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The neurobiological markers of acute alcohol's subjective effects in humans

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The ingestion of alcohol yields acute biphasic subjective effects: stimulation before sedation. Despite their predictive relevance to the development of alcohol use disorders (AUD), the neurobiological markers accounting for the biphasic effects of alcohol remain poorly understood in humans. Informed by converging lines of evidence, this study tested the hypothesis that alcohol ingestion acutely increases gamma-aminobutyric acid (GABA)-mediated inhibition, which would positively and negatively predict the feeling of stimulation and sedation, respectively. To do so, healthy participants ($n = 20$) ingested a single dose of 94% ABV alcohol (males: 1.0 ml/kg; females: 0.85 ml/kg) in a randomized placebo-controlled cross-over design. The alcohol's biphasic effects were assessed with the Brief-Biphasic Alcohol Effects Scale, and non-invasive neurobiological markers were measured with transcranial magnetic stimulation, before and every 30 min (up to 120 min) after the complete ingestion of the beverage. Results showed that acute alcohol ingestion selectively increased the duration of the cortical silent period (CSP) as compared to placebo, suggesting that alcohol increases non-specific GABAergic inhibition. Importantly, CSP duration positively and negatively predicted increases in the feeling of stimulation and sedation, respectively, suggesting that stimulation emerges as GABAergic inhibition increases and that sedation emerges as GABAergic inhibition returns to baseline values. Overall, these results suggest that modulations of GABAergic inhibition are central to the acute biphasic subjective effects of alcohol, providing a potential preventive target to curb the progression of at-risk individuals to AUD.

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INTRODUCTION

The ingestion of ethyl alcohol produces characteristic acute biphasic effects [1, 2]. Specifically, the ingestion of alcohol (~0.8 g of pure alcohol per kg of body weight) quickly induces euphoria and a subjective high (~5 to 10 min) [1, 2], which then gradually returns to baseline values to give way to an increasing feeling of sedation minutes later (~45 to 60 min) [1, 2]. Interestingly, individual sensitivity to these subjective properties is associated with drinking habits [3], binge drinking [4–6], and the future development of alcohol use disorders (AUD) [7]. The neurobiological effects of alcohol underlying these biphasic subjective properties, however, remain poorly understood in humans [1]. Since AUD is the most prevalent substance use disorder [8] and there is currently no effective medical treatment for the condition [9, 10], gaining a better understanding of the relationship between the behavioral and neurobiological effects of acute alcohol consumption could be an important step in the development of preventive and therapeutic interventions for AUD [9, 10].

Of the two subjective effects of alcohol, the feeling of stimulation has received more attention than the feeling of sedation [1]. On the one hand, in humans, the stimulant properties of alcohol have been shown to predict increased binge drinking propensity [4–7], an effect believed to be mediated by the rewarding alcohol-induced acute increases in dopaminergic (DAergic) [11–14] and adrenergic/noradrenergic (NAergic) activity [15–17]. Animal studies indicate that the type A ionotropic γ-aminobutyric acid ($GABA_A$) receptor agonist properties of alcohol, by triggering the disinhibition of DAergic and NAergic neurons, are responsible for the increases in DAergic and NAergic activity [18, 19]. This finds partial support in neuroimaging studies conducted in humans showing that acute alcohol ingestion modulates GABAergic activity [20–24]. Whether modulations of GABAergic activity can account for the feeling of stimulation, however, remains unclear [25–29]. On the other hand, lower sedative responses following acute alcohol ingestion are also predictive of increased binge drinking propensity [5–7], but uncertainty remains as to what accounts for their delayed-onset in humans [1, 2]. One possibility is that sedation arises because of the $GABA_A$ agonist properties of alcohol, considering that $GABA_A$ agonist drugs, including benzodiazepines and barbiturates [30, 31], induce a feeling of sedation with a time-course similar to that of alcohol [32, 33]. Still, animal studies show that alcohol promptly (~5 min) increases $GABA_A$ activity [19, 34, 35], which then gradually returns to baseline values ~60 min following its administration [36], which is inconsistent with the delayed-onset feeling of sedation observed in humans (~45 to 60 min) [1, 5, 37].

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As a result, whether or not alcohol-induced increases in GABAergic activity [20–24] contribute to the feeling of sedation in humans remains unclear [25–29].

The objective of this study was to investigate the neurobiological markers associated with alcohol's acute biphasic effects. Twenty healthy adults ingested a beverage that either contained alcohol or not (placebo) over two distinct experimental visits in a counterbalanced order. The subjective and neurobiological effects of alcohol were evaluated with validated questionnaires [38] and transcranial magnetic stimulation (TMS) [39], respectively, before and every 30 min (up to 105 min) following complete ingestion of the beverage (Fig. 1). For decades, TMS has been routinely used to evaluate the cortical silent period duration and—when delivering paired-pulses—short intracortical inhibition, intracortical facilitation, and long intracortical facilitation, measures shown by pharmacological-TMS studies to reflect non-specific GABAergic, $GABA_A$, N-Methyl-D-Aspartate (NMDA), and metabotropic $GABA_B$ receptor activity, respectively [40, 41]. Here, TMS was used to determine if these systems were modulated by alcohol ingestion, as animal evidence has shown [18, 19]. It was expected that the ingestion of alcohol would yield its acute biphasic effects [1] and enhance TMS measures of GABAergic inhibition [21, 23] as compared to placebo. Given the expected time-course of stimulation and sedation in humans (promptly stimulates, then gradually sedates) [1, 2] and the expected time-course of $GABA_A$ inhibition as reported in animal studies (promptly increases, [19, 34, 35] then gradually returns to baseline values [36]), it was hypothesized that GABAergic inhibition would positively and negatively predict stimulation and sedation, respectively.

METHODS

Participants

Twenty medication-free and neurologically healthy young adults gave their written informed consent to participate in this study ($n = 20$; $F = 9$). An a priori analysis was conducted in G*Power v.3.1.9.2. [42] and revealed that 18 participants would be required to achieve an R^2 of 0.2 when conducting random linear models (assuming 2 predictors, two-tailed statistical significance, an alpha of 0.05, and 80% power). This R^2 value was the smallest effect size of interest in the context of this work [43]. To ensure sufficient statistical power, this study had a final sample size of 20 participants.

All participants were of legal drinking age for alcohol consumption (mean ± 95% CI: 22 ± 0.93 years old) and had already experienced alcohol in the past. Participants were not eligible for the study if they scored 8 or above on the Alcohol Use Disorders Identification Test (AUDIT) [44, 45]. Namely, participants had a mode and median total AUDIT score of 5, the lowest and highest scores being 2 and 7, respectively (see Additional Table 1 for individual AUDIT items). Female participants took an over-the-counter pregnancy test before each experimental visit to ensure non-pregnancy at the moment of testing. Participants were also screened for any contraindication to the use of TMS [46], the absolute contraindication being the presence of metallic particles/hardware in the head region and a major one being epileptic seizure history [47]. Ethics approval was obtained from the local institutional ethics review board and complied with the 1964 Declaration of Helsinki.

Protocol overview

An overview of the protocol is shown in Fig. 1. This work used a within-subject placebo-controlled design to control for individual differences in subjective [48] and physiological responses to alcohol [49, 50], as well as environmental, biological, and genetics individual differences in alcohol metabolism [51]. Namely, participants took part in two experimental visits where they either consumed an alcohol-containing or a placebo beverage. The order between the two visits was fully counterbalanced and separated by at least 48 h. For each participant, the two visits occurred within an interval of 1 to 2 weeks and both visits occurred at the same time of day. The latter was to control for a potential effect of circadian rhythms on cortical excitability [52–54] and alcohol metabolism [55].

Upon arrival at the study site, participants were asked if they had taken any over-the-counter drugs in the preceding 48 h (e.g., alcohol, cannabis, acetaminophen, antihistamines, etc.). The case being, participants were rescheduled to a later date to meet this inclusion criterion [56]. To control

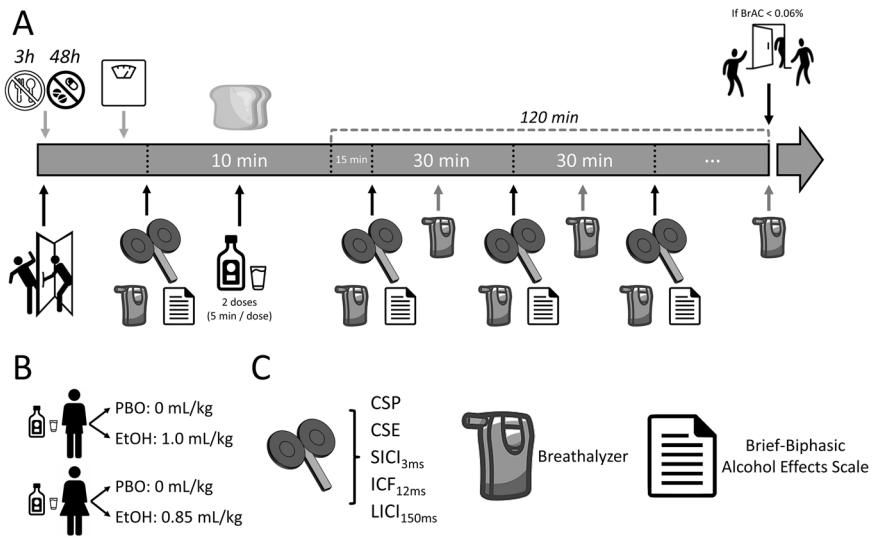

Fig. 1 Overview of the within-subject and placebo-controlled paradigm. A *Timeline of a typical experimental visit*. Session order was fully counterbalanced across participants. From start to finish, a typical session lasted ~ 3 h. The first set of measurements was taken before beverage ingestion. Starting 15 min after complete beverage ingestion, 4 subsequent measurements were taken every 30 min up to 105 min, except for breath alcohol concentrations (BrAC), which were measured every 15 min up to 120 min. Participants could then leave the laboratory if their BrAC returned below 0.06%. **B** *Body weight- and gender-controlled quantity of 94% ABV alcohol administered*. Females ingested 15% less alcohol than males. **C** *Measurements taken*. To evaluate cortical excitability, the following variables were measured: cortical silent period (CSP) duration, corticospinal excitability (CSE), short intracortical inhibition ($SICI_{3ms}$), intracortical facilitation (ICF_{12ms}), and long intracortical inhibition ($LICI_{150ms}$). Pharmacological-TMS studies have shown that CSP duration, SICI, ICF, and LICI reflect non-specific GABAergic inhibition, $GABA_A$-mediated inhibition, NMDA-mediated activity, and $GABA_B$-mediated inhibition, respectively (see ref. [40]). To evaluate BrACs, participants blew in a breathalyzer. To evaluate the subjective feelings of stimulation and sedation, the Brief-Biphasic Alcohol Effects Scale 6-item questionnaire was administered.

for gastric emptying and absorption rate, participants had to fast for 3 h before an experimental visit to arrive on an empty stomach. Participants were blinded as to which beverage they were to consume; however, verbal reports indicated that all of them were able to determine if they ingested the alcohol-containing or placebo beverage, presumably because of their familiarity with the subjective feelings of alcohol.

Participants were weighed on a medical-grade weight scale, which was used to adjust the quantity of alcohol and volume of liquids that needed to be ingested. Participants were then prepared for the TMS measurements. After the localization of the motor hotspot and resting motor threshold (RMT), data collection began. Breath alcohol concentrations (BrAC) were assessed using the BACtrack mobile device (Breathalyzer.ca ®) [57] before and every 15 min up to a total of 120 min following complete ingestion of the beverage. The Brief-Biphasic Alcohol Effects Scale (B-BAES) questionnaire evaluating the biphasic subjective effects of alcohol [38] and TMS measurements were taken before and every 30 min up to a total of 105 min following complete ingestion of the beverage (see Fig. 1A). At each measurement, the above were always assessed in the same order (BrAC, B-BAES, then TMS).

To allow for alcohol to be absorbed and not contaminate the BrAC readings, 15 min separated the end of the beverage's complete ingestion and the first BrAC, B-BAES, and TMS measurements. Participants were allowed to leave the laboratory 120 min after ingestion of the beverage and if BrAC readings were below 0.06%.

Alcohol administration and beverage ingestion

Ninety-four percent (94%) alcohol by volume (ABV) ethyl alcohol was dosed to induce a maximal BrAC reading of 0.095% using a variation of the Widmark formula which included the duration of ingestion [58, 59]. This BrAC was chosen to approximate a level of intoxication that would be reached in a social setting and to minimize the adverse reactions to alcohol, which tend to appear above a BrAC of ~0.15% [60, 61]. To induce a peak BrAC of 0.095%, the 94% ABV alcohol doses were body weight- and gender-controlled. Namely, males ingested 1 mL/kg of body weight while females ingested 0.85 mL/kg of body weight (See Fig. 1 panel B). Females ingested 15% less alcohol than males because they typically reach higher BrAC readings even after correcting for body weight [62–64].

To control participants' absorption of alcohol, participants had 5 min to drink the first and second half of the beverage (total of 10 min). A solution of 1 part 94% ABV alcohol for 3 parts of orange juice was provided for the alcohol-containing beverage. The placebo beverage contained an identical liquid volume but was composed of orange juice only. To improve taste, a Mango Peach-flavored liquid flavor enhancer was added to both the alcohol-containing and placebo beverages. Given that participants arrived on an empty stomach, ad libitum pieces of toast (with a choice of peanut butter and/or strawberry jam) were prepared for the participants to eat while drinking their beverage. This was to minimize the emergence of any gastric discomfort during the experiment. The number of pieces of toast eaten was identical for both visits for a given participant.

To evaluate the subjective feelings of sedation and stimulation, the Brief-Biphasic Alcohol Effects Scale (B-BAES) [38] was administered (Fig. 1). The B-BAES contained a total of 6 items (Energized, Excited, Up; Sedated, Slow Thoughts, Sluggish) that participants rated on a scale from 0 (not at all) to 10 (extremely) [38]. For statistical analyses, B-BAES scores for stimulation and sedation were separately summed. Then, these scores were normalized by subtracting the pre-measurement (baseline) scores from the following measurements (see Fig. 2B, C). A single administration of the B-BAES required less than 30 s to complete.

Neuronavigated-ppTMS data acquisition

Electromyographic (EMG) data were recorded in a tendon-belly arrangement from the right first dorsal interosseous (FDI) muscle. The ground and reference electrodes were placed on the ulnar styloid process and the distal phalanx of the right index, respectively. The EMG signal was amplified using a 1940 CED amplifier (Cambridge, UK) with a gain of 1000, bandpass filtered between 20 Hz and 1000 Hz (notch filter at 60 Hz; the notch filter was removed to assess the CSP), and digitized at a sample rate of 5000 Hz. Each EMG epoch lasted 500 ms, with the first TMS pulse occurring 100 ms after the epoch onset.

The motor hotspot was defined as the optimal location to induce motor evoked potentials (MEPs). Once the motor hotspot was localized, it was recorded with a neuronavigation stereotactic system (BrainSight; Rogue Research; Montreal, Canada) to ensure the reliable and stable positioning of the coil on the head. The RMT was defined as the percentage of maximum stimulator output required to induce at least 5 MEPs of a minimum of 50 µV peak-to-peak amplitude out of 10 consecutive pulses [47].

In animals, alcohol has been reported to increase $GABA_A$ and inhibit NMDA activity [19, 34], which should be reflected in TMS-measured increases in short intracortical inhibition (SICI) and decreases in intracortical facilitation (ICF), respectively [40, 41]. SICI and ICF were assessed using a conditioning stimulus (CS) intensity set at 70% of the RMT and a test-stimulus (TS) intensity set at 125% of the RMT. These intensities were maintained constant for the experimental session, as pilot data ($n = 5$) and previous studies indicated no change in RMT [21, 23] or corticospinal excitability (CSE) following alcohol consumption [21, 23, 24]. For SICI and ICF, the inter-pulse interval (IPI) was set at 3 ms and 12 ms, respectively [40, 41]. For subsequent analyses, peak-to-peak amplitudes of the MEP were calculated and averaged separately for SICI and ICF at each measurement and participant. To account for the possibility that alcohol also increases the activity of metabotropic $GABA_B$ receptors [65], TMS-measured modulations of long intracortical inhibition (LICI) were recorded [40, 41]. To measure LICI, both pulses were set at TS intensity with an IPI of 150 ms [41, 66]. To normalize the LICI measurements, the amplitude of the second MEP was divided by the amplitude of the first one on a trial-per-trial basis. The resulting ratios were then averaged for each measurement and participant. The MEP induced by the first pulse of the LICI was used to assess CSE since this pulse was delivered at TS intensity and was not conditioned by a prior pulse. To normalize the SICI and ICF measurements, the average SICI and ICF amplitude values were divided by average CSE amplitude values to obtain percent changes for each measurement and participant. A total of 30 pseudo-randomized trials was recorded for each ppTMS variable to ensure reliable assessment at each measurement [67, 68].

Cortical silent period (CSP) duration was assessed by stimulating the motor hotspot at TS intensity while participants maintained an isometric contraction of the FDI corresponding to 30% of their maximal voluntary contraction. To optimize the reliable assessment of the CSP, 10 trials were recorded [41]. CSP duration was calculated as the time difference between the positive peak of the MEP and the returning to baseline values of the EMG signal with a threshold based on a 2 ms-wide sliding time window (10 samples). Specifically, the threshold used to determine if the EMG signal returned to baseline values was set as the value corresponding to 50% of the standard deviation of the first 60 ms of the epoch EMG signal (300 samples; similar to refs. [69, 70]).

To obtain percent changes as a function of the baseline values, the 15 min, 45 min, 75 min, and 105 min TMS measurements were divided by pre-measurement (baseline) data for each condition and participant (see Fig. 3). Overall, the above procedures resulted in the recording of a total of 500 TMS trials per experimental visit per participant. Each TMS measurement required ~20 min to be completed. Because of software malfunctions during data acquisition, a total of 3 EMG measurements (out of a total of 200) for distinct participants were lost.

Adverse reactions

One male participant vomited approximately 45 min after the complete ingestion of the beverage, causing the immediate cessation of data collection. The last BrAC value measured before vomiting was 0.064%. The participant underwent the placebo before the alcohol condition, implying that data points were missing in the alcohol condition only. Because the present statistical analyses can accommodate missing data (see below), this participant's data were included in the analyses. No other adverse event occurred.

Statistical analyses

Main analyses were conducted using Mixed Linear Models (MLMs) [71–73] because these models can accommodate missing data [74] and can be used to determine the presence of linear associations in repeated measures data (e.g., repeated measure correlation; see ref. [75]), which is required to test this work's hypotheses. The random coefficients included in the models were determined based on the most recent recommendations (see ref. [74] for a comprehensive review). Namely, the manipulated factors (i.e., Condition and Time) were systematically included in the model as Fixed Effects. Regarding the random coefficients, maximally complex models (random intercepts for Participants and random slopes for all of the Fixed Effects and Interactions, wherever the data allowed their inclusion)

were built [74]. The random coefficients that best minimized the model's information loss, as determined via model-specific lowest relative Akaike Information Criterion (AIC) values, were chosen to analyze the data and report the results [74]. The random coefficients included in the models are reported below each statistics table. The Benjamini-Hochberg procedure (i.e., false discovery rate) was used to correct for multiple comparisons [76]. To provide an assessment of the direction, size, and plausibility of the discovered effects [77, 78], β regression coefficients and their 95% confidence intervals are reported in addition to p values. For conciseness, the acronyms EtOH and PBO are used to refer to the alcohol and placebo conditions, respectively. Unless otherwise specified, all of the reported descriptive statistics represent the mean ± 95% confidence intervals (CIs). The above analyses were conducted using the Linear [Mixed] Models module in *jamovi* (v1.6.23) [79]. Microsoft Office Excel and PowerPoint (2013) were used for graphic preparation and figure building. Statistical significance was set at an alpha of 0.05.

RESULTS

Alcohol increased BrACs and induced subjective biphasic stimulation/sedation effects

Concerning BrAC data, the results revealed a Condition × Time interaction ($p < 0.0001$; see Additional Table 2 and Fig. 2A). As expected, the breakdown of the Condition × Time interaction revealed that BrAC readings were higher in the EtOH condition as compared to PBO at every measurement from 15 min ($\beta = 0.102 \pm 0.004$; $p < 0.0001$) to 120 min ($\beta = 0.058 \pm 0.004$; $p < 0.0001$). No difference was observed at the pre-measurement between the two conditions ($p = 1.0000$) or against zero ($p = 1.0000$). This confirms that participants arrived sober to both experimental visits and that alcohol increased BrACs.

Concerning the subjective feeling of stimulation assessed with the B-BAES, the results revealed a Condition × Time interaction ($p < 0.0001$; see Additional Table 3 and Fig. 2B). Breakdown of the interaction revealed that the subjective feeling of stimulation increased at 15 min ($\beta = 6.700 \pm 2.381$; $p < 0.0001$) and remained higher at 45 min ($\beta = 5.400 \pm 2.381$; $p < 0.0001$) and 75 min ($\beta = 2.993 \pm 2.401$; $p = 0.0251$) in the EtOH condition as compared to PBO. The feeling of stimulation did not differ between EtOH and PBO at 105 min ($\beta = 1.686 \pm 2.417$; $p = 0.1788$). These results confirm that alcohol quickly induced a transient feeling of stimulation that progressively returned to baseline values (Fig. 2B).

Concerning the subjective feeling of sedation assessed with the B-BAES, the results revealed a Condition × Time interaction ($p = 0.0193$, see Additional Table 4 and Fig. 2C). Breakdown of the interaction revealed that the subjective feeling of sedation did not differ at 15 min ($\beta = 1.200 \pm 2.379$; $p = 0.3275$) between PBO and EtOH. However, the feeling of sedation was greater at 45 min ($\beta = 2.650 \pm 2.379$; $p = 0.0447$), 75 min ($\beta = 2.662 \pm 2.403$; $p = 0.0457$), and 105 min ($\beta = 4.085 \pm 2.425$; $p = 0.0023$) in the EtOH condition as compared to PBO. These results confirm that alcohol induced a feeling of sedation that emerged as a function of the time after its ingestion (Fig. 2C).

Normalized TMS results: alcohol increased CSP duration but did not alter CSE, SICI, ICF, or LICI

Concerning CSP data, the results revealed a Condition × Time interaction ($p < 0.0001$; Table 1 and Fig. 3A). Breakdown of the interaction revealed that the CSP duration was greater at 15 min ($\beta = 0.174 \pm 0.076$; $p < 0.0001$), 45 min ($\beta = 0.194 \pm 0.077$; $p < 0.0001$), 75 min ($\beta = 0.186 \pm 0.077$; $p < 0.0001$), and 105 min ($\beta = 0.111 \pm 0.078$; $p = 0.0082$) in the EtOH condition as compared to PBO. These results suggest that alcohol increased GABAergic inhibition [80, 81].

Concerning CSE data, the results revealed no Condition × Time interaction ($p = 0.3202$), no effect of Condition ($p = 0.5365$), but showed an effect of Time ($p = 0.0058$; Table 2 and Fig. 3B). Breakdown of the effect of Time revealed that CSE was greater at 15 min compared to Pre ($\beta = 0.138 \pm 0.115$; $p = 0.0495$), 45 min ($\beta = 0.142 \pm 0.116$; $p = 0.0430$), 75 min ($\beta = 0.138 \pm 0.116$; $p = 0.0510$), and 105 min ($\beta = 0.227 \pm 0.116$; $p = 0.0005$). No other comparison was significant (all $\beta < 0.089 \pm \sim 0.116$; all $p > 0.2250$). These results indicate that the mere passage of time, but not alcohol, modulated CSE.

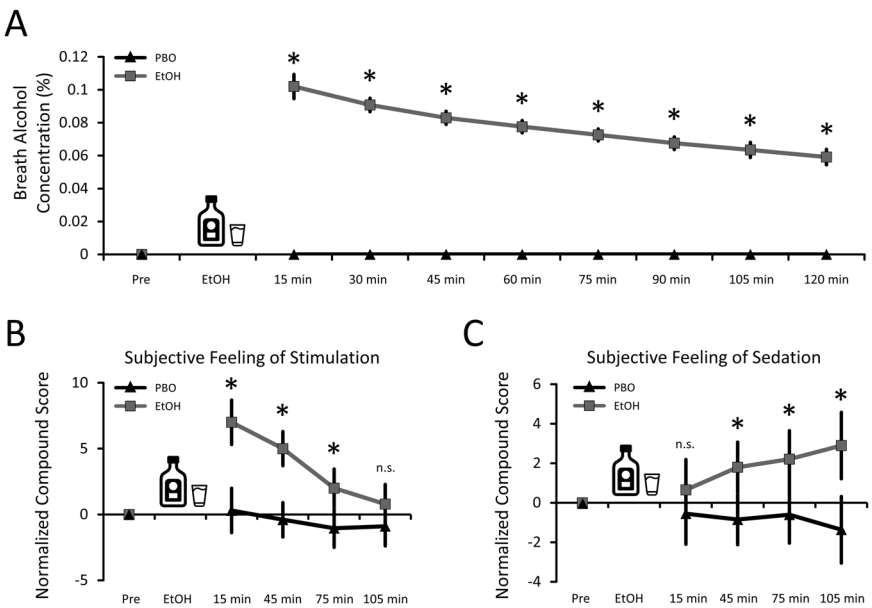

Fig. 2 BrAC and B-BAES results. A *Breath alcohol concentration (BrAC) as a function of time*. As expected, the ingestion of alcohol promptly increased BrACs in the EtOH condition whereas BrACs remained at zero in the PBO condition. BrACs peaked 15 min after the complete alcohol-containing beverage ingestion and monotonically decreased afterward. **B** *Subjective Feeling of Stimulation as a function of time*. The feeling of stimulation of the EtOH condition increased at 15 min, but decreased in the following measurements to reach values similar to the PBO condition at 105 min. **C** *Subjective Feeling of Sedation as a function of time*. The feeling of sedation monotonically increased from 15 min to 105 min in the EtOH as compared to the PBO condition. The differences reached significance from the 45 min measurement onwards. The markers represent within-subject conditions means and error bars depict within-subject 95% CIs. Asterisks (*) indicate significant differences ($p < 0.05$) and "n.s." means "non-significant" ($p > 0.05$).

Table 1. Cortical silent period duration.

Model info	AIC	R^2 marginal	R^2 conditional	ICC
	−262.4	0.353	0.753	0.336
Model results	*F*	Num df	Den df	*P* value
Condition	18.069	1	19.07	0.0004
Time	13.962	4	22.96	<0.0001
Condition × Time	9.781	4	111.18	<0.0001

The random effects that minimized the relative AIC value were the inclusion of participants as random intercepts and both Condition and Time as random slope coefficients.

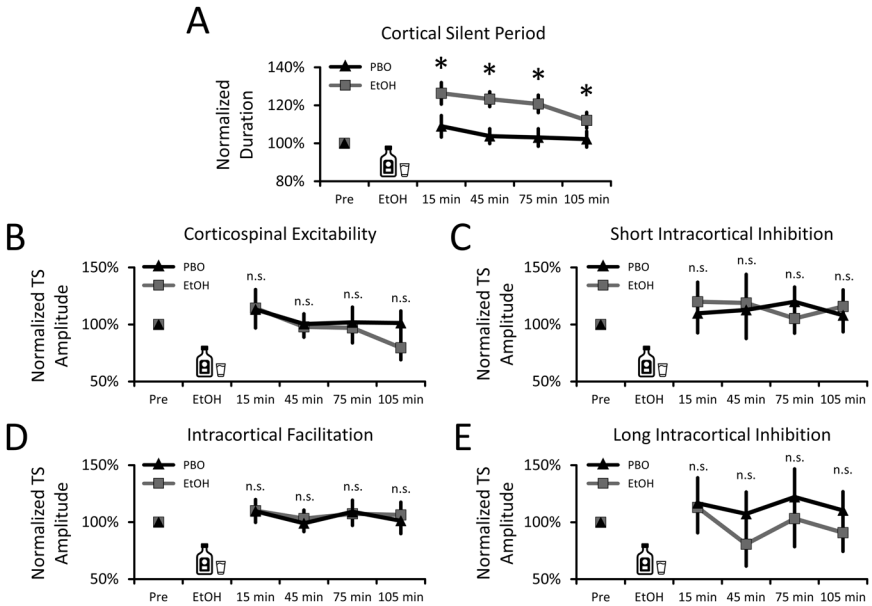

Fig. 3 TMS results. A *Cortical Silent Period (CSP)*. The results revealed that alcohol increased the duration of the CSP as compared to placebo. **B** *Corticospinal Excitability (CSE)*. Alcohol did not alter CSE as compared to placebo. **C** *Short Intracortical Inhibition (SICI)*. Alcohol did not alter SICI as compared to placebo. **D** *Intracortical Facilitation (ICF)*. Alcohol did not alter ICF as compared to placebo. **E** *Long Intracortical Inhibition (LICI)*. Alcohol did not alter LICI as compared to placebo. The markers represent within-subject conditions means and error bars depict within-subject 95% CIs. Asterisks (*) indicate significant differences ($p < 0.05$) and "n.s." means "non-significant" ($p > 0.05$).

Table 2. Corticospinal excitability.

Model info	AIC	R^2 marginal	R^2 conditional	ICC
	107.7	0.061	0.459	0.261
Model results	F	Num df	Den df	*P* value
Condition	0.396	1	19.22	0.5365
Time	3.790	4	148.67	0.0058
Condition × Time	1.184	4	148.67	0.3202

The random effects that minimized the relative AIC value were the inclusion of participants as random intercepts and Condition as a random slope coefficient.

Concerning SICI, ICF, and LICI data, the results revealed no statistically significant Condition × Time interaction (all $p > 0.3092$) and no effect of Condition (all $p > 0.3143$; see the Additional Tables 5–7 in the Supplementary Results for statistical details; Fig. 3C–E). Globally, the ingestion of alcohol did not meaningfully alter any ppTMS variable as compared to the ingestion of the placebo.

CSP positively and negatively predicted stimulation and sedation, respectively, while release from SICI predicted sedation

MLMs allow determining if time-varying repeated measurements are linearly associated, therefore acting as a repeated measure correlation (see ref. [75]). Here, this property was used to determine if the above neurobiological markers predicted alcohol's biphasic effects. Specifically, CSP duration, CSE, SICI, ICF, and LICI were separately included as covariates in the MLMs conducted on the feeling of stimulation and sedation (see Additional Tables 3, 4) to determine if they significantly predicted alcohol's biphasic effects. As in the MLMs conducted above, the fixed factors (Condition, Time) and the random coefficients minimizing the relative AIC value were included in each model. For further statistical details, readers are referred to Additional Tables 8–17 in the Supplementary Results.

Concerning the feeling of stimulation, the results revealed that CSP duration was positively associated with stimulation

(β = 4.479 ± 3.908; $p = 0.0262$; see Additional Table 8). However, including CSP as a covariate did not account for the Condition × Time interaction initially reported in the feeling of stimulation, as it remained significant ($p < 0.0001$; see Additional Tables 3, 8). Further results revealed that neither CSE (β = 0.039 ± 1.482; $p = 0.9592$; see Additional Table 9), SICI (β = 0.815 ± 1.070; $p = 0.1381$; see Additional Table 10), ICF (β = 0.300 ± 1.880; $p = 0.7552$; see Additional Table 11), nor LICI (β = 0.005 ± 0.850; $p = 0.9903$; see Additional Table 12) was associated with the feeling of stimulation. Overall, these results suggest that the feeling of stimulation increased as CSP duration increased. They also suggest that CSE, SICI, ICF, and LICI were not associated with the feeling of stimulation.

Concerning the feeling of sedation, the results revealed that CSP duration was negatively associated with sedation (β = −6.250 ± 4.252, $p = 0.0046$; see Additional Table 13). However, including CSP as a covariate did not account for the Condition × Time interaction initially reported in the feeling of sedation, as it remained significant ($p = 0.0011$; see Additional Tables 4, 13). The results also revealed that SICI was positively associated with sedation (β = 1.310 ± 1.211; $p = 0.0358$; see Additional Table 15) and also did not account for the Condition × Time interaction initially reported in the feeling of sedation ($p = 0.0110$; see Additional Tables 4, 15). Further results also revealed that neither CSE (β = −0.223 ± 1.670; $p = 0.7939$; see Additional Table 14), ICF (β = 0.682 ± 2.119; $p = 0.5295$; see Additional Table 16), nor LICI (β = 0.312 ± 0.987; $p = 0.5363$; see Additional Table 17) was associated with the feeling of sedation. Overall, these results suggest that the feeling of sedation increased as CSP duration decreased and SICI was released. They also suggest that CSE, ICF, and LICI were not associated with the feeling of sedation.

DISCUSSION

The present work tested the hypothesis that increases in GABAergic inhibition would positively and negatively predict the stimulant and sedative properties of alcohol, respectively. Data confirmed the expected biphasic properties of alcohol. Furthermore, alcohol increased the duration of the CSP—but did not alter CSE, SICI, ICF, or LICI—as compared to placebo treatment. This suggests that alcohol alters human nervous system functioning through non-specific GABAergic mechanisms rather than selectively through $GABA_A$, NMDA, or $GABA_B$ inhibition. Additionally, CSP duration positively and negatively predicted alcohol-induced stimulation and sedation, respectively, suggesting that stimulation increases with GABAergic inhibition while sedation emerges when GABAergic inhibition returns to baseline values. Finally, although SICI was not modulated by alcohol ingestion, it positively predicted the feeling of sedation. Given that SICI is thought to reflect $GABA_A$ inhibition [40, 41], this indicates that a *release* from $GABA_A$ inhibition contributes to sedation, suggesting that alcohol does neither alter human brain functioning nor induce sedation through increased $GABA_A$ inhibition per se.

Alcohol increased CSP duration, which was associated with its biphasic effects

One important finding of the present study is that alcohol increased CSP duration as compared to placebo treatment, which is in line with previous TMS work [21, 23]. Increased CSP duration reflects increased inhibition of neural activity [40, 41] but its unclear origins make it difficult to determine whether this inhibition is mediated by $GABA_A$ or $GABA_B$ receptors in cortical or spinal structures. For example, although CSP duration is believed to predominantly reflect $GABA_B$-mediated inhibition [40, 41], studies have shown that administration of a $GABA_B$ agonist drug (Baclofen) does not affect CSP duration [82, 83]. Moreover, the administration of $GABA_A$ agonists (Diazepam and Lorazepam) has been shown to decrease [82, 84] or increase [85] CSP duration, suggesting that $GABA_A$ inhibition contributes to the modulation of CSP duration. In light of this conflicting evidence, one parsimonious interpretation for the present results is that CSP duration increases reflect non-specific enhancements of GABAergic inhibition [65]. Given that alcohol diffuses to virtually every biological tissue [86, 87], it can be reasonably assumed that these GABAergic enhancements occur in both supraspinal and spinal structures involved in the generation of a CSP [81].

The key novel finding of the present work is that CSP duration positively and negatively predicted the feeling of stimulation and sedation, respectively. These results suggest that stimulation arises when GABAergic inhibition increases and that sedation emerges when GABAergic inhibition returns to baseline values. The positive association between SICI disinhibition and the feeling of sedation further emphasizes the idea that sedation emerges when GABAergic inhibition diminishes. It should be noted, however, that CSP duration and SICI data are unlikely to fully account for the feeling of stimulation and sedation, as the effects of alcohol on the latter remained significant after including CSP and SICI as covariates in the analyses. Although future confirmatory work is required to formally test if CSP and SICI are mediators of stimulation and sedation, this observation nonetheless suggests that the biphasic effects of alcohol are mediated by multifaceted mechanisms [1, 2, 19]. In further support, alcohol increased both stimulation and sedation at the 45 and 75 min marks as compared to placebo treatment, where stimulation was decreasing while sedation was increasing. This temporal overlap but with opposite directions suggests that at least two parallel systems mediate the subjective effects of alcohol, further arguing for multifaceted mechanisms. One aspect awaiting further clarification is the extent to which DAergic activity contributes to the increases in CSP duration found in the present study. Previous TMS work has shown that the administration of the DAergic agonists L-Dopa and Pergolide increases CSP duration [88, 89], suggesting that increased GABAergic inhibition is not the only factor underlying CSP duration increases and their association with alcohol-induced stimulation [1, 90–92]. However, determining the respective contribution of GABAergic and DAergic activity to the present results may be challenging as animal work indicates that GABAergic and DAergic activity covary [18, 19]. Nevertheless, the present results indicate that increased alcohol-induced neural inhibition, as assessed by CSP duration, is associated with increased stimulation. The contribution of additional neuromodulatory systems—such as acetylcholine [19]—to alcohol-induced stimulation remains a query for future studies.

With regards to sedation, the present results suggest that increases in GABAergic inhibition are not a direct contributor to the emergence of sedation. One alternative mechanistic account is an alcohol-induced increase in β-endorphin levels [93]. β-endorphin levels increase to mitigate alcohol-induced physiological stress responses [94, 95]. In turn, increased β-endorphin levels could induce sedation through their analgesic and inhibitory properties [96]. Interestingly, animal work points to an alcohol-induced increase in β-endorphin levels that qualitatively matches the delayed-onset alcohol-induced feeling of sedation (~60 min) [97]. Additionally, alcohol-induced reductions in cerebral glucose metabolism [98–101], increases in serotonin levels [102–104], and increases in pro-neuroinflammatory activity [105–109] could also be involved in the sedative properties of alcohol, as they are believed to contribute to the onset of central fatigue [110–114]. Future studies are needed to determine if these biological mechanisms—and their time-course—can account for the delayed-onset emergence of alcohol-induced sedation.

Alcohol did not alter CSE, SICI, ICF, and LICI

Alcohol did not alter CSE, SICI, ICF, and LICI as compared to placebo treatment. While the lack of CSE alteration is in line with previous TMS studies [21, 23, 24], the absence of significant modulation of SICI and ICF following alcohol ingestion is at odds with a study by Ziemann et al. [21], which reported increased SICI and reduced ICF

30 min after alcohol ingestion. This discrepancy could be explained by the absence of a placebo condition, a small sample size ($n = 6$) [115], and the low number of TMS measures per variable ($n = 10$) [67, 68] in the original study [21]. Nevertheless, given that SICI and ICF are believed to represent $GABA_A$ and NMDA receptors activity, respectively [40, 41], and that animal models indicate that alcohol potentiates $GABA_A$ and inhibits NMDA receptor activity [18, 19], increases in SICI and decreases in ICF could reasonably have been expected in the present study. One possibility is that the net stimulant NAergic effects of alcohol opposes its inhibitory properties in humans, summing up to a null effect when assessed at the systems level with TMS. In support, TMS studies have shown that the administration of a NAergic agonist (i.e., the NA reuptake inhibitor Reboxetine) reduces SICI and increases ICF [116–119], suggesting that the agonist NAergic properties of alcohol could oppose the expected increases of SICI and decreases of ICF.

LIMITATIONS

One limitation of the present study is the absence of detailed demographic, psychometric, and alcohol consumption pattern characterization of the participants. Future work will gain from determining if the present neurobiological markers can be associated with sex differences, individual demographic and psychometric factors as well as alcohol consumption patterns. Another limitation is the absence of a dose-response design, in which an intermediate alcohol dose (e.g., peak BrAC of ~0.05%) or multiple smaller alcohol doses distributed over a longer ingestion (similar to ref. [120]) period could have been administered. Future work reinvesting the present results to investigate these open questions is bound to be insightful. Also, it should be noted that the present TMS measures are bound to excitability changes in cortical motor areas and corticospinal tract. Using multimodal pharmacological-neuroimaging means (see ref. [121]) to determine how the present results translate to brain regions outside of these structures remains a query for future work.

CONCLUSION

This work investigated the relationship between the subjective biphasic and neurobiological effects of acute alcohol consumption using TMS in humans. The results revealed that acute alcohol ingestion selectively increased CSP duration as compared to placebo treatment, which suggests that alcohol increases non-specific GABAergic inhibition. Importantly, the results revealed that increases in the feeling of stimulation are accompanied by increases in GABAergic inhibition and that the feeling of sedation gradually emerges as GABAergic inhibition returns to baseline values. Interestingly, human evidence suggests that drugs with GABAergic agonists properties (e.g., topiramate [122] and baclofen [123]) and non-invasive brain stimulation interventions (e.g, transcranial direct current stimulation [124, 125] and repetitive TMS [126–128]) improve AUD symptoms by decreasing alcohol consumption, relieving withdrawal symptoms, and preventing relapses (although see refs. [129, 130]). As the present results show that GABAergic inhibition predicts alcohol's biphasic effects—themselves predicting AUD development [4–7]—one possibility is that preventive interventions seeking to alter GABAergic inhibition while drinking could curb the progression of at-risk individuals to AUD.

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AUTHOR CONTRIBUTIONS

RH designed the experiment, conducted the analyses, prepared the figures, and wrote the manuscript. OD collected the data, conducted the analyses, prepared the figures, and helped to write the manuscript. CB and MLR collected the data. HT and PMB revised the manuscript. JFL helped to design the experiment and revised the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

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