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**Advancing flavor perception research with EEG microstate analysis: A dynamic approach to understanding brain responses to alcoholic stimuli**

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**Abstract:** Understanding how our brain's perceptual system related to sensory evaluation of food can be affected by alcohol concentration is essential for both neuroscience and food science. This study applied EEG microstate analysis to characterize dynamic brain activity across seven alcohol levels (water, 5%, 10%, 20%, 40%, 53% ABV, and Baijiu). Unlike traditional EEG analyses, microstate analysis provides a temporally resolved perspective on large-scale neural dynamics. Four microstates (A, B, C, D) were identified, with microstates B and C predominantly involved in sensory-emotional processing. Lower alcohol levels ( $\leq 20\%$  ABV) enhanced sensory focus, whereas higher concentrations ( $\geq 40\%$  ABV) induced frequent sensory re-evaluation and attentional shifts. These results reveal concentration-dependent neural adaptations, demonstrating that alcohol modulates both sensory and cognitive processing through dynamic brain state transitions. These findings enhance our understanding of alcohol-induced sensory and cognitive processing, providing insights for both neuro-flavor research and food science applications.

**Keywords:** Baijiu, EEG, Microstates, Alcohol, Brain Activity, Neurophysiology

## 1. Introduction

Alcoholic beverages captivate consumers through their sensory appeal and social significance. Among the many factors shaping drinking behavior, flavor perception is widely recognized as a primary driver of purchase decisions (Bruwer & Buller, 2012).

The sensory attributes of alcoholic beverages arise from the complex interplay of multiple volatile and non-volatile flavour compounds, including alcohols, esters, acids, ketones, aldehydes, and nitrogen- and sulfur-containing compounds. Among these, ethanol, as the predominant component apart from water, plays a crucial role in defining the drinking experience by modulating mouthfeel, texture, and overall flavour perception. Alcohol concentration significantly influences these sensory characteristics, yet its effects are not a simple linear function of quality. Beverages with higher alcohol content are typically associated with increased viscosity, warmth, and mouthfeel complexity, whereas lower alcohol levels may enhance acidity, fruitiness, or aromatic nuances, depending on the beverage matrix (King et al., 2013).

While these effects on sensory perception are well-documented, the underlying mechanisms by which alcohol concentration shapes flavor perception, particularly at the neural level, remain largely unexplored. Understanding these neural processes is crucial for bridging the gap between sensory experience and cognitive perception.

Building on these distinctions, we hypothesize that neural responses to alcoholic beverages exhibit concentration-dependent variations, reflecting the perceptual and cognitive demands associated with different alcohol levels. Investigating these neural

mechanisms will provide new insights into how alcohol concentration influences sensory perception, with implications for beverage formulation and consumer experience.

Drinking is inherently a multisensory experience, engaging inputs from taste, smell, touch, temperature, and even visual and auditory cues. The integration of these sensory inputs not only influences conscious (explicit) perception, such as flavor intensity and mouthfeel, but also subconscious (implicit) processing mechanisms that shape overall drinking behavior (Spence, 2015; Spence et al., 2014). Traditional sensory evaluation methods, such as self-reported questionnaires and structured sensory panels, are widely used to assess flavor perception. While these approaches provide valuable insights into consumer preferences, they are inherently limited by cognitive biases, inter-individual variability, and an inability to capture real-time neural responses (Lawless & Heymann, 2010; Prescott, 2015). To overcome these limitations, neuroimaging techniques such as electroencephalography (EEG) offer an objective and time-sensitive means to examine the neural mechanisms underlying flavor perception (Danner et al., 2014; Hinojosa-Aguayo et al., 2022; Songsamoe et al., 2019; Gao et al., 2023). EEG's high temporal resolution makes it particularly well-suited for capturing rapid neural responses to taste stimuli, providing a real-time, quantitative measure of brain activity (Anbarasan et al., 2022; Han, 2021). Previous studies have successfully employed EEG to investigate how basic taste stimuli, such as bitterness, saltiness, sourness, and sweetness, modulate neural activity. For

instance, Yang et al. applied EEG power spectral density (PSD) analysis to assess neural responses to four basic taste stimuli and found that high-concentration taste stimuli significantly increased EEG power in the  $\alpha$  and  $\theta$  bands, particularly in sensory and emotional processing regions such as the insula and orbitofrontal cortex (Yang et al., 2023). Similarly, Wu et al. examined EEG responses to three distinct umami compounds and reported that each compound elicited unique spectral changes, highlighting EEG's capability in differentiating fine-grained gustatory stimuli (Wu et al., 2022). However, while EEG has been increasingly explored in basic taste perception, its use in understanding how alcohol concentration influences neural activity remains limited. Prior research suggests that alcohol modulates neural responses, with higher concentrations eliciting stronger activations in the  $\delta$  and  $\alpha$  frequency bands, particularly in the frontal and right temporal regions (Wang et al., 2025). These findings demonstrate that EEG can effectively capture neural responses to varying alcohol concentrations. However, existing studies have focused primarily on spectral power changes, lacking insight into the dynamic transitions between large-scale neural networks involved in alcohol flavor perception. Flavor perception is not a static process but rather a real-time, dynamic integration of sensory inputs across large-scale neural networks (Bressler & Menon, 2010; Engel et al., 2001)

Conventional EEG analyses, which focus primarily on localized power changes, cannot capture these transient interactions, limiting our understanding of how the brain encodes and processes multisensory information (Engel et al., 2001; Iannilli et

al., 2015; Moore et al., 2015). To address these limitations, this study employs EEG microstate analysis, a method that characterizes transient, large-scale neural configurations over time. By applying microstate analysis to alcohol-induced sensory perception, this study aims to provide novel insights into the temporal evolution of neural responses to varying alcohol concentrations. This approach moves beyond static power-based measures, offering a dynamic perspective on the neural mechanisms underlying alcohol flavor perception.

EEG signals exhibit rapid, non-stationary fluctuations over time, yet underlying stable patterns can be observed when analyzed over short temporal intervals. Microstate analysis, first introduced by Dietrich Lehmann and colleagues in 1987, provides a data-driven approach to characterizing these transient brain states. Unlike traditional EEG analyses that focus on localized spectral power, microstate analysis identifies recurring scalp potential configurations, offering a more comprehensive representation of large-scale neural activity (Lehmann et al., 2009). Each microstate remains stable for approximately 80 to 120 milliseconds before transitioning to a new configuration, reflecting dynamic shifts in neural processing. This method has been widely used to study cognitive and perceptual processes, as it captures the temporal evolution of brain states rather than isolated power fluctuations (Khanna et al., 2014; Musso et al., 2010). Research has consistently identified four dominant microstates (A, B, C, and D), each associated with distinct functional networks and cognitive processes (Liu et al., 2023; Milz et al., 2016). Microstate A is oriented from right-

frontal to left-posterior and is thought to be linked to self-referential thoughts and attentional processes. Microstate B, displaying an opposite orientation from left-frontal to right-posterior, is associated with the task-positive network, involved in goal-directed behavior and cognitive control. Microstate C presents a symmetrical pattern along the anterior-posterior axis and is linked to the visual network, playing a role in processing visual information. Microstate D, also symmetrical but with a stronger frontal midline peak, is associated with the salience network, which aids in detecting and responding to important sensory stimuli (Santarnecchi et al., 2017). These microstates collectively explain approximately 80% of total EEG variance and have been consistently identified across different studies with notable similarity among subjects (Khanna et al., 2015). Their spatial and temporal characteristics have been linked to various cognitive and behavioral states, reinforcing the notion that dynamic transitions among microstates form the foundation of complex neural processing (Antonova et al., 2022; Milz et al., 2017; Musso et al., 2010). Given that flavor perception is a dynamic and multisensory process, understanding how neural activity evolves over time is critical. Unlike traditional EEG approaches that focus on localized power changes, microstate analysis allows for the characterization of transient brain states, offering a dynamic perspective on neural coordination. Despite its potential, this method remains largely unexplored in the context of flavor perception, particularly in alcohol-related sensory processing (Okamoto & Dan, 2013). By employing microstate analysis, this study provides a novel framework to

examine how alcohol influences the temporal evolution of neural states, bridging a critical gap in sensory neuroscience and food science.

The present study aims to investigate the feasibility of using EEG microstates to decode flavor perception, providing a novel framework for understanding whole-brain dynamic responses to alcohol stimuli. The primary objectives are: (1) to apply microstate-based analysis to examine brain responses to varying alcohol concentrations and distilled spirits, identifying distinct microstate patterns associated with different alcohol levels; (2) to evaluate overall differences in microstate dynamics across stimuli by analyzing variance in microstate characteristics; (3) to investigate the influence of alcohol concentration on key microstate temporal features, including transition probabilities, coverage, duration, and occurrence. By mapping alcohol-induced flavor perception onto EEG microstates, this study not only offers new insights into the temporal evolution of sensory and cognitive processing but also advances our understanding of how the brain integrates multisensory information during alcohol consumption. These findings provide a methodological foundation for future applications of EEG microstate analysis in sensory neuroscience and food science.

## **2. Materials and methods**

### **2.1. Chemicals and Samples**

Food-grade alcohol (95% pure ethanol, HuaLin, Yunnan, China) was used, with the remaining 5% composed of residual water and naturally occurring trace

components inherent to commercially available ethanol. No additional flavoring agents or volatile compounds were present. The ethanol was diluted using ultrapure water (NW10VF water purification system, Heal Force Development Ltd., Hong Kong, China) to achieve the required concentrations, ensuring that the stimuli reflected only the effect of ethanol concentration without additional sensory influences.

Ethanol content varies significantly across different types of alcoholic beverages. For example, beer typically has an alcohol by volume (ABV) ranging from 2.9% to 8.5% ABV, with an average of 5% ABV; wine generally contains 11% to 14% ABV; while spirits such as Chinese Baijiu, Tequila, Whiskey, Rum, and Brandy usually range from 40% to 60% ABV (DiLoreto et al., 2012). To investigate the impact of ethanol concentration on taste perception, ethanol solutions were prepared by mixing ethanol with purified water to achieve final concentrations of 5%, 10%, 20%, 40%, and 53% ABV. Purified water served as both the control and the base for ethanol solutions, ensuring that no additional taste or olfactory compounds influenced sensory perception. Additionally, Baijiu (53% ABV) was included to compare taste perception between a complex alcoholic beverage and a pure ethanol solution with the same alcohol concentration.

## 2.2. Participants

This study was approved by the Ethics Review Committee of Zhejiang University (Approval Reference No. 2024-2) and adhered to the ethical guidelines for human

experimentation outlined in the Declaration of Helsinki by the World Medical Association. A total of 20 right-handed Chinese participants (10 females and 10 males), all in good physical and mental health, were recruited. The sample size was determined based on prior EEG studies on sensory processing, ensuring data reliability while maintaining methodological feasibility (Wu et al., 2022). Participants were required to be familiar with the taste of alcohol and not allergic to it. They were instructed to refrain from eating, drinking, or smoking for at least 2 hours prior to the test. The experiments were conducted in a dedicated EEG testing room, ensuring a safe, quiet, and odor-neutral environment, with the room temperature maintained at  $21 \pm 1$  °C. Prior to the experiment, participants received detailed instructions, completed a questionnaire to familiarize themselves with the procedure and the expected time commitment, and disclosed any previous participation in EEG studies. All participants provided informed consent before the experiments began and were compensated monetarily for their participation.

### 2.3. Procedure

The detection apparatus was set up according to the provided instructions. Participants wore an EEG cap, ensuring a secure fit between the cap and the scalp. They were instructed to close their eyes, take deep breaths, exhale slowly, and maintain emotional stability. At the start of each trial, participants rinsed their mouths three times with ultrapure water within 30 seconds to establish a baseline. The experiment assistant then presented seven stimuli, including water (as a blank), Baijiu,

and five alcohol solutions with varying concentrations, in 5 mL aliquots. The stimuli were presented in a randomized order. Participants followed the training instructions, tasting each sample slowly without swallowing and avoiding head movements to minimize motion-related artifacts.

#### 2.4. EEG recording

EEG data were recorded using the Curry 9 system with a 64-channel Quik-Cap for the SynAmps 2/RT amplifier, procured from Compumedics Europe GmbH. The reference electrode was placed at the FCz channel. Scalp impedance was kept below 5 k $\Omega$ , and the sampling rate was set to 1000 Hz. The SynAmps2/RT amplifier was configured for Quick-cap64, operating in DC mode with a low-pass filter set at 400 Hz, and a resolution of 24 nV/LSB. The EEG test began when the instructor signaled "start," marking the onset with "0" in the EEG recording system. Participants were instructed to relax for 10 seconds to establish a calm baseline. Once the EEG signal stabilized, each 5 mL sample was presented in a standardized tasting cup and gently delivered by a trained experimenter into the participant's mouth while they remained still with their mouth slightly open. The administration of the test stimulus was marked as "1", officially initiating data recording. Participants held the sample in their mouth for 5 seconds (marked as "2"), after which they spat out the liquid and rinsed their mouth with purified water. Participants were advised to rinse their mouths and rest briefly to minimize any lingering aftertaste. A 60-second break followed, allowing participants to relax and return EEG signals to baseline levels. Once stabilized, the baseline was

recorded in a 10-second stimulus-free state before the next stimulus was presented. To minimize perceptual interference from high-intensity alcohol stimuli, the tasting order was arranged from lowest to highest alcohol concentration, ensuring that stronger stimuli did not influence the perception of lower-intensity samples. Extended rest periods were provided when necessary to ensure full sensory recovery before the next test. This procedure was repeated for each sample, maintaining consistency across control and experimental conditions. The trial was replicated three times, and EEG signals were recorded throughout the entire process (Figure 1). Electrode placement and recording site selection followed the methodology described in previous studies, ensuring consistency with established EEG recording protocols (Wu et al., 2022; Wang et al., 2025).

Prior to the main EEG experiment, participants underwent pre-tests to assess EEG signals under various conditions, including a quiet state, with eyes open or closed, swallowing, eye movements, smiling, and emotional responses. These pre-tests ensured the stability of resting EEG and accounted for potential emotional fluctuations. Participants were also trained on the preparation procedures, emphasizing the importance of keeping their eyes closed unless otherwise instructed. Before the experiments, participants were briefed on the test details (e.g., using food-grade chemicals) and instructed to taste the samples in a randomized, blind manner. They were reminded to follow instructions, remain still, and focus solely on taste perception, avoiding unnecessary movements or talking. To mitigate the impact of negative

emotions or satiety, EEG measurements were conducted in the morning (10-11 a.m.) and afternoon (3-4 p.m.), without formal emotional assessments.

## 2.5. EEG pre-processing and analysis

The raw EEG signals were pre-processed using MATLAB software (The MathWorks, USA). The pre-processing steps included channel location mapping, data selection, and down-sampling. Signals were then filtered to remove noise, followed by epoch extraction for further analysis. Artifact rejection was performed to eliminate signals affected by eye movements, muscle activity, and other non-neural interference. Independent Component Analysis (ICA) was applied to separate sources of noise from brain activity. After re-referencing the data to a common electrode, components associated with artifacts were identified and removed to ensure clean signal analysis (Figure 1). These pre-processing steps were critical for obtaining high-quality EEG data for subsequent analysis (Delorme & Makeig, 2004; Wang et al., 2025).

## 2.6. Microstate analysis

The standard EEG microstate analysis procedure was adopted using Cartool64, a powerful software designed for EEG data analysis, particularly for microstate analysis, connectivity, and source localization. The procedure was carried out in five stages: (1) Segment into microstates, (2) Individual-level clustering, (3) Group-level clustering, (4) Backfitting microstates onto EEG, and (5) Calculation of microstate statistics (Figure 1). In the Segment into microstates stage, the Global Field Power (GFP) curve was calculated, and the peak points were selected as potential microstates. These potential

microstates were then extracted as candidate templates for the two-step clustering process (individual-level clustering followed by group-level clustering). Both clustering steps were performed using K-means. The first clustering step was conducted at the individual participant level, involving 20 participants, each with 3 parallel trials, resulting in a total of 60 datasets that were clustered separately. This process was considered the initial clustering phase. After obtaining the best results from the first clustering, these results were subjected to a second K-means clustering, which yielded the optimal clustering result of 4 microstates, labeled as A, B, C, and D. These four microstates are commonly used in studies involving healthy adult participants, representing distinct brain networks responsible for specific neural processing, and are considered the fundamental building blocks of cognition. To assess clustering performance, the Global Explained Variance (GEV) values were calculated. For backfitting, the Pearson correlation between the four microstate templates and each peak point in the GFP curve was computed. Each peak point was then assigned to the microstate class with the highest correlation. Finally, four commonly used EEG microstate features were extracted from the microstate sequence in each trial: transition probability, coverage, duration, and occurrence. The specific definitions of these features are as follows: (1) Transition probability: The percentage of transitions from each of the four microstate classes to any other class. For example, the transition probability from microstate A to microstate B is defined as the percentage of transfer times from A to B over the total transfer times from A to any of the other three

microstates. (2) Coverage: The proportion of time a microstate class occupies in the total recording time. (3) Duration: The average time a segment of a microstate class lasts, measured in milliseconds. (4) Occurrence: The frequency at which a microstate class appears, measured in times per second. It is noted that transition probability and coverage are expressed as percentages, while duration and occurrence are measured in milliseconds and times per second, respectively (Kleinert et al., 2024; Koenig et al., 2024; Musso et al., 2010). In summary, a total of 24 microstate features were obtained per trial for each participant (12 features for transition probability, 12 features for coverage, duration, and occurrence). These features were averaged across trials within the same experimental condition, and the output feature data were organized into a matrix of 20 participants  $\times$  7 conditions  $\times$  24 microstate features.

## 2.7. Statistical Analysis.

To examine the influence of the different flavor stimuli on the set of microstate features, Analysis of Variance (ANOVA) was applied to determine whether the various flavor stimuli were associated with distinct patterns in microstate feature representation. ANOVA, along with Spearman's correlation test, was performed using IBM SPSS Statistics (version 17.0, Chicago, IL, USA), with a significance level set at  $p < 0.05$ , indicating statistically significant differences. All figures were generated using GraphPad Prism (version 14, GraphPad Software, San Diego, CA, USA).

## 3. Results and discussion

### 3.1. Microstate topologies

EEG microstate analysis captures dynamic neuronal activity through quasi-stable topographies that persist for 80-120 ms before transitioning. These microstates offer valuable insights into how the brain processes sensory stimuli, providing a framework for understanding the underlying neural mechanisms. In this study, we identified four distinct microstates (A, B, C, D), each reflecting a unique neural configuration. Notably, the clustering results of these microstates align with those reported in classical literature, confirming the reliability of these templates in capturing dominant neural states (Michel & Koenig, 2018). While there is limited research on the application of microstates to flavor perception, the consistency of these findings with existing literature supports the potential of microstate analysis in investigating alcohol-induced sensory processing and, more broadly, its applicability to the study of flavor perception. The overall GEV of 65.45% further reinforces the utility of these microstate templates in understanding alcohol-induced sensory processing, offering a novel approach to studying the neural dynamics underlying alcohol-related sensory experiences (Chen et al., 2021; Michel & Koenig, 2018).

Microstate A accounts for 12.4% of the total GEV and is characterized by a right frontal to left posterior orientation. This microstate is typically associated with attention reorientation and basic cognitive integration, processes essential during the early stages of sensory perception. In the context of alcohol-induced flavor processing, microstate A likely facilitates the initial allocation of attentional resources to the various components of the alcohol-related flavor stimuli. Its moderate GEV

contribution suggests a preparatory role in organizing sensory inputs, setting the stage for more complex evaluations of the sensory experience. This aligns with existing research suggesting that microstate A is involved in structuring sensory information, thereby enabling the brain to focus on and organize sensory stimuli for further processing (Khanna et al., 2015).

Microstate B, contributing 17.3% to the GEV, exhibits a left frontal to right posterior orientation and is strongly linked to emotional salience and sensory evaluation (Milz et al., 2016). This microstate is engaged when the brain processes emotionally significant or hedonic stimuli. In our study, the higher GEV of microstate B suggests a more pronounced role in evaluating the emotional and sensory qualities of alcohol-related flavor stimuli (Seitzman et al., 2017). Given alcohol's strong sensory and emotional effects, microstate B likely plays a key role in assessing the hedonic value and potential aversiveness of different alcohol concentrations. This finding highlights microstate B's central role in the emotional evaluation of sensory experiences, particularly in the context of alcohol, which elicits both sensory pleasure and emotional responses.

Microstate C, contributing the highest GEV proportion (53.7%), is characterized by a midline frontal-to-occipital orientation and is frequently associated with the default mode network (DMN). The DMN is involved in introspective thought and sensory re-evaluation, and the dominance of microstate C in our study suggests that alcohol stimulation engages the brain in deeper re-assessment of sensory inputs,

driven by the increased complexity of alcohol-induced flavor perception (Seitzman et al., 2017). The high GEV of microstate C underscores its critical role in the continuous processing and introspective evaluation of complex sensory stimuli. This finding aligns with existing research on the DMN's involvement in continuous sensory re-evaluation, reinforcing microstate C's role in managing intricate sensory information, particularly in response to alcohol-induced flavor stimuli (Milz et al., 2016; Santarnecchi et al., 2017).

Microstate D, contributing 16.6% to the GEV, is associated with attentional reallocation and cognitive control. This microstate is crucial for managing attention shifts and allocating cognitive resources, especially when processing complex or demanding stimuli (Michel & Koenig, 2018). In our study, the increased engagement of microstate D in response to alcohol stimulation suggests that the brain needs to allocate additional cognitive resources to manage the complexity of flavor processing. This is consistent with prior studies that have shown microstate D's involvement in tasks requiring flexible attention and cognitive control, particularly as sensory stimuli become more complex or intense (Yao et al., 2024). The results from our study further emphasize microstate D's role in modulating attentional and cognitive processes in response to alcohol-induced flavor stimuli.

The distinct contributions of microstates A (12.4%), B (17.3%), C (53.7%), and D (16.6%) to alcohol-induced flavor perception provide insights into the brain's adaptive responses. Rather than representing a fixed sequential order, these

microstates emerge dynamically as distinct, recurring patterns of large-scale neural activity. Among these states, microstate C exhibited the highest prevalence and longest duration, particularly at higher ethanol concentrations. This pattern suggests that increased alcohol intensity demands greater cognitive resources for sensory discrimination and attentional control. These results highlight the dynamic nature of alcohol-induced sensory processing and support the hypothesis that ethanol concentration modulates large-scale neural networks involved in flavor perception. By characterizing alcohol-induced sensory dynamics, this study provides a foundation for future research on the neural mechanisms of flavor perception. The methodological approach used here can be expanded to investigate more complex flavor interactions in alcoholic beverages, integrating both ethanol concentration and additional gustatory and olfactory components.

### 3.2. The overall microstate difference across alcoholic stimuli.

Turning to the ANOVAs on individual microstate features, Figure 2 presents the detailed results, including F statistics and p-values in brackets. Thirteen out of the 24 features showed significant differences among the alcohol stimuli ( $p < 0.05$ , FDR corrected), further supporting the utility of microstate analysis for evaluating alcohol-induced sensory processing.

Among the microstate features, four transition probabilities exhibited significant differences in response to alcohol stimulation. Specifically, the transition from microstate C to B was significantly influenced by alcohol stimulation ( $F=12.07$ ,

$p=0.002$ ). This shift from sensory re-evaluation (C) to emotional evaluation (B) indicates that alcohol stimulation engages the brain in a heightened emotional processing of flavor stimuli. Microstate C, linked to introspection and sensory re-evaluation, transitions to microstate B, which is involved in emotional salience and evaluation. This transition suggests that alcohol amplifies the emotional impact of sensory stimuli, reinforcing the idea that alcohol increases the emotional response to flavor perception, consistent with the experiment's goal of examining the interaction between sensory and emotional processing (Antonova et al., 2022; Liu et al., 2023; Milz et al., 2016). In addition, the transition from microstate D to C ( $F=11.38$ ,  $p=0.002$ ) demonstrated a shift in attentional control towards sensory re-evaluation. Microstate D, associated with attentional regulation and cognitive control, showed significant shifts towards microstate C as alcohol stimulation increased, suggesting that as sensory complexity grows, the brain reallocates cognitive resources to engage more deeply in sensory processing. This finding aligns with the interpretation that alcohol-induced flavor perception necessitates a dynamic reallocation of cognitive resources to manage increased sensory complexity. Notably, this transition reflects the brain's adaptive flexibility in shifting between cognitive integration and sensory re-assessment, as previously observed in studies involving challenging sensory tasks (Musso et al., 2010; Pan et al., 2020; Zanesco et al., 2021). These findings underscore how alcohol modulation affects neural transitions, particularly in the interaction between sensory re-evaluation and emotional processing. Transitions from Microstate

C to B and D to C when alcohol concentration increased highlight key adaptive shifts in brain activity, emphasizing the brain's capacity to handle the complexity of sensory stimuli and emotional evaluation under alcohol exposure. These transitions reflect flexible reconfigurations of neural networks in response to ethanol stimuli, which supports the hypothesis that ethanol concentration modulates large-scale neural networks involved in flavor perception. By establishing a neural framework for alcohol-induced sensory dynamics, this study lays the groundwork for future research integrating multimodal neuroimaging (e.g., EEG-fMRI) and expanding to complex gustatory stimuli. This approach may enhance our understanding of how ethanol and other flavor compounds interact within large-scale brain networks.

The temporal characteristics of microstates, specifically coverage, duration, and occurrence, provide valuable insights into how alcohol stimulation influences sensory and emotional processing in the brain. These features represent distinct aspects of the brain's neural activity: coverage refers to the proportion of time spent in a given microstate, duration reflects how long the brain stays in that state, and occurrence indicates the frequency with which the brain transitions into a specific state.

Coverage analysis revealed significant differences in microstates B and C ( $F=18.64$ ,  $p=0.001$  for B;  $F=12.83$ ,  $p=0.001$  for C). These findings suggest that alcohol stimulation shifts the brain's engagement towards emotional evaluation (B) and sensory re-evaluation (C). Specifically, the significant changes in the coverage of B and C indicate that alcohol enhances the brain's focus on emotional and sensory

processing. The significant changes in B coverage reflect greater emotional processing, while the significant changes in C coverage highlight the brain's increased involvement in sensory re-evaluation. This suggests that alcohol shifts the brain's processing priorities towards emotional and sensory dimensions of flavor perception.

For duration, significant differences were observed in microstates A ( $F=17.35$ ,  $p=0.001$ ) and B ( $F=15.38$ ,  $p=0.005$ ). Duration reflects how long the brain remains engaged in a specific microstate. The significant change in microstate A suggests that alcohol prolongs cognitive processing, indicating that the brain continues to integrate sensory information over an extended period. In contrast, the significant change in microstate B shows that alcohol extends emotional evaluation. This implies that alcohol influences the brain's emotional processing by increasing the time spent assessing the emotional aspects of the sensory stimuli. Duration thus provides insight into the sustained engagement of the brain with both cognitive and emotional processing, reflecting how alcohol alters the brain's ability to process complex stimuli over time (Liu et al., 2023; Milz et al., 2017).

The occurrence frequencies of microstates B ( $F=15.02$ ,  $p=0.002$ ) and C ( $F=24.77$ ,  $p=0.001$ ) showed significant changes with alcohol stimulation. Occurrence indicates how often a microstate is activated during a given period. The significant changes in the occurrence of microstate B suggest that emotional evaluation becomes more frequent under alcohol exposure, meaning the brain engages with emotional assessments of sensory stimuli more often. Similarly, the significant changes in the

occurrence of microstate C indicate that sensory re-evaluation happens more frequently. These findings suggest that alcohol increases the frequency with which the brain engages in emotional and sensory assessments, leading to more recurrent cycles of emotional evaluation and sensory re-evaluation. Unlike duration, which highlights how long the brain stays in a specific state, occurrence focuses on how often these processes are engaged over time.

Taken together, these findings highlight how alcohol stimulation alters the brain's engagement with sensory and emotional processing. Coverage analysis shows that alcohol shifts the brain's focus towards emotional and sensory processing, with increased engagement in microstates B and C. Duration analysis reveals that alcohol extends the engagement with cognitive processing (A) and emotional evaluation (B), indicating prolonged involvement with these processes. Occurrence analysis shows that alcohol increases the frequency of emotional and sensory evaluations, indicating that alcohol induces more frequent cycles of emotional and sensory assessments. These results demonstrate that alcohol significantly alters the brain's processing priorities, with greater emphasis on emotional and sensory evaluations of flavor perception.

### 3.3. Microstate duration, occurrence, and coverage under seven alcoholic stimuli

To further investigate the ANOVA results, which indicated that microstates B and C are the most significant indicators of brain responses to alcohol stimulation, we conducted a detailed analysis of microstate duration, occurrence, and coverage across

the seven alcoholic stimuli: water, 5%, 10%, 20%, 40%, 53% ABV, and Baijiu (Figure 3). In the coverage analysis (Figure 3a), we observed that microstate C exhibited higher coverage at low alcohol concentrations (water, 5%, 10%, and 20% ABV). This suggests that C, associated with sensory re-evaluation, is more strongly engaged during low alcohol exposure. However, at higher alcohol concentrations (40%, 53% ABV, and Baijiu), C's coverage began to decrease, indicating a shift in brain processing priorities as alcohol concentration increases. Conversely, microstate B did not show a clear pattern across the different alcohol concentrations, with no significant increase or decrease in its coverage across the different alcohol stimuli (Antonova et al., 2022; Musso et al., 2010).

Next, we examined microstate duration (Figure 3b), and the results mirrored some of the trends observed in coverage. At lower alcohol concentrations (water, 5%, 10%, and 20% ABV), microstates B and C exhibited longer durations, suggesting a prolonged engagement in emotional evaluation (B) and sensory re-evaluation (C). This aligns with the notion that moderate alcohol exposure amplifies sensory and emotional processing, requiring extended neural commitment to evaluating ethanol-related stimuli. However, at higher alcohol concentrations (40% ABV and above), we observed a reduction in the duration of both microstates, suggesting that the brain spends less time in each occurrence of these states. Despite the decrease in individual microstate durations, their overall coverage increased, indicating that these states were engaged more frequently. This suggests that higher alcohol concentrations drive more

rapid yet repetitive cycles of sensory integration and cognitive re-evaluation, rather than extended processing within each activation. This aligns with prior findings that increasing stimulus intensity enhances attentional and sensory network engagement while promoting more transient but frequent shifts between brain states (Michel & Koenig, 2018). These findings highlight the adaptive nature of neural processing under alcohol exposure and suggest that ethanol modulates perceptual and cognitive responses through both intensity-dependent engagement and dynamic neural state transitions.

Finally, the occurrence analysis (Figure 3c) showed that microstates B and C were activated more frequently in response to low alcohol concentrations (water, 5%, 10%, and 20% ABV). This increase in occurrence implies that alcohol stimulates more frequent engagement with emotional evaluation (B) and sensory re-evaluation (C) at lower concentrations. The heightened frequency of these microstates suggests that the brain is engaging in iterative cycles of emotional and sensory assessments to evaluate the flavor stimuli. However, at higher alcohol concentrations, both microstate B and microstate C showed a reduction in their occurrence frequencies, further reinforcing the idea that alcohol shifts brain processing dynamics at higher concentrations, possibly due to changes in neural resource allocation or the complexity of the sensory stimuli.

These findings underscore the dynamic nature of the brain's response to alcohol stimulation, with alcohol concentration significantly modulating brain activity.

Microstates B and C are central to sensory re-evaluation and emotional evaluation during alcohol exposure. At low alcohol concentrations (water, 5%, 10%, and 20% ABV), both microstates B and C exhibit increased coverage, duration, and occurrence, suggesting that the brain prioritizes emotional and sensory processing. However, as alcohol concentration increases, both microstate B and microstate C show a decrease in coverage, duration, and occurrence, indicating a shift in brain activity and a reallocation of neural resources. This suggests that higher alcohol concentrations reduce the brain's engagement with emotional and sensory processing, possibly due to the increased complexity of sensory stimuli and the need to redistribute cognitive resources. In conclusion, alcohol concentration significantly shifts the brain's neural processing priorities, with microstates B and C remaining crucial for alcohol-induced flavor perception. As alcohol concentration rises, the brain's processing of emotional and sensory information becomes more complex and less prolonged, reflecting the brain's adaptive response to varying levels of alcohol exposure.

#### 3.4. The transition probability under seven alcoholic stimuli

Figure 4 presents the transition probabilities between different microstates across seven alcohol concentrations: water, 5%, 10%, 20%, 40%, 53% ABV, and Baijiu. The data reveal that transition probabilities do not follow consistent patterns across the alcohol conditions. Instead, transitions fluctuate without clear or predictable trends, suggesting that the relationship between alcohol concentration and neural dynamics in

flavor perception is more complex than expected. Despite variability, no systematic changes in transitions between microstates A, B, C, and D were reliably linked to specific alcohol concentrations. This lack of clear trends suggests that alcohol concentration does not uniformly affect neural processes underlying flavor perception. For instance, patterns such as smoother cognitive-emotional integration at lower concentrations or more pronounced sensory re-evaluation at higher concentrations were not observed.

Several factors may explain this. Alcohol's complexity likely plays a role, as its effects on the brain are multifaceted, involving complex interactions with neurotransmitter systems and neural networks. These interactions may result in unpredictable transitions between microstates. Additionally, as alcohol concentration increases, the sensory complexity of the stimuli also rises, further complicating the identification of consistent patterns. The absence of clear patterns in transition probabilities is not unexpected, suggesting that traditional statistical methods are insufficient for capturing the dynamic shifts in brain activity during alcohol-induced flavor perception. Advanced techniques such as machine learning (e.g., clustering algorithms or SVM) are better suited to detect complex, non-linear relationships within the data. These methods could uncover hidden patterns that are not apparent in simpler analyses. In conclusion, while Figure 4 does not reveal consistent patterns, it highlights the complex relationship between alcohol concentration and neural processing during flavor perception. These results suggest that alcohol affects brain

dynamics, but its effects may be subtle and complex. Future studies should refine experimental designs and apply machine learning techniques to reveal deeper insights into how the brain processes alcohol-induced sensory stimuli.

#### **4. Conclusion**

Overall, this study demonstrates the utility of EEG microstate analysis in investigating alcohol-induced flavor perception across seven alcohol concentrations (water, 5%, 10%, 20%, 40%, 53% ABV, and Baijiu). Our findings reveal that microstate analysis effectively captures the neural dynamics of alcohol-induced sensory processing, making it a valuable tool for studying alcohol perception and broader flavor mechanisms. Specifically, four distinct microstates (A, B, C, D) were identified, with microstates B and C playing pivotal roles in sensory and emotional processing. Notably, microstate C, associated with sensory re-evaluation, showed the highest engagement during alcohol exposure, emphasizing its dominant role in flavor perception under alcohol stimulation. Furthermore, we observed that alcohol concentration modulates the engagement of these microstates. At lower alcohol concentrations (water, 5%, 10%, and 20% ABV), B and C exhibited greater coverage, duration, and occurrence, suggesting that the brain prioritizes emotional and sensory processing at these levels. In contrast, at higher concentrations (40%, 53% ABV, and Baijiu), the engagement of B and C decreased, reflecting a shift in brain processing priorities as alcohol concentration increases. These findings highlight the complexity of alcohol's effects on flavor perception and underscore how alcohol concentration

alters neural resource allocation across sensory and emotional evaluations. This study not only enhances our theoretical understanding of alcohol-induced sensory perception but also provides valuable insights for beverage development and neuro-flavor research. By establishing a robust methodological foundation for exploring neural responses to various flavor stimuli, it opens new avenues for future studies. To build on these findings, future research should incorporate a wider range of flavor stimuli, including assessments of consumer preferences and emotional responses. Expanding the scope of research will deepen our understanding of how neural signals relate to subjective perception, thereby enriching our insights into the sensory and emotional reactions triggered by flavor stimuli.

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#### **AUTHOR CONTRIBUTIONS**

**Guangnan Wang:** Conceptualization, Writing - Original Draft; **Xiaolei Wang:** Software, Visualization; **Tianyi Zhang:** Investigation, Methodology; **Zihan Qin:** Software, Visualization; **Fuping Zheng:** Conceptualization, Writing - Review & Editing; **Xingqian Ye:** Conceptualization, Writing - Review & Editing; **Baoguo Sun:** Resources, Supervision; **Huan Cheng:** Resources, Supervision;

#### **NOTES**

The authors announce that they have no conflicts of interest concerning the publication of this manuscript.

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## Figure Captions

Figure 1: The Workflow of EEG Microstate Collection and Analysis.

Figure 2: ANOVA results of 24 microstate features, including (a) transition probabilities and (b) coverage, duration, and occurrence. Heatmaps display F statistics, with significant results marked by asterisks.

Figure 3: Mean and standard error of each microstate feature across different alcohol stimuli, organized by coverage, duration, and occurrence. Microstate A is typically associated with attention reorientation and basic cognitive integration; microstate B is engaged when the brain processes emotionally significant or hedonic stimuli; microstate C is involved in introspective thought and sensory re-evaluation; microstate D is associated with attentional reallocation and cognitive control.

Figure 4: Mean and standard error of each microstate feature across different alcohol stimuli for transition probability.

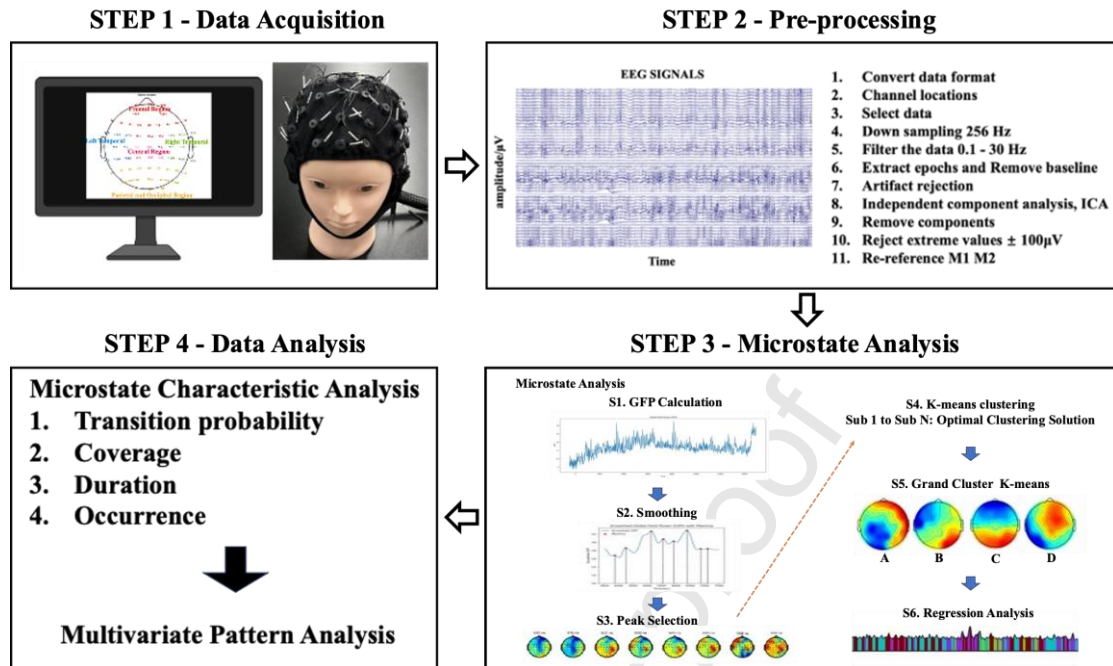


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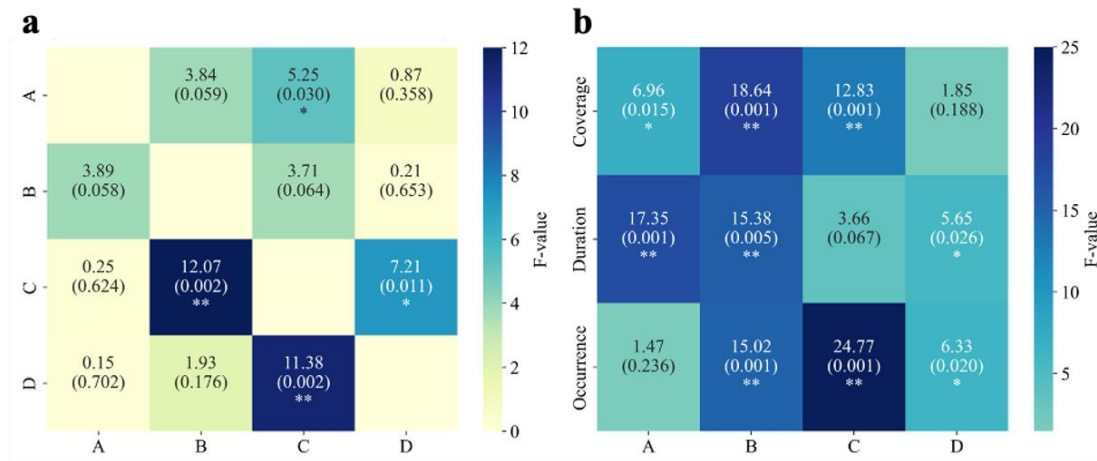


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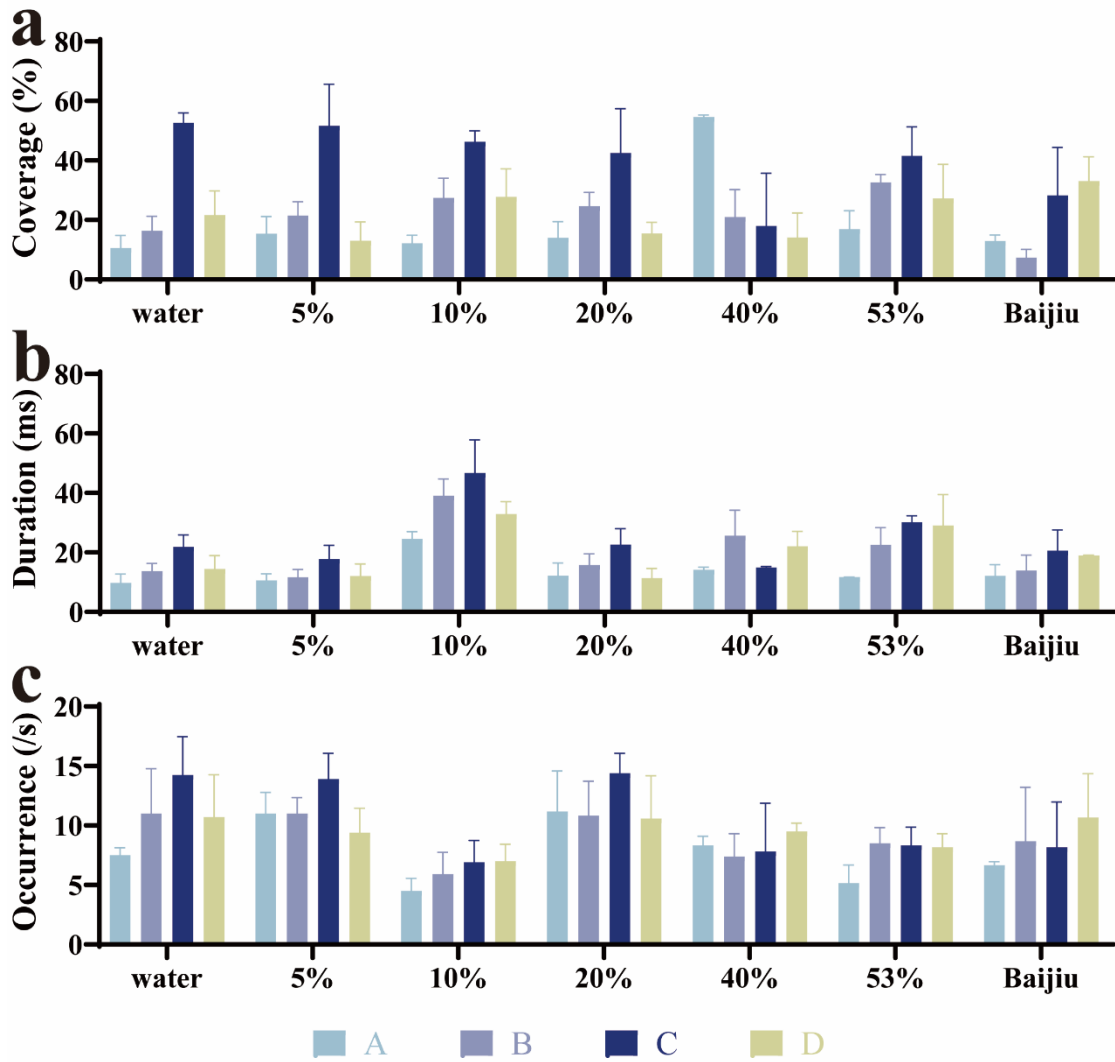


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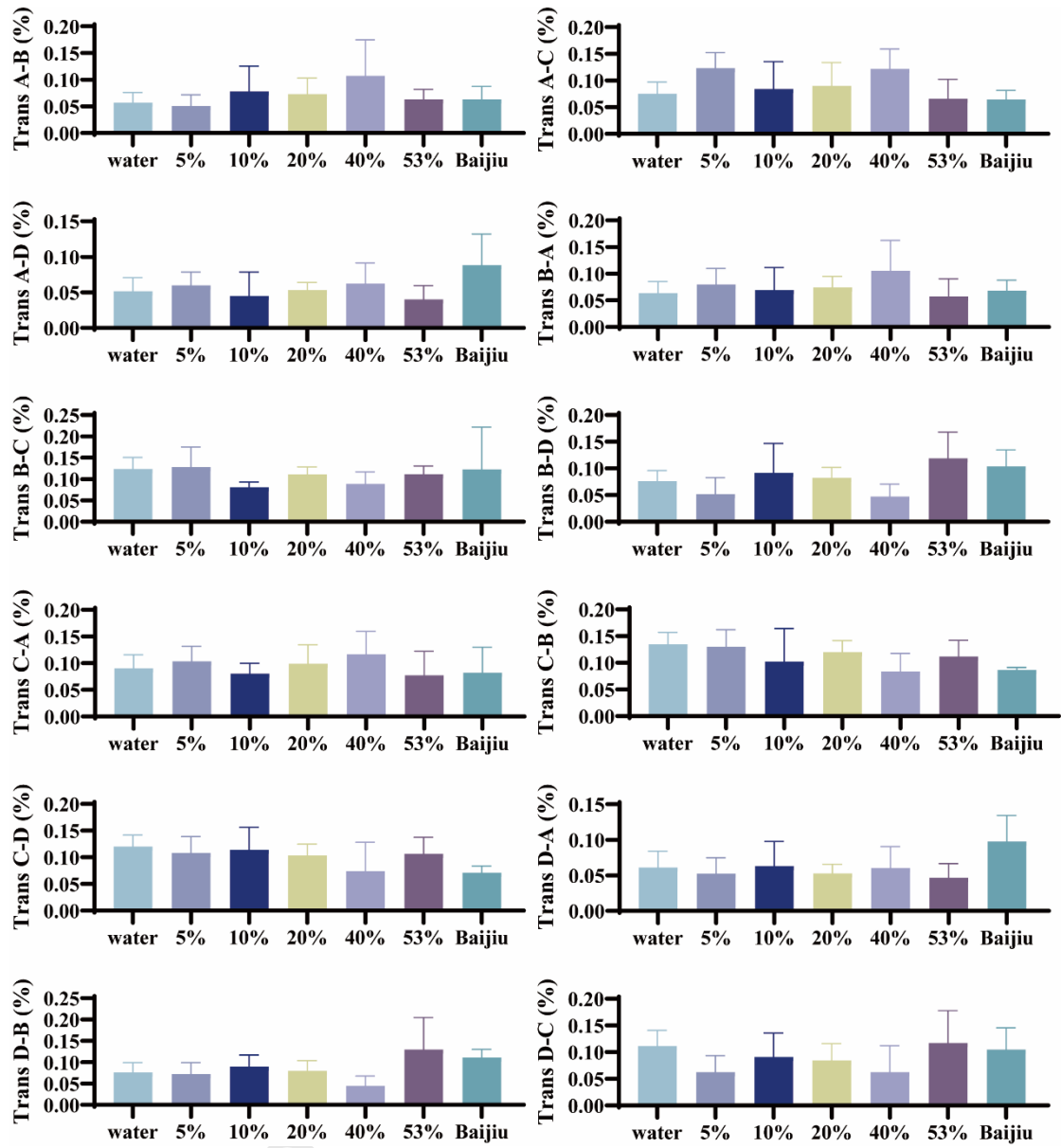
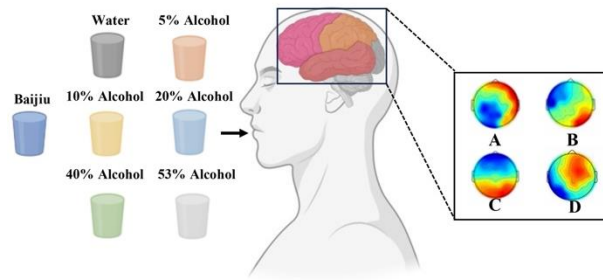


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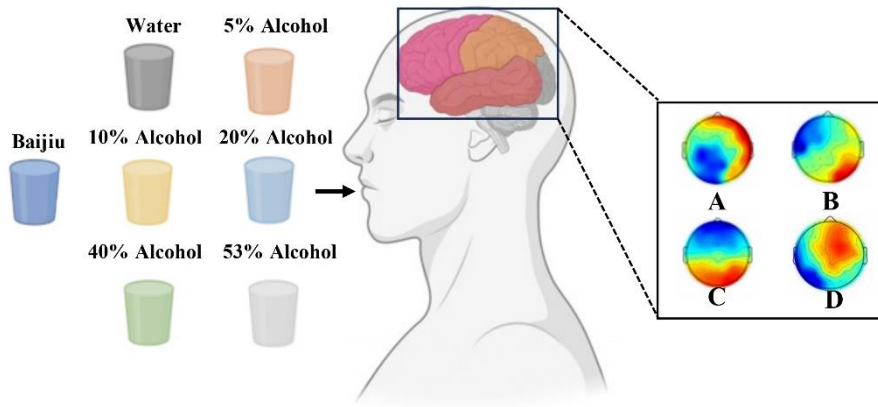
Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Graphical abstract



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1. Alcohol alters brain responses, with microstates B and C pivotal in processing.
2. Lower alcohol ( $\leq 20\%$ ) enhances sensory focus; higher (40%+) prompts re-evaluation.
3. Alcohol shifts microstate dynamics, revealing neural adaptation to concentration.

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