDonnell et al., 2006) receptor-mediated inhibitory neurotransmission, as largely demonstrated by the pharmacological effects on these measures. On the opposite, intracortical facilitation (ICF) induces a facilitation of the MEP and originates from excitatory postsynaptic potentials transmitted by *N*-methyl-D-aspartate (NMDA) glutamate receptors (Ziemann et al., 1998). Hence, these protocols indirectly assess the specific activity of GABA-A, GABA-B and NMDA receptors within M1.

Finally, MEPs can also serve as valuable readouts of cognitive processes which, although occurring outside M1, induce measurable state-changes (Bestmann and Duque, 2016). In particular, inhibitory control, defined as the ability to suppress inappropriate actions in favor of goal-directed behavior, has been related to the drastic suppression of motor excitability classically reported during action preparation (Duque et al., 2017; Quoilin et al., 2018, 2021b). Such MEP suppression is usually investigated using instructed-delay choice reaction time (RT) tasks, in which participants have to choose between two hand responses and to withhold their movement until the onset of an imperative signal. When probed before the imperative, MEPs elicited in task-relevant and task-irrelevant muscles of both hands are strongly suppressed relative to resting conditions (Grandjean and Duque, 2020; Greenhouse et al., 2015; Labruna et al., 2019; Quoilin et al., 2016, 2021a), thanks to non-specific inhibitory processes thought to help inhibit premature or inappropriate responses (Derosiere et al., 2020; Duque et al., 2017). Importantly, as action preparation also entails excitatory processes to prepare the motor system for the forthcoming action, the MEP suppression can be reduced, or even absent, in the selected effector (i.e., the muscle involved in the response), especially when the tendency to act is particularly strong (Quoilin et al., 2016; van Elswijk et al., 2007; Wilhelm et al., 2016). Therefore, whereas the excitatory influences can be indirectly detected in MEPs probed in selected effectors, it is ideal to focus on MEPs probed in task irrelevant muscles, preferentially away from the prime-mover (e.g. in the non-selected hand) to tackle inhibitory influences related to inhibitory control, as they are spared from the excitatory drive associated with the planned response (Grandjean and Duque, 2020; Quoilin et al., 2021a). Finally, note that efficient inhibitory control has also been related to GABAergic function (Boy et al., 2011; Coxon et al., 2006; Hermans et al., 2018).

Whereas, on one hand, gut microbiota composition and, on the other hand, cortical excitability at rest or during inhibitory control have been extensively studied, they have always been investigated separately, despite a growing literature highlighting the impact of the gut microbiota on these brain processes. Here, we aimed at joining these two fields of research to elucidate the relationship between both aspects. To do so, fecal samples and TMS measurements were obtained in healthy volunteers and patients suffering from alcohol use disorder (AUD). Moreover, behavioral inhibition was assessed in all participants, using a neuropsychological task. The population of AUD patients (AUDs) was chosen based on prior works revealing that these patients suffer from significant microbial changes (Amadieu et al., 2022a, 2022b; Leclercq et al., 2014), an altered balance between inhibitory and excitatory neurotransmission (Gilpin and Koob, 2008) and a lack of inhibitory control (Quoilin et al., 2018; Smith et al., 2014), providing us with a highly heterogeneous sample. Overall, the approach followed in this study has the potential to identify bacteria that might significantly affect excitability and inhibitory states associated with motor behavior.

2. Material and methods

2.1. Participants

Fifteen recently detoxified AUDs and thirteen healthy controls (HCs) participated in the study. AUDs were recruited during the third week of their alcohol detoxification program (St. Luc Academic Hospital, Université catholique de Louvain, Brussels, Belgium). They were tested between day 18 and day 20 of abstinence and were no longer on withdrawal medication. The mean alcohol consumption before detoxification was 11.8 alcohol units (SD = 5.25) per day (an alcohol unit = 10 g of pure ethanol) and the mean duration of AUD was 13.0 years (SD = 7.65). The severity of AUD was evaluated using the DSM-5 criteria: 13 patients had been diagnosed as severe AUDs (presence of at least 6 symptoms out of 11) and 2 as moderate AUDs (presence of 5 symptoms); the mean number of DSM-5 criteria was 7.5 (SD = 1.85). AUDs were matched for age, gender and body mass index (BMI) with HCs with no AUD (Alcohol use disorders test [AUDIT] score <8 in males and <7 in females); their mean alcohol consumption was 0.8 units (SD = 1.04) per day. According to the Edinburg Handedness Inventory (Oldfield, 1971), two subjects in each group were left-handed.

Exclusion criteria for both groups included any chronic inflammatory diseases, cirrhosis or significant liver fibrosis, cancer, metabolic disorders such as obesity (BMI ≥30 kg/m2) or diabetes and bariatric surgery. Subjects who had been taken antibiotics, probiotics or prebiotics in the 2 months prior to enrolment, or non-steroidal anti-inflammatory drugs or glucocorticoids in the previous month were not included. Finally, all participants were free of any medical or

neurological disorder and of any drug treatment that could influence neural activity, including benzodiazepine. They had no history of other substance use disorder (except nicotine); nicotine dependence was more prevalent among AUDs (n = 13) than HCs (n = 3).

All participants gave written informed consent, following protocols approved by the Biomedical Ethic Committee of the Saint-Luc University Hospital, Université catholique de Louvain (2017/04JUL/354, 2014/14AOU/438 and 2018/22MAI/219). A final compensation of €70 was

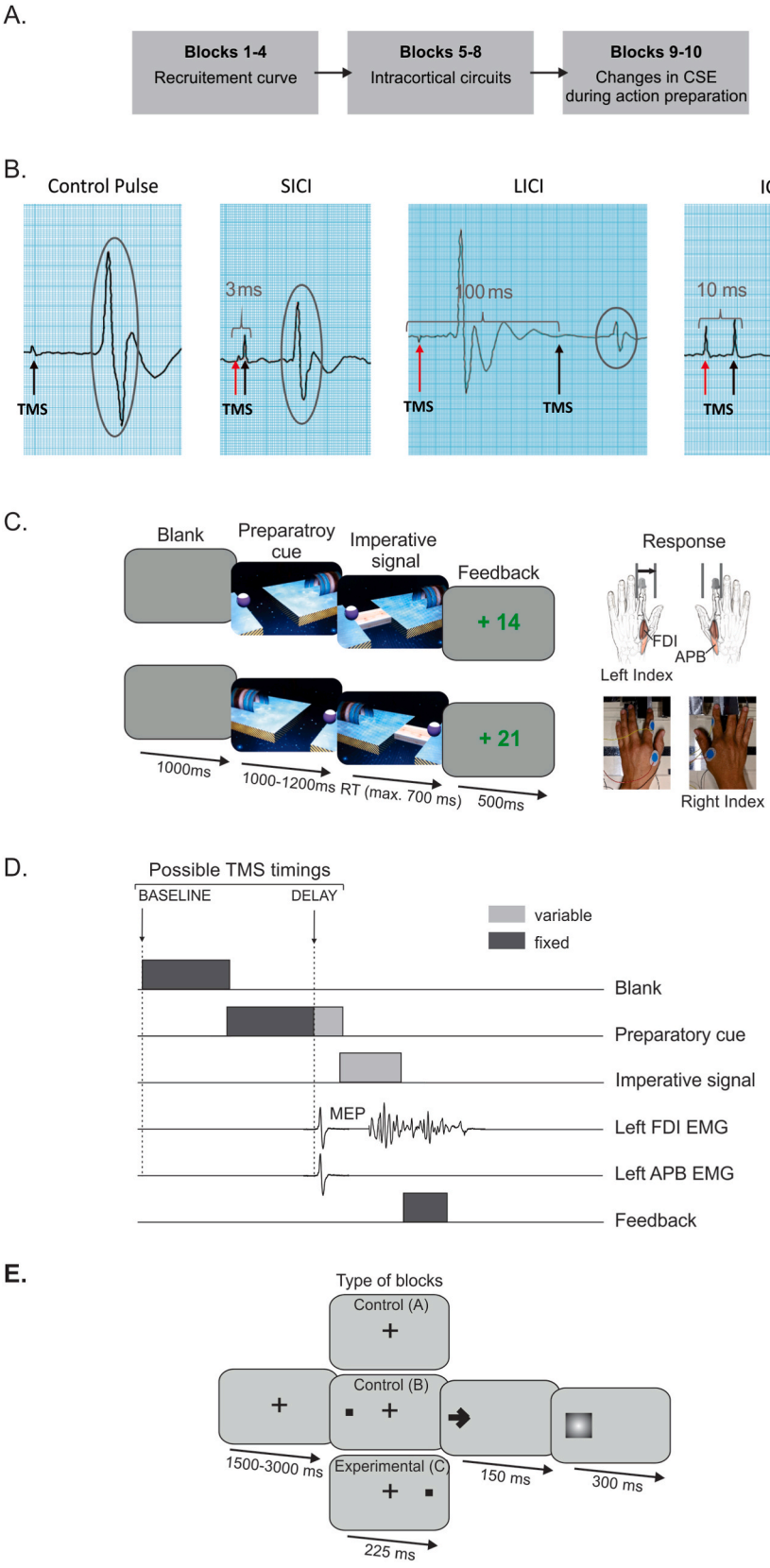

Fig. 1. Experimental procedure. (A) Time course of the transcranial magnetic stimulation (TMS) session. CSE = corticospinal excitability. **(B)** Measure of intracortical circuits. Paired-pulse TMS was used to assess short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and intracortical facilitation (ICF). To do so, the impact of a first conditioning stimulus (red arrow) on the motor-evoked potential (MEP; encircled in grey) elicited by a second test stimulus (black arrow) was evaluated. The mean amplitude of MEPs in each condition was then compared to the mean amplitude of MEPs elicited by a single, control, pulse. **(C)** Rolling ball task. Subjects performed an instructed-delay choice reaction time task, requiring to them to choose between an abduction movement of the left (non-dominant, upper trace) or right (dominant, lower trace) index finger depending on the position of a preparatory cue (i.e., the ball), and to withhold their response until the onset of an imperative signal (i.e., the bridge). Then, they were required to release their response as fast as possible. Index finger responses were recorded using a home-made response device. **(D)** TMS timings. One single TMS pulse was delivered in each trial over the left or the right primary motor cortex (separate blocks) at two possible timings: either at the onset of the blank screen ($TMS_{BASELINE}$) or 950 ms after the onset of the preparatory cue (TMS_{DELAY}). **(E)** Anti-saccade task. Participants successively performed three different blocks, in which they had to indicate the direction of an arrow presented very briefly (i.e., 150 ms). The A and B blocks consisted in control blocks, while the block C represented the experimental block, in which participants had to inhibit an automatic saccadic eye movement towards a visual disturbing cue in order to correctly process the arrow. By contrasting performance in the experimental and control blocks, this design allowed to obtain the anti-saccade cost, both in terms of reaction times and accuracy. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

only provided to HCs, in accordance with ethical regulations.

2.2. Self-reported measures

Mood status was measured using French versions of the Spielberger State Trait Anxiety Inventory (STAI Trait and State) and the Beck Depression Inventory (BDI-II).

2.3. Brain measures

2.3.1. Measures of motor excitability

All participants underwent one single TMS session (Fig. 1A). During the whole experiment, subjects sat on a chair with their arms semi-flexed and both hands resting palm-down on a table, and were explicitly asked to stay still and to keep their eyes open. First, four blocks were performed to obtain the recruitment curve, as a global measure of corticospinal excitability at rest. A paired-pulse paradigm was then used in the four following blocks to probe intracortical motor inhibitory and excitatory circuits. In particular, SICI, LICI and ICF were assessed as an indirect measure of GABA-A (Ziemann et al., 1996a), GABA-B (McDonnell et al., 2006) and NMDA (Ziemann et al., 1998) mediated neurotransmission, respectively. Blocks lasted approximatively 4 min, and participants were given a short break between each of them. Finally, participants performed two blocks of the "rolling ball" task to evaluate changes in corticospinal excitability during action preparation (see below). For each block type, corticospinal excitability measures were obtained for the non-dominant (ND) and dominant (D) hand on separate blocks, and the order of stimulation (ND vs D) was counterbalanced between subjects.

TMS protocol. Single- and paired-pulse TMS (biphasic pulses, Posterior/Anterior – Anterior/Posterior current direction) were delivered through a 75 mm figure-of-eight coil (*C*–B60, MagVenture, Denmark) connected to a MagPro X100 magnetic stimulator (MagVenture, Denmark). The coil was placed tangentially on the scalp over the D or ND M1 with the handle pointing backwards and laterally at a 45° angle away from the midline, approximately perpendicular to the central sulcus. For each M1, the optimal coil placement for eliciting MEPs in the contralateral first dorsal interosseous (FDI) was identified and marked on a head cap placed on the participant's scalp to provide a reference mark throughout the experiment (Vandermeeren et al., 2009). The resting motor threshold (rMT) was determined as the minimal TMS intensity required to evoke MEPs of 50 μV peak-to-peak in the relaxed FDI muscle in 5 out of 10 consecutive stimulations. Across HCs, the rMT corresponded to 51.8 ± 1.79% and 52.2 ± 2.29% of the maximum stimulator output for the D and ND M1, respectively. In AUDs, the rMT equaled 51.5 ± 3.28% and 51.5 ± 2.73% in the corresponding conditions. Importantly, those values were equivalent in both groups ($F_{1,25} = 0.14$; $p = 0.71$). Note that because finger representations have a large degree of overlap in M1 (Schieber, 2001), TMS pulses applied over the FDI hotspot can also elicit reliable MEPs in other finger muscles. So, during the rolling ball task, MEPs were also recorded in the abductor pollicis brevis (APB) of both hands, as successfully done in past studies (Grandjean and Duque, 2020; Márquez et al., 2018; Quoilin et al., 2021a), allowing us to obtain measures of corticospinal excitability in a task-irrelevant muscle during the rolling ball task (see below).

Recruitment curve. To evaluate the recruitment curve in both FDIs, single-pulse TMS was delivered on the D and ND M1 (separate blocks) at different intensities, ranging from 100 to 160% of the rMT (increments of 10%). Twelve trials for each experimental condition were randomly recorded with an inter-trial interval of 5–7 s (168 trials in total, divided in 4 blocks of 42 trials). The variables of interest were the mean MEP amplitude (mV) at each intensity as well as the Area Under the Recruitment Curve (AURC). The later was obtained by calculating the trapezoidal integration of the curve ($AURC = \sum_{i=I_1}^{I_6} \frac{MEP_i + MEP_{i+1}}{2} X\ 10$, with MEP_i being the MEP amplitudes for each $_i$ intensity); the higher the AURC, the higher the overall corticospinal excitability (Chaves et al., 2020; El-Sayes et al., 2020).

Intracortical circuits. Intracortical inhibition (SICI and LICI) and facilitation (ICF) were evaluated using paired-pulse TMS, in which two magnetic stimuli are delivered through the same stimulating coil in order to assess the effect of the first (conditioning) stimulus on the second (test) stimulus (Kujirai et al., 1993; Valls-Solé et al., 1992; Ziemann et al., 1996b) (Fig. 1B). The intensity of the conditioning stimulus was set either at 80% (SICI and ICF) or 120% (LICI) of the rMT, while the intensity of the test stimulus was always set at 120%. Four conditions were presented. In the control condition, the test stimulus was given alone. In the other conditions, the conditioning stimulus was given prior to the test stimulus at an inter-stimulus interval of 3 ms (SICI), 10 ms (ICF), or 100 ms (LICI). For each M1 (separate blocks), 16 trials of each paired-pulse condition and 24 trials of the control condition were recorded in a random order (72 trials in total, divided in 2 blocks of 36 trials, for each M1). SICI, LICI and ICF were calculated by expressing the mean amplitude of the conditioned MEPs (MEPs elicited by the test stimulus in the paired-pulse condition) in percentage of the mean amplitude of the unconditioned MEPs (MEPs elicited in the control condition). Hence, a value below 100% reflects intracortical inhibition, while a value above 100% indicates intracortical facilitation.

Motor excitability during action preparation. Changes in corticospinal excitability during action preparation were evaluated by applying single-pulse TMS over the D and ND M1 (separate blocks) when participants were performing an instructed-delay choice RT task, which was implemented with Matlab 7.5 (Mathworks, Natick, Massachusetts, USA) using the Psychophysics Toolbox extensions (Brainard, 1997; Pelli, 1997). It consisted in a computer-generated "rolling ball" game previously used in other studies (Grandjean et al., 2019; Quoilin et al., 2020; Vassiliadis et al., 2018, 2020), requiring participants to choose between responding with a left or a right index finger abduction according to the position of a preparatory cue that appeared on a computer screen (i.e., a left or right-side ball separated from a goal by a gap), and to provide their response as fast as possible after the onset of an imperative signal (i.e. a bridge connecting the ball and the goal). Once a correct response was detected, the ball rolled over the bridge to reach the goal. Importantly, responses provided before the onset of the imperative caused the ball to fall into the gap. Between the trials, participants were asked to stay still with forearms resting in a semi-flexed position and hands placed palms down on the response device.

The sequence of events of a typical trial is shown on Fig. 1C. Each trial started with the presentation of a blank screen for 1000 ms. Then, a preparatory cue was displayed, allowing participants to prepare their movement. After a random delay of 1000–1200 ms, the imperative signal appeared and remained visible until a finger response was detected (700 ms max). We purposely varied the duration of the delay to decrease the subjects' tendency to respond prematurely (i.e., before the imperative signal). For the same reason, each block involved some trials in which the bridge did not appear (i.e., catch trials – 6 per block), for which subjects were required not to respond. If they did, the ball fell into the gap. Finally, a feedback score appeared for 500 ms: correct responses led to positive scores (inversely proportional to RT, ceiling at + 100), while errors resulted in a fixed negative score (- 75). Note that when subjects succeeded not to respond on a catch trial, they received 75 points. The inter-trial interval was set at 2300 ms.

Each subject performed 2 blocks of 66 trials during which single-pulse TMS (at 120% of the individual rMT for each hemisphere) was applied over the D or ND M1 to elicit MEPs in the contralateral hand (MEP_{ND} and MEP_D, respectively). The TMS pulse could be delivered at one of two possible timings (Fig. 1D). To establish a baseline measure of corticospinal excitability, the TMS pulse occurred at the onset of the blank screen, eliciting MEPs at rest ($TMS_{BASELINE}$; 20 MEPs/block). In other trials, TMS was delivered 950 ms after the onset of the preparatory cue, when subjects were withholding their response (TMS_{DELAY}; 40 MEPs/block). At the latter timing, MEPs could either occur in a hand

cued for the forthcoming response (selected condition; e.g. MEP_{ND} in ND hand trials; 20 MEPs/block) or in the non-cued hand (non-selected condition; e.g. MEP_{ND} in D hand trial; 20 MEPs/block). The remaining trials (6/block) did not include any TMS pulse, preventing participants from anticipating TMS pulses at TMS_{DELAY} when it had not occurred at $TMS_{BASELINE}$.

The variables of interest were the MEPs elicited at TMS_{DELAY} in the FDIs of the selected hand, which was exploited as an indirect measure of the excitatory drive associated with the tendency to act, and the MEPs elicited at TMS_{DELAY} in the task-irrelevant APBs of the non-selected hand, which best capture the inhibitory drive related to inhibitory control. Importantly, those MEPs were expressed in percentage of MEPs probed at $TMS_{BASELINE}$; a value below one hundred reflects MEP suppression, while a value above one hundred indicates MEP facilitation.

EMG recording. EMG activity was recorded from surface electrodes (Ambu Blue Sensor NF-50-K Neuroline, Medicotest, Oelstykke, Denmark) placed over the FDI and APB muscles of both hands. EMG data were collected for 3200 ms on each trial, starting always 200 ms before the TMS pulse. The raw EMG signals were amplified (gain, 1 K), band-pass filtered online (10–500 Hz, Neurolog; Digitimer) and digitized at 2000 Hz for offline analysis. The latter consisted in extracting the peak-to-peak amplitude of MEPs recorded in both FDIs, as well as in the-task irrelevant APB during the rolling ball task. In order to prevent contamination of MEP measurements by significant fluctuations in background EMG, trials with visible EMG activity prior to the TMS pulse were removed (Quoilin et al., 2019). Moreover, during the task, trials in which subjects had provided the wrong response were also discarded; the task was so easy that these trials remained rare and errors were not analyzed. The remaining MEPs were then classified according to the experimental condition within which they were elicited. For each condition, we excluded trials with peak-to-peak MEP amplitudes exceeding 2.5 SD around the mean. Following data cleaning, a minimum of 8 MEPs per condition were left.

2.3.2. Measure of behavioral inhibition

The anti-saccade task (adapted from Roberts et al., 1994) was used to assess behavioral inhibition. Following the experimental procedure recommended by Miyake et al. (2000), participants successively performed two control blocks (parts A and B) and an experimental block (part C), which were identical in their structure and requirements except that parts A and B did not involve executive functions while part C did. As shown on Fig. 1E, each trial started with the presentation of a fixation cross in the middle of the screen for 1500–3500 ms, followed by the onset of a target stimulus. This stimulus was an arrow displayed for 150 ms on the left or the right side of the screen, before being masked by a grey cross-hatching square. The participant's task was to indicate the orientation of the arrow (towards the left, the right or upwards) by pressing the corresponding key on a keyboard. In the non-executive block A (40 trials, no cue condition), the sequence of events for each trial occurred as describe above. In the non-executive block B (40 trials, congruent condition), a visual cue was presented for 225 ms between the fixation cross and the target stimulus on the same side of the arrow. Finally, the executive block C (80 trials, incongruent condition) involved trials in which the visual cue was systematically displayed on the side opposite to the target stimulus, and thus requiring participants to inhibit the automatic saccadic eye movement towards the cue to be able to correctly identify the orientation of the arrow. This design enabled to calculate the so-called anti-saccade cost, which was based on the extraction of the specific executive subcomponent while controlling for visual and motor involvements. Accordingly, this measure was computed by calculating the difference between the average scores obtained in the executive block C and the average scores recorded in the non-executive blocks A and B, for both accuracy (in percentage of correct responses) and RTs (in ms).

2.4. Measures of the gut microbiota composition

On the same day as the TMS session, fecal samples were collected in a sterile container and immediately stored at −20 °C then transferred for long-term storage at −80 °C (within 6 h). Genomic DNA was extracted using QIAamp DNA stool kit (Qiagen, Germany) and following Protocol Q (Costea et al., 2017). The composition of the gut microbiota was analyzed by Illumina sequencing of the 16 S rRNA gene as described in (Amadieu et al., 2022a, 2022b). Briefly, the V3–V4 region of the 16 S RNA gene was amplified by PCR. The amplicons were purified, quantified and sequenced using an Illumina Miseq to produce 2x300-bp sequencing products at the University of Minnesota Genomics Center. Subsequent bioinformatics analyses were performed using FROGS (Escudié et al., 2018). Those analyses allowed to obtain measures of the α-diversity (observed species, Chao 1, Shannon's and Simpson's indices) as well as the relative abundances of the phylotypes. In order to avoid considering taxa whose relative abundance was too sparse (Pötgens et al., 2021), phyla, families and genera with a relative abundance lower than 0.1% in more than a quarter of the participants were discarded.

2.5. Statistical analyses

Self-reported measures. Demographic variables and current mood status were compared in HCs and AUDs using independent sample t-tests.

Brain measures. To evaluate the recruitment curve, an analysis of variance (ANOVA) was computed on the raw amplitudes of MEPs (mV), with MEP-SIDE (ND, D) and TMS-INTENSITY (1–7) as within-subject factors and GROUP (HCs, AUDs) as the between subject factor. Moreover, a two-way ANOVA was run on the AURC. Intracortical circuits were evaluated by performing an ANOVA on conditioned MEPs (expressed in percentage of unconditioned MEPs), with MEP-SIDE (ND, D) and CONDITION (SICI, LICI, ICF) as the within-subject factors and GROUP (HCs, AUDs) as the between-subject factor. Regarding the rolling ball task, we first analyzed behavior by performing an ANOVA on the RTs in trials in which MEPs were elicited at baseline (i.e. trials in which the RT is not influenced by the TMS pulse). Second, we considered MEPs elicited at TMS_{DELAY} (expressed in percentage of MEPs elicited at $TMS_{BASELINE}$). MEPs probed in the selected FDI, which best capture the excitatory drive associated with the tendency to act, and those elicited in the APB of the non-selected hand, which best capture the inhibitory drive related to impulse control, were analyzed using two separate ANOVAs, with MEP-SIDE (ND, D) as the within-subject factor and GROUP (HCs, AUDs) as the between-subject factor. Additionally, to assess the presence of changes in corticospinal excitability during action preparation in each condition, one-sample t-tests were used to compare these values to 100 (i.e. to $TMS_{BASELINE}$). Finally, to compare behavioral inhibition in both groups, independent sample t-tests were performed on the anti-saccade cost (in terms of accuracy and RTs).

Measures of gut microbiota composition. Measures of α-diversity and the relative abundance of each phylum, family and genus were compared between HCs and AUDs using non-parametric Mann-Whitney U tests for independent samples.

Relationship between brain measures and gut microbiota. Spearman rank-order correlations were performed between measures of α-diversity, relative abundance of bacteria at the phylum, family and genus levels and our main brain measures [i.e., global corticospinal excitability (AURC), intracortical circuits (SICI, LICI and ICF), changes in corticospinal excitability during action preparation (selected ND and D FDI %MEPs and non-selected ND and D APB %MEPs), and the anti-saccade cost (in terms of accuracy and RTs)]. Those correlations were conducted in all participants (AUDs and HCs). As our results indicated a lack of effect of the factor MEP-SIDE for the TMS measures recorded at rest, AURC, SICI, ICF and LICI were pooled across both hands for the correlational analyses.

ANOVAs were followed the by Fisher's Least Significant Difference

(LSD) methods to run post-hoc comparisons. The statistical significance was set at $p < 0.05$. When appropriate, Bonferroni corrections were applied to control for multiple comparisons, with the exception of the correlational analyses. Due to the exploratory nature of these analyses and the small sample size, here, we lowered the significance level to $p < 0.01$, to reduce the risk of type I error, instead of using Bonferonni corrections. All analyses were carried out using Statistica 10 (StatSoft, Cracow, Poland).

3. Results

3.1. Demographic and psychopathological measures

As illustrated in Table 1, analyses confirmed that both groups were matched for age ($t_{26} = -0.13$; $p = 0.90$), gender ($X^2_1 = 2.18$; $p = 0.14$) and BMI ($t_{26} = -1.10$; $p = 0.28$). In addition, they did not significantly differ for state anxiety ($t_{26} = 0.74$; $p = 0.47$), trait anxiety ($t_{26} = 1.41$; $p = 0.17$) and depression ($t_{26} = 0.94$; $p = 0.36$).

3.2. Characterization of brain measures in both groups

3.2.1. Motor excitability

Recruitment curve. The recruitment curves obtained in both groups are shown on Fig. 2A. Unsurprisingly, and as evident on the figure, MEP amplitudes significantly increased with TMS-INTENSITY ($F_{6,144} = 82.69$; $p < 0.001$), regardless of MEP-SIDE (MEP-SIDE x TMS-INTENSITY interaction; $F_{6,144} = 0.70$; $p = 0.65$). Importantly, this effect depended on the group (GROUP x TMS-INTENSITY interaction; $F_{6,144} = 2.22$; $p < 0.05$). Hence, in HCs, a significant increase in the amplitude of MEPs elicited by two adjacent intensities was only observed between 110 and 120% ($p < 0.05$) as well as between 120 and 130% of the rMT ($p < 0.05$). By contrast, in AUDs, increasing the pulse intensity by 10% resulted in significantly larger MEP amplitudes from 100% to 140% of the rMT (all $p < 0.05$). In other words, the recruitment curve seemed steeper in AUDs relative to controls, as it started to increase with lower intensities (i.e. at 110% in AUDs versus 120% of the rMT in HCs) and reached a plateau at a higher intensity (i.e. at 140% versus 130% of the rMT). This higher global corticospinal excitability in AUDs was also apparent in the figure depicting the AURC (Fig. 2B), even though the difference between both groups did not reach statistical significance ($t_{24} = 1.70$; $p = 0.10$).

Intracortical circuits. During the paired-pulse blocks, one AUD subject displayed too small MEP amplitudes in the control condition (i.e., mean of 0.09 mV in the D FDI) to obtain reliable measures of intracortical circuits (Ibáñez et al., 2020), and was consequently excluded from the subsequent analyses. The ANOVA performed on conditioned MEPs (expressed in percentage of unconditioned MEPs) revealed a main effect of the factor CONDITION ($F_{2,48} = 86.35$; $p < 0.001$). Unsurprisingly, and as shown on Fig. 3, MEPs recorded in the ICF condition were significantly larger than those probed in the SICI and LICI conditions (both $p < 0.001$). Furthermore, LICI was more pronounced than SICI ($p < 0.05$). No other main effect or interaction was significant (all $F < 0.88$ and all $p > 0.35$). Hence, ICF, SICI and LICI were comparable in both groups.

Table 1
Demographic and self-reported measures in healthy controls (HCs) and patients with alcohol use disorders (AUDs) [Mean *(SE)*].

	HCs (n = 13)	AUDs (n = 15)
emographic and psychopathological measures		
Age ^{NS}	46.0 *(3.03)*	45.6 *(2.08)*
Gender (n women) ^{NS}	6	3
BMI (kg/m^2) ^{NS}	23.9 *(0.87)*	22.6 *(0.90)*
State anxiety ^{NS}	31.5 *(2.60)*	34.5 *(3.14)*
Trait anxiety ^{NS}	37.2 *(3.64)*	43.9 *(3.07)*
BDI ^{NS}	5.8 *(2.49)*	9.1 *(2.36)*

NS = non-significant; BMI = Body Mass Index; BDI = Beck Depression Inventory.

Motor excitability during action preparation. During the rolling ball task, the mean RT in HCs was 331.2 (SE = 21.70) and 328.1 (SE = 19.48) ms for responses performed with the left and the right hand, respectively. The corresponding means in AUDs were 341.1 (SE = 20.20) and 350.7 (SE = 18.13) ms. Analyses performed on these data revealed neither an effect of RESPONDING-SIDE ($F_{1,26} = 0.23$; $p = 0.64$) nor an effect of GROUP ($F_{1,26} = 0.35$; $p = 0.56$). Hence, HCs and AUDs performed equally in the task, consistent with past studies (Quoilin et al., 2018, 2021a).

Changes during action preparation in the selected FDI, a condition in which MEPs better reflect excitatory inputs, are displayed on Fig. 4A. Analyses showed a main effect of MEP-SIDE ($F_{1,25} = 6.43$; $p < 0.05$), due to larger MEP_D relative to MEP_{ND}, regardless of the group (GROUP x MEP-SIDE interaction; $F_{1,26} = 0.27$; $p = 0.61$). This effect was confirmed by the t-tests (comparisons to a constant value of 100): while a MEP suppression was noticeable in the ND FDI (both $|t| > 2.95$ and $p < 0.05/4$), this suppression was absent for MEPs probed in the D effector (both $|t| < 0.45$ and $p > 0.66$). Hence, and consistent with prior works (Quoilin et al., 2016; Wilhelm et al., 2016), the excitatory drive associated with the tendency to act was mainly manifest when the forthcoming response entailed the dominant hand (see Fig. 5).

MEPs probed in the APB of the non-selected hand, which represent the most appropriate probe of inhibitory influences, are shown on Fig. 4B. As can be seen on the figure, here, MEPs probed during action preparation tended to be larger in AUDs than in HCs, even though this effect did not reach significance ($F_{1,25} = 3.13$; $p = 0.08$). In line with this tendency, the t-tests showed that MEPs were significantly suppressed relative to baseline in both hands of HCs, whereas a significant suppression was not reported for the D hand in AUDs. Finally, contrary to what was observed in the selected FDI, the effect of MEP-SIDE was not significant ($F_{1,25} = 0.10$; $p = 0.75$).

3.2.2. Behavioral inhibition

In the anti-saccade task, even though the figures suggest a larger cost in AUDs than in HCs, our analyses did not reveal any significant difference between both groups, neither for the percentage of correct responses ($t_{26} = 1.18$; $p = 0.25$, i.e. $> 0.05/2$; Fig. 2A), nor for the RTs ($t_{26} = 1.17$; $p = 0.25$, i.e. $> 0.05/2$; Fig. 2B). Hence, AUDs did not significantly display more difficulties than HCs to inhibit the initial reflexive saccade towards the incongruent visual cue.

3.3. Characterization of gut microbiota composition in both groups

Following the 16s rDNA sequencing, 486 operational taxonomic units (OTUs) in total were observed across all samples. As shown on Fig. 6A, no significant difference in fecal microbiota richness (Observed species, Chao 1) or diversity (Simpson's and Shannon's indices) were found between HCs and AUDs (*all* $U < 78$ and all $p > 0.08$). Yet, taxon-based analyses revealed some between-group differences at the phylum, the family and the genus level. Hence, we found that the *Firmicutes* phylum was more abundant in HCs ($U = 28$; $p < 0.01$; i.e. $< 0.05/5$), while the Bacteroidota phylum was more abundant in AUDs ($U = 28$; $p < 0.01$; i.e. $< 0.05/5$) (Fig. 6B). Some differences between both groups were also observed at the family level, with Christensenellaceae and Tannerellaceae being less and more abundant in AUDs relative to HCs, respectively ($U = 29$ and $U = 20$, respectively; both $p < 0.0026$; i.e. $< 0.05/19$). Finally, at the genus level, the relative abundance of *Christensenellaceae R-7 group* was significantly higher in HCs ($U = 29$; $p < 0.0019$; i.e. $< 0.05/26$), whereas *Parabacteroides* were more abundant in AUDs ($U = 20$; $p < 0.0019$; i.e. $< 0.05/26$).

3.4. Correlations between gut microbiota composition and brain measures

In order to explore the relationships between the gut microbiota composition and brain processes, correlational analyses were first performed between measures of α-diversity and our different brain

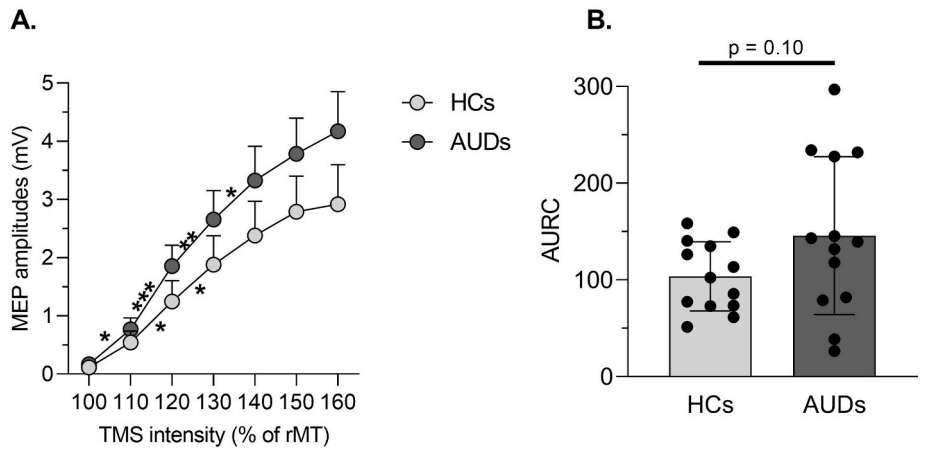

Fig. 2. Recruitment curve. (A) Raw amplitudes (mV) of motor evoked-potentials (MEP) elicited at different TMS intensities (expressed in percentage of resting motor threshold, rMT) in HCs (light grey) and AUDs (dark grey). **(B)** Mean area under the curve (AURC) in each group. Data from both hands were comparable and thus pooled together. *p < 0.05, **p < 0.01 and ***p < 0.001: significantly different.

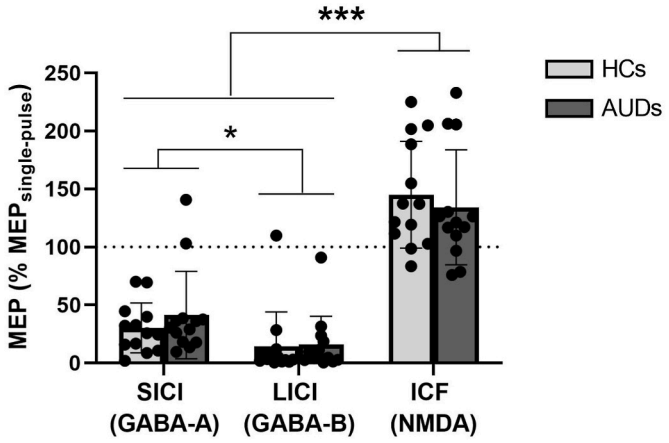

Fig. 3. Intracortical circuits. Short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and intracortical facilitation (ICF) in HCs (light grey) and AUDs (dark grey). The data represent the mean amplitude of the conditioned motor-evoked potentials (MEPs) (MEPs elicited by the test stimulus in the paired-pulse conditions) in percentage of the mean amplitude of the unconditioned MEPs (MEPs elicited in the control condition). Data from both hands were comparable and thus pooled together. *p < 0.05 and ***p < 0.001: significantly different. Data are mean ± SD.

measures obtained with the TMS and with the neuropsychological task. As shown in Fig. 7, a significant negative correlation was observed between the Shannon index and non-selected APB MEPs probed at TMS_{DELAY} during the task ($r_s = -0.60$; $p < 0.01$). Hence, a higher bacterial diversity was associated with stronger inhibitory influences. None of the other correlations with α-diversity indices was statistically significant (all $|r_s| < 0.42$ and all $p > 0.02$; i.e. > 0.01).

Then, in order to identify candidate bacteria influencing brain functions, we investigated correlations with the relative abundance of bacteria at the phylum, family and genus levels. Although no significant correlations were found at the phylum level, several correlations were observed at the family and the genus levels. Among the TMS measures obtained at rest, our analyses revealed a positive correlation between *Anaerostipes* and the AURC ($r_s = 0.58$; $p < 0.01$), indicating that a higher relative abundance of this bacteria was associated with a higher level of corticospinal excitability. Correlations were also found regarding changes in corticospinal excitability during action preparation. Interestingly, these effects were only evident for MEPs elicited in the APB of the non-selected hand, and therefore specifically concerned inhibitory influences. On the one hand, a higher relative abundance of *Lachnoclostridium* ($r_s = 0.52$; $p < 0.01$) and *Roseburia* ($r_s = 0.56$; $p < 0.01$) genera, which both belong to the *Lachnospiraceae* family, was associated with higher MEPs, suggesting that these bacteria might have a deleterious effect. On the other hand, the *Oscillospiraceae* ($r_s = -0.63$; $p < 0.001$) and *Christensenellaceae* ($r_s = -0.61$; $p < 0.001$) families seemed to have a beneficial impact on the strength of inhibitory influences, an effect driven by the relative abundance of *UCG-002* ($r_s = -0.50$; $p < 0.01$) and *Christensenellaceae R-7 group* ($r_s = -0.61$; $p < 0.001$), respectively. Finally, *Lachnospira* ($r_s = -0.61$; $p < 0.001$), *Faecalibacterium* ($r_s = -0.51$; $p < 0.01$), *Bifidobacterium* ($r_s = -0.50$; $p < 0.01$) and *Sutterella* ($r_s = -0.49$; $p < 0.01$) genera were negatively correlated with the anti-saccade cost (in terms of accuracy); the higher the relative abundance, the better the behavioral inhibition abilities. Altogether, these preliminary results support the hypothesis that the microbiota

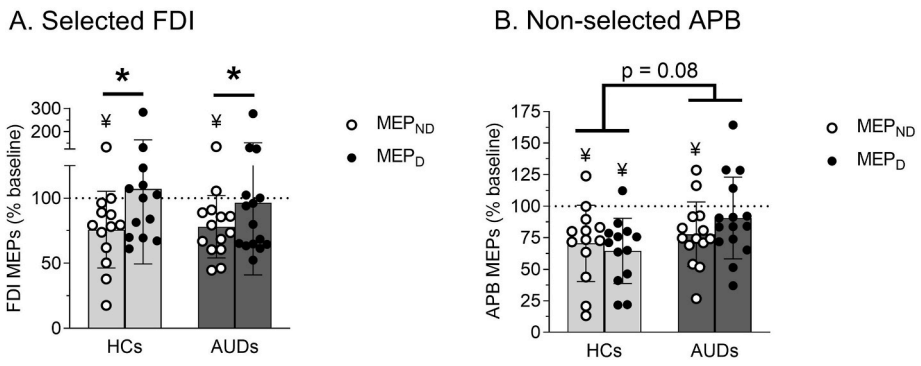

Fig. 4. Changes in corticospinal excitability during action preparation. Amplitudes of motor-evoked potentials (MEPs) recorded at TMS_{DELAY}, expressed in percentage of MEPs elicited at $TMS_{BASELINE}$, in the non-dominant (MEP_{ND}) and the dominant (MEP_D) hand of HCs (light grey) and AUDs (dark grey). Data are shown for the selected task-relevant first dorsal interosseous (FDI; A) and the non-selected task-irrelevant abductor pollicis previs (APB; B), as a measure of excitatory influences associated with the tendency to act and of inhibitory influences related to inhibitory control, respectively. ¥ = significantly different from MEPs probed at $TMS_{BASELINE}$ ($p < 0.05/4$). *p < 0.05: significantly different. Data are mean ± SD.

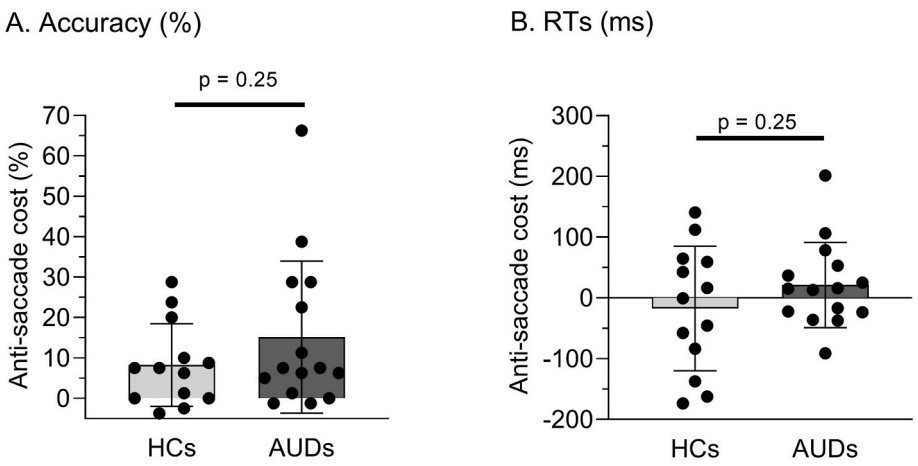

Fig. 5. Behavioral inhibition. Anti-saccade cost, defined as the difference between the scores in incongruent and control trials, both for accuracy **(A)** and reaction times (RTs) **(B)** in HCs (light grey) and AUDs (dark grey). Data are mean ± SD.

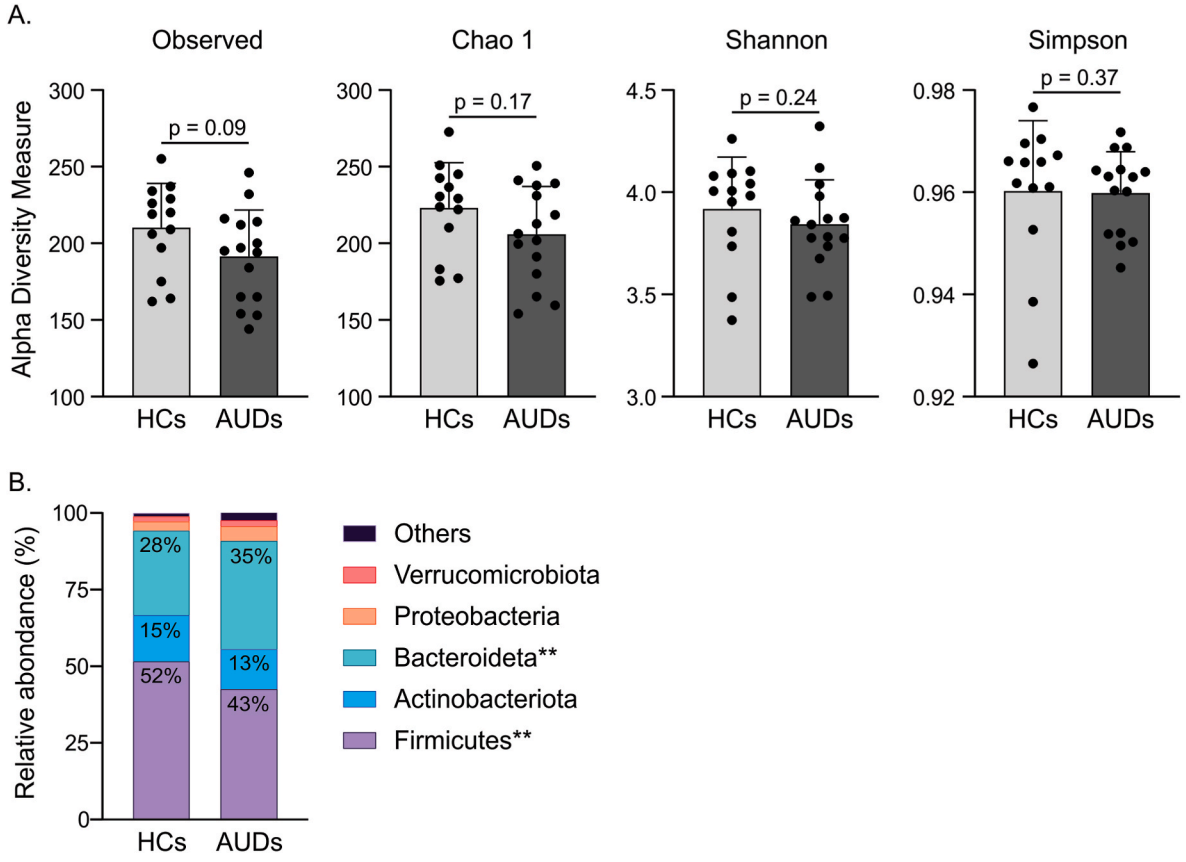

Fig. 6. Gut microbiota composition. **A:** Microbial α-diversity in HCs (light grey) and AUDs (dark grey). **B:** Relative abundance (%) of bacterial phyla present in the fecal samples of HCs and AUDs. **p < 0.01: significantly different between both groups. Data are mean ± SD.

composition may influence cerebral processes.

4. Discussion

Based on the increasingly recognized impact of the gut microbiota on brain and behavior, the primary objective of this research paper was to explore the potential relationships between the gut microbiota composition, motor excitability at rest and during action preparation, and behavioral inhibition. To do so, we investigated correlations between the abundance of intestinal bacteria and brain measures obtained using TMS and neuropsychological tasks. While few fMRI studies have recently started to tackle this question, the present work is the first one to use TMS, an electrophysiological method with the strong advantage to allow dissociating inhibitory and excitatory activity. Overall, our results highlight several interesting correlations. Although correlation is not causation, this research is a first step in the identification of potential bacterial targets to develop microbiota-based therapies.

One of the most interesting brain measures addressed here concerns the modulatory changes in the excitability of the corticospinal pathway probed during action preparation, with those MEPs representing

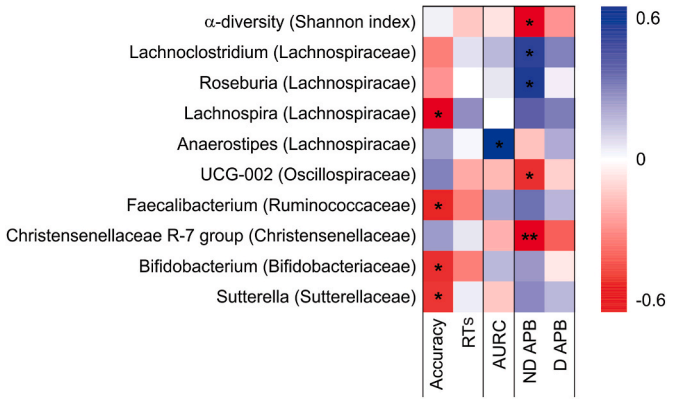

Fig. 7. Relationships between gut microbiota composition and brain measures. Heatmap showing Spearman correlations between α-diversity as well as relative abundance of gut bacteria and brain measures in all participants. On the left side of the graph, each row depicts the name of the genus, and the family to which it belongs is in brackets. Significant correlations were found for the anti-saccade cost (in terms of accuracy, but not in terms of reaction times (RTs)), the area under the curve (AURC), and changes in corticospinal excitability during action preparation probed in the task-irrelevant abductor pollicis brevis (APB) of the non-selected non-dominant (ND) hand, but not of the dominant (D) hand. *$p < 0.01$ and **$p < 0.001$.

objective and physiological biomarkers of upstream cognitive processes. As such, while MEPs elicited in the muscle involved in the forthcoming action indirectly assess the tendency to act, MEPs probed in a task-irrelevant muscle of the non-selected hand reflect inhibitory influences dedicated to suppress inappropriate actions, and thus ensuring some sort of inhibitory control. Interestingly, significant correlations with the gut microbiota composition were found only regarding the latter, indicating that associations specifically concerned inhibitory abilities.

The first striking finding was that a higher alpha-diversity, usually reflecting better health outcomes (Valdes et al., 2018), was associated with stronger inhibitory influences. More particularly, at the genus level, higher relative abundances of *UGC-002* and *Christensenellaceae R-7 group* were both correlated with better inhibitory control abilities. While the role of *UCG-002* is still unclear, the beneficial impact of *Christensenellaceae R-7 group* was consistent with the current literature. Hence, Christensenellaceae has shown compelling associations with metabolic parameters as well as with healthy dietary habits (Waters and Ley, 2019). It is depleted in obesity and in inflammatory bowel disease patients and increased in human dietary interventions involving prebiotic fibers (Waters and Ley, 2019). Regarding brain health, significant decrease in *Christensenellaceae R-7 group* has been linked to dysfunctional cognitive, learning and memory performance in a mouse model of Alzheimer disease (Zhan et al., 2018), while its higher abundance has been related to better cognition in healthy subjects (Karamujić-Čomić et al., 2020). By contrast, we found that two members of the Lachnospiraceae family, *Roseburia* and *Lachnoclostridium*, had a detrimental effect on the strength of inhibitory influences. Interestingly, in a recent paper (Arnoriaga-Rodríguez et al., 2021), Lachnospiraceae was negatively associated with performance on the Stroop Task, suggesting a negative influence of this bacterial family on inhibitory control. Furthermore, *Lachnoclostridium* has been involved in neurodevelopmental diseases such as attention-deficit hyperactivity disorder and autism spectrum disorder (Ma et al., 2019; Tengeler, 2020).

By examining performance at the anti-saccade task, we also identified several bacteria that were positively correlated with behavioral inhibition abilities. Hence, higher relative abundances of *Bifidobacterium, Faecalibacterium, Sutterella* and *Lachnospira* were associated with a lower anti-saccade cost. As negative correlations with *Bifidobacterium* and *Faecalibacterium* were observed whether the cost was measured in terms of accuracy or RTs, we were particularly confident in the significant effect of these bacteria. Coherently, a growing body of literature has unveiled their beneficial effect of cognitive processes. For instance, a positive correlation between *Bifidobacterium* and cognitive functions has been reported in a murine model exploring the influence of maternal high fat diet on the offspring cognitive development (Sanguinetti et al., 2019). In addition, two recent randomized, double-blind, placebo-controlled clinical trials demonstrated a positive effect of a 12-week supplementation with probiotics containing *Bifidobacterium* on cognitive function (Kim et al., 2021; Kobayashi et al., 2019). *Faecalibacterium*, a butyrate-producing bacterium known for its anti-inflammatory properties (Sokol et al., 2008), was more abundant in cognitively unimpaired subjects compared to subjects with low cognitive performance (Bajaj et al., 2016) and has been associated with good performance on cognitive tests in post-traumatic stress disorder veterans (Bajaj et al., 2019). Finally, while the positive impact of *Sutterella* and *Lachnospira* was less clear as it only concerned the accuracy cost, it was in line with recent studies demonstrating their beneficial effects in other contexts. Hence, *Sutterella* is thought to confer protection against demyelinating autoimmune diseases such as multiple sclerosis (Berer et al., 2017; Miller et al., 2015) and has been shown to be more abundant in healthy subjects compared to major depression disorder patients (Zheng et al., 2016). Furthermore, in a study investigating the role of the gut microbiome in dementia, *Lachnospira* has been associated with healthy mental state (Stadlbauer et al., 2020).

Whereas the role of gut microbiota in the modulation of GABA and NMDA signaling in the brain has been shown in preclinical studies (Bravo et al., 2011; Janik et al., 2016; Savignac et al., 2013), we did not find any significant correlation between intestinal bacteria and the strength of intracortical inhibitory (SICI, LICI) and excitatory (ICF) circuits. Importantly, those TMS protocols reflect phasic, postsynaptic neurotransmission mediated by the activity of receptors specifically localized within M1. By contrast, those prior studies revealed an impact of the gut microbiota on receptors expression in other brain regions (Bravo et al., 2011; Savignac et al., 2013) or on the levels of ambient extracellular neurotransmitters measured by MRS (Janik et al., 2016). Yet, those extracellular concentrations are uncorrelated with TMS measures such as SICI (Dyke et al., 2017; Stagg et al., 2011; Tremblay et al., 2013). Interestingly, when looking at a more global measure of motor excitability, we identified a relationship with *Anaerostipes*: the higher the relative abundance of the bacterium, the higher the area under the recruitment curve. As MEPs induced by single-pulse TMS represent the summation of multiple excitatory and inhibitory cortical, subcortical and spinal inputs acting at the same time on the motor output pathway (Derosiere and Duque, 2020), this greater area may reflect a biased brain excitation-inhibition balance, in the form of more excitatory influences. In line with this result, a recent study showed that mice colonized with the gut microbiota of individuals with attention-deficit hyperactivity disorder, known to have altered cortical excitability (Gilbert et al., 2019), displayed a higher abundance of *Anaerostipes* relative to control mice (Tengeler, 2020).

Altogether, the present study identified several bacterial correlates of inhibitory influences at play during action preparation, behavioral inhibition and global corticospinal excitability at rest. Unsurprisingly, various genera were found depending on the measured components, the latter representing different brain processes. For example, previous studies showed that performance at the anti-saccade task and MEPs probed during action preparation were not correlated and relied on distinct brain networks, demonstrating that they reflect different facets of inhibitory control (Quoilin et al., 2018, 2021b).

Although this question was not addressed directly, we might put forward some hypotheses regarding the pathways mediating the gut-brain relationships reported here. In particular, we posit that a potential candidate would be the inhibitory neurotransmitter GABA, a microbiota-derived neuromodulatory metabolite. As such, the relative abundance of Christensenellaceae and Oscillospiraceae, which were in this study associated with stronger inhibitory influences at play during

action preparation, positively correlates with fecal glutamate (Beaumont et al., 2021), which is the precursor of GABA. Furthermore, *Bifidobacterium*, found to be related to behavioral inhibition, has the functional ability to metabolize glutamate to produce GABA (Barrett et al., 2012). As greater levels of extracellular GABA measured by MRS have been linked to better inhibitory abilities (Hermans et al., 2018; Quetscher et al., 2015; Silveri et al., 2013), we believe that GABA production is a plausible mechanism by which bacteria influence inhibitory control. Future studies, combining TMS, neuropsychological tasks, MRS and 16 S rDNA gene sequencing should help clarify this point.

In view of the well-known microbiota dysbiosis and impaired inhibition characterizing AUDs, HCs and AUDs were included in an attempt to improve inter-individual variability and consequently to better identify candidate bacteria that might be related to brain processes. As such, and in agreement with prior works (Leclercq et al., 2014; Mutlu et al., 2012), we observed an alteration of the gut microbiota composition in AUDs in comparison with controls. Regarding TMS measures obtained at rest, our results indicate that the slope of the recruitment curve was steeper in AUDs than in HCs, reflecting higher global corticospinal excitability in the former, while the strength of intracortical circuits was similar in both groups. Interestingly, those data are consistent with the steeper slope previously found in chronic cocaine users (Hanlon et al., 2015) as well as with the cortical hyperexcitability identified in individuals at high-risk for developing AUD (Muralidharan et al., 2008). In addition, normal SICI and ICF within M1 have already been reported in detoxified AUDs (Naim-Feil et al., 2016; Nardone et al., 2010). Finally, MEPs recorded in the non-selected task-irrelevant muscle and the anti-saccade cost tended to be larger in AUDs than in controls, suggesting that motor inhibitory influences and behavioral inhibition would be weaker in AUDs. Yet, the between-group differences were not statistically significant, contrasting with our prior work (Quoilin et al., 2018). This discrepancy might be due to several factors. For instance, as the magnitude of the inhibitory defect has been related to addiction severity (Quoilin et al., 2018), it might be that patients recruited in this study suffered from a less severe form of AUD. In line with this assumption, two out of the 15 patients were diagnosed with moderate AUD, while the level of anxiety and depression did not significantly differ between both groups, contrary to many other works (Leclercq et al., 2014; Quoilin et al., 2018). Importantly though, this lack of difference was actually advantageous, allowing us to avoid strong confounding effects related to the links between anxiety, depression and gut microbiota (Bercik et al., 2011; Leclercq et al., 2014; Zheng et al., 2016).

In summary, the present study indicates that excitatory and inhibitory brain processes might be partially attributable to gut microbiota. Although we proposed a potential contribution of GABA, the mechanisms underlying the gut-brain interactions are complex and remain to be elucidated. As such, we plan to evaluate immunological factors (endotoxins, pro-inflammatory cytokines) as well as metabolic components, especially microbiota-derived metabolites, that could drive the behavioral effects. This future work, analyzing the fecal and blood metabolites and their association with TMS measures, will shed light on the potential neuroactive properties of specific bacterial metabolites such as the short-chain fatty acids, the aryl hydrocarbon receptors ligands and bacterially produced neurotransmitters. Although this study was exploratory due to the small sample size, it represents the first attempt to investigate the relationships between gut microbiota and excitatory and inhibitory influences assessed with TMS. By highlighting the role of specific bacteria, this work opens new avenues regarding the use of psychobiotics (Sarkar et al., 2016) as a novel approach to improve altered brain processes associated with AUD.

While this study investigated the link between the gut microbiota and brain activity, the gut microbiota-brain axis is less studied in the direction from the brain to the gut. An interesting perspective would be to investigate the effect of neuromodulation interventions, such as repetitive TMS, on the gut microbiota composition (Korenblik et al., 2022; Seewoo et al., 2022).

Author contributions

Conceptualization; CQ, PdT, JD, SL.Data curation; CQ, CA, SL. Formal analysis; CQ, SL.Funding acquisition; PdT, NMD, JD.Investigation; CQ, CA, FF, SL.Methodology; CQ, JD.Project administration; CQ, SL.Resources; NMD, PdT, JD.Software; JD, SL.Supervision; NMD, PdT, JD, SL.Writing original draft; CQ, SL.Writing review & editing; CQ, CA, FF, NMD, PdT, JD, SL.All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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