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The colour and taste of a placebo mouth rinse alters sweetness perception, neural responses and performance in trained cyclists

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Abstract

We investigated whether the colour of a non-caloric, artificially sweetened mouth-rinse modulates (1) visual and gustatory sweetness perception, (2) prefrontal cortical activity using electroencephalography (EEG) in healthy individuals and trained cyclists, and (3) cycling performance in trained cyclists. Experiment 1 identified red as a sweetness-congruent colour and green as incongruent and confirmed that the congruent solution elicited higher perceived sweetness intensity despite identical sweetener content. Experiment 2 showed colour-dependent modulation of prefrontal activity exclusively in trained cyclists: the congruent solution reduced the theta–beta ratio (TBR), a proxy of attentional engagement and executive control, before taste stimulation, while both coloured solutions reduced TBR after stimulation relative to a colourless control. In Experiment 3, cyclists completed a 4-km time trial faster following the incongruent solution. These findings demonstrate that colour cues can modulate perception, neural dynamics, and exercise performance through neurocognitive mechanisms in the absence of metabolic input.

Introduction

The colour of a drink can affect perception, expectations, behaviour and overall tasting experiences [1, 2, 3]. Red hues are consistently associated with sweetness, whereas green is more frequently linked to sourness or bitterness, indicating a connection between visual and gustatory information [4]. Flavour perception is a multisensory experience, integrating visual cues with gustatory and cognitive processing, including beliefs, prior information, expectations, and learning [5, 6, 7]. Multiple brain regions contribute to taste perception, including the primary somatosensory cortex, the orbitofrontal cortex, and the ventromedial prefrontal cortex. Orbitofrontal cortex activation occurs even before the taste stimulus, reflecting the expected value and the hedonic (pleasurable) aspects of the anticipated flavour. The dopaminergic and frontostriatal systems also participate in anticipatory flavour responses while expectation and predictive processing modulate effects on taste [7, 8, 9].

Studies suggest that colour might also influence exercise performance through placebo effects. Brown et al. [10] reported that a pink, non-caloric, artificially sweetened mouth rinse improved self-selected running speed and total distance covered during a 30-min run, while increasing feelings of pleasure during exercise. Similarly, Painelli et al. [11] found that ingesting a pink, non-caloric, artificially sweetened solution enhanced strength endurance performance, even in the absence of notable psychological changes. These pink-coloured beverages were chosen because they are often perceived as sweeter, and this sweetness is associated with ergogenic benefits signalled by gustatory properties and their association to carbohydrate supplementation. Nonetheless, no study to date has investigated whether the colour of a solution incongruent with sweetness (i.e., associated with bitterness) might also influence exercise performance due to its association with ergogenic compounds such as caffeine.

The mechanisms underlying placebo effects are fundamentally neurocognitive [12]. Although evidence indicates that colour can influence sweetness perception and may enhance exercise performance

through expectancy-related placebo pathways, the underlying neurophysiology is unknown. No studies have systematically examined whether the colour of a solution modulates sweetness perception and the temporal dynamics of prefrontal activity during anticipation and taste. Furthermore, it is unclear whether these responses differ according to training status which is a well-established moderator of placebo responsiveness [13]. Importantly, early anticipatory prefrontal brain activity, frequently discussed within predictive processing accounts of placebo effects [14, 15, 16], has not yet been investigated in the context of colour-driven sweetness perception. Likewise, it remains unknown whether the colour of a non-caloric, artificially sweetened placebo mouth rinse influences exercise performance during cycling.

In this context, we conducted three interrelated experiments involving 168 participants (82 healthy adults and 86 cyclists). We first established colour sweetness correspondence by having participants rank and taste solutions congruent and incongruent with sweetness based upon their colour (Experiment 1; *n*=119). We then examined prefrontal EEG spectral dynamics; theta (4–8 Hz) and beta (13–30 Hz) band power and their Theta/Beta Ratio (TBR), computed using Welch's method over two 1-s time windows across two prefrontal electrode clusters: 1 s before taste stimulus over the anticipatory cluster (Fp1–Fp2–F5–F6; expectancy formation) and 1 s after taste stimulus over the gustatory cluster (Fp1–F3–F5; decision and action), to determine whether colour and taste modulate prefrontal oscillatory activity as a function of training status (Experiment 2; *n*=20). Finally, we tested whether colour-driven placebo manipulation altered exercise performance in a randomised, counterbalanced, double-blind crossover 4-km cycling time trial (Experiment 3; *n*=29).

Results

Experiment 1

Part A. Pink (41.7%), red (27.8%), and purple (13.9%) received the highest sweetness-association rankings, whereas brown (33.3%), yellow (20.8%) and green (19.4%) were most frequently rated as least sweet-associated (Fig. 2a). Ordinal logistic regression showed a significant main effect of colour (likelihood ratio $\chi^2 = 112.8$, $p < 0.0001$), with no significant colour × sex ($\chi^2 = 12.2$, $p = 0.056$) or colour × training-status interactions ($\chi^2 = 1.9$, $p = 0.92$).

Although interactions were not statistically significant, predicted-probability curves revealed consistent patterns: pink remained the highest-ranked colour among women, whereas red ranked highest among men. Among cyclists, red showed the strongest association with sweetness, whereas green consistently received the lowest probability across subgroups (Fig. 2b).

Based on these distributions and model estimates, **red** was selected as the *congruent* colour and **green** as the *incongruent* colour for Experiments 1B, 2, and 3.

Part B. The mixed-effects model revealed a significant main effect of colour on sweetness ratings ($F = 30.3$, $p < 0.0001$) and a significant effect of training status ($F = 10.0$, $p = 0.003$), with no effect of sex ($F = 1.1$, $p = 0.29$) (Fig. 2c).

Exploratory interaction models detected no colour × training ($F = 1.00$, $p = 0.32$) or colour × sex interaction ($F = 0.58$, $p = 0.45$).

Participants rated CS as significantly sweeter than IS (mean difference = 1.75 cm, 95% CI 1.11–2.38; $p < 0.001$). Non-cyclists provided higher sweetness ratings overall than cyclists (mean difference = 1.83 cm, 95% CI 0.67–2.99; $p = 0.002$). Ordinal logistic regression showed that CS was less likely to receive low sweetness ratings (score 2: $\Delta P = -0.12\%$, 95% CI −0.23 to −0.01; $p = 0.04$) and more likely to receive higher ratings (score 4: $\Delta P = +0.20\%$, 95% CI 0.02–0.38; $p = 0.03$). Colour significantly affected hedonic acceptability ($\chi^2 = 5.08$, $p = 0.02$), but not purchase intention ($\chi^2 = 1.21$, $p = 0.27$) (Fig. 2d–e).

Experiment 2

In the 1-s prior to the trigger (approach), cyclists showed no significant differences in theta power between conditions (CS: median = 0.21, IQR: 0.17–0.25; IS: median = 0.23, IQR: 0.19–0.27; median = 0.21, IQR: 0.17–0.26; all $p \geq 0.525$, Fig. 3a–b). However, beta power was significantly higher in CS (median = 0.004, IQR: 0.001–0.007; $p = 0.0007$) compared with colourless (median = 0.002, IQR: 0.001–0.005), but not IS (median = 0.003, IQR: 0.001–0.006; $p = 1$). Cyclists exhibited a significantly lower TBR in the congruent condition (median = 37.83, IQR: 19.82–53.11) relative to colourless (median = 80.94, IQR: 42.38–121.00; $p = 0.026$; Fig. 3c), reflecting an increase in fast-frequency beta activity without a concurrent change in slow-frequency theta activity, consistent with greater prefrontal cortical engagement over the Fp1–Fp2–F5–F6 electrode cluster. No significant differences across conditions were observed in non-cyclists for any of these measures.

In the 1-s post-trigger (taste), cyclists exhibited lower theta power in both CS (median = 0.207, IQR: 0.180–0.244; $p = 0.04$) and IS (median = 0.213, IQR: 0.187–0.256; $p = 0.03$) relative to colourless (median = 0.318, IQR: 0.280–0.458; Fig. 3d), accompanied by higher beta power in CS (median = 0.0065, IQR: 0.0029–0.010; $p = 0.004$) and IS (median = 0.0068, IQR: 0.003–0.010; $p = 0.03$) compared with colourless (median = 0.0035, IQR: 0.002–0.008; Fig. 3e). These concurrent spectral shifts were reflected in significantly lower TBR values for both CS (median = 26.43, IQR: 18.88–54.01; $p = 0.047$) and IS (median = 29.52, IQR: 17.28–50.39; $p = 0.049$) relative to colourless (median = 47.37, IQR: 22.48–96.34; Fig. 3f), reflecting a shift in prefrontal oscillatory state towards higher-frequency activity and enhanced prefrontal responsiveness to colour stimuli over the Fp1–F5–F3 electrode cluster. No significant differences across conditions were observed in non-cyclists for any measure.

Cyclists rated the congruent solution sweeter (median 4.75 cm) than the incongruent (3.30 cm) and colourless conditions (4.00 cm). Non-cyclists exhibited the same pattern. The mixed-effects model confirmed a significant effect of condition ($F(2,278) = 5.53$; $p = 0.004$), with CS sweeter than IS ($\Delta = 0.48$ cm; $p = 0.039$) and colourless ($\Delta = 0.56$ cm; $p = 0.017$). IS did not differ from colourless ($p = 0.906$). Training status, sex, and all interactions were non-significant ($p \geq 0.13$).

Experiment 3

There was a significant effect of condition on time-to-completion ($F=2.77$, $p=0.046$) along with a main effect of sex ($F=29.63$, $p<0.0001$), but the interaction was not significant ($F=1.55$, $p=0.20$). Time-to-completion in IS was 11.2 s (95% CI 1.0, 21.4, $p=0.026$) faster than control, independent of sex (Fig. 4a). No other comparisons were significant. An exploratory model including post-exercise belief scores showed that perceived treatment influence did not predict performance ($F = 0.88$, $p = 0.35$).

For RPE, the condition × distance interaction was not significant ($F = 2.53$, $p = 0.555$), and the simpler main-effects model provided the best fit. Both condition ($F = 11.79$, $p < 0.0001$) and distance ($F = 2352$, $p < 0.0001$) influenced RPE. All active mouth-rinse conditions elicited higher RPE than control (colourless: $\Delta = 0.73$; CS: $\Delta = 0.43$; IS: $\Delta = 0.47$; all $p \leq 0.003$), with no differences among active conditions (Fig. 4b). Sweetness ratings did not differ between conditions ($F = 1.91$, $p = 0.13$) or sexes ($F = 1.02$, $p = 0.39$), with similar median scores across all solutions (Fig. 4c).

For blood glucose, both condition ($F = 3.19$, $p = 0.024$) and time ($F = 7.99$, $p = 0.0004$) had significant effects. Across the protocol, IS produced lower glucose values compared with CS and the colourless solution (-0.32 mg·dL^{-1}; $p \leq 0.046$) (Fig. 4d). Glucose increased 5-min post-exercise relative to pre-exercise ($+0.41$ mg·dL^{-1}; $p < 0.001$). For blood lactate, condition was not significant ($F = 1.77$, $p = 0.152$), whereas time had a large effect ($F = 416.83$, $p < 0.0001$). Lactate increased from pre-exercise to 5-min post-exercise ($+9.20$ mmol·L^{-1}; $p < 0.001$) and decreased at 5 min (-1.86 mmol·L^{-1}; $p < 0.001$) (Fig. 4e). Dietary intake did not differ between conditions (Supplementary Table 2).

Discussion

The present study examined whether colour modulates sweetness perception and prefrontal neural activity in healthy individuals and recreational cyclists, and whether a colour-based placebo mouth rinse can influence cycling performance. Across three experiments, colour systematically shaped sweetness judgements, altered prefrontal neural dynamics during both the anticipatory and oral sensory phases of taste processing, and influenced cycling performance in trained cyclists. Specifically, sweetness-congruent and incongruent colours differentially affected perceived sweetness and hedonic responses, while EEG analyses revealed colour-dependent modulation of prefrontal activity in cyclists but not in non-cyclists. Notably, a sweetness-incongruent mouth rinse improved 4-km time-trial performance despite no differences in perceived sweetness during exercise. Together, these findings provide converging evidence that colour functions as a meaningful contextual cue that interacts with taste to shape perceptual processing over time and can elicit placebo-like effects on exercise performance.

A central motivation for this work was to determine whether colour can modulate sweetness perception and prefrontal neural processing, and ultimately whether colour-based placebo effects can influence exercise performance. Visual cues such as colour generate anticipatory responses that shape expectations about upcoming sensory input, thereby influencing perceived intensity. Placebo effects during perception engage neural systems involved in multisensory integration and top-down cognitive processes, including prior beliefs, expectation formation, and predictive modulation of sensory input [17,

[18, 19]. Within predictive processing frameworks, expectations are proposed to shape perception by modulating precision weighting and perceptual inference[20, 21]. Given the susceptibility of sensory experiences to contextual influences, extending placebo mechanisms to colour–sweetness interactions provides a coherent framework for interpreting the expectancy-driven effects observed in the present study, including their modulation by training status and prior sensory experience.

Experiment 1 replicated robust colour–taste correspondences [4, 22]. Red was consistently judged as congruent with sweetness, whereas green was perceived as incongruent in our population. These findings align with extensive psychophysical evidence demonstrating that colour cues can bias flavour interpretation independently of chemical composition [1, 2, 23, 24]. Despite identical sucralose concentrations, the congruent red solution elicited higher perceived sweetness intensity and greater hedonic acceptability than the incongruent green solution. This pattern supports the notion that colour establishes sensory expectations that subsequently modulate perceptual judgments via top-down influences [21, 25, 26]. Interestingly, cyclists rated all solutions as less sweet than non-cyclists, which suggests that consistent repeated exposure to carbohydrate-rich sports beverages may attenuate gustatory sensitivity or alter perceptual calibration.

Experiment 2 examined whether colour modulates neural processing during sweetness perception. To minimise direct chemosensory and reward-related activation, the lowest sucralose concentration was selected (0.024%) [27, 28, 29], increasing the likelihood that observed neural differences reflected expectancy-related processes rather than chemical stimulation. Among cyclists, colour modulated prefrontal spectral activity. Specifically, beta and theta band power and their TBR were altered both before and during taste stimulation. In the second preceding taste onset, the congruent solution elicited lower TBR values relative to the colourless control, reflecting an increase in fast-frequency band activity (beta) alongside a reduction in slow-frequency band activity (theta), indicating accelerated cortical electrical activity and enhanced prefrontal responsiveness to colour stimuli. The observed increase in prefrontal beta power in response to colour stimuli is consistent with evidence that beta oscillations are elicited by reward-related visual feedback over frontal areas [30], serve as a carrier for attentional activation during anticipatory periods of visual tasks[31], and have been identified as neurophysiological predictors of placebo responsiveness in frontal electrodes[32]. Together, these findings suggest that the sweetness-congruent colour-induced increase in beta power observed in the present study may reflect the activation of reward-related prefrontal circuits in anticipation of a contextually meaningful sensory stimulus, consistent with expectancy-based mechanisms of placebo effects.

The ratio between slow wave theta (4–7 Hz) and fast wave beta (13–30 Hz) band power, known as the theta/beta ratio (TBR), has been employed as a valuable index of brain activity, particularly in the context of attentional control and cognitive demand [33]. Van Son et al. [34] demonstrated that greater executive control network connectivity during focused states compared to mind wandering was associated with lower TBR, suggesting that reduced TBR reflects enhanced attentional engagement. In contrast, Tan et al. [35] reported positive associations between theta power and cognitive performance in task-related EEG

paradigms. In this context, resting-state TBR has been associated with executive engagement, given that frontal theta oscillations serve as a mechanism for cognitive control [36]. However, TBR has also been shown to fluctuate dynamically within sessions in response to momentary cognitive state changes, with lower TBR observed during periods of focused attention compared to mind wandering [34, 37]. The condition-dependent reduction in TBR observed in the present study may therefore reflect a state-level shift in prefrontal oscillatory activity driven by colour cues, characterised by the same spectral pattern of increased beta and reduced theta power described above, consistent with enhanced prefrontal engagement during expectancy formation. The selective presence of this effect in cyclists and not non-cyclists suggests that repeated sensory exposure and training history may refine predictive models, facilitating greater prefrontal responsiveness to contextually meaningful cues.

During taste stimulation, both coloured solutions elicited lower TBR values relative to the colourless condition, albeit through potentially distinct mechanisms. In the congruent condition, the reduction in TBR over left-lateralised prefrontal regions may reflect facilitated sensory integration when incoming gustatory input aligns with prior colour-based predictions, a pattern consistent with reduced prediction error and efficient perceptual inference [20]. In contrast, modulation in the incongruent condition emerged exclusively during taste stimulation, suggesting a prediction error response in which the mismatch between colour-driven expectancy and actual gustatory input engages left-lateralised dorsolateral prefrontal and anterior insular regions implicated in belief updating and expectancy revision[8, 31, 32]. The differential engagement of right-lateralised reward circuits during anticipation and left-lateralised executive circuits during taste stimulation therefore maps onto the two-phase architecture of placebo processing proposed in the neurocognitive literature [19], providing electrophysiological evidence that colour functions as a contextually meaningful cue capable of sequentially engaging expectancy formation and expectancy updating mechanisms within prefrontal networks. The absence of neural modulation in non-cyclists further supports the role of prior experience in shaping sensitivity to contextual cues such as colour and may also explain the discrepancies in relation to previous studies showing performance benefits of pink-coloured drinks (i.e., congruent with sweetness) in habitually active[10] and strength-trained individuals[11].

The spatial distribution of these effects across distinct electrode clusters further informs the interpretation of these findings. The two electrode clusters analysed in the present study were selected based on established associations between prefrontal and lateral frontal EEG activity and specific cognitive processes[31, 32], and were applied to distinct temporal windows reflecting different phases of the placebo response, consistent with neuroimaging evidence that visual cues and gustatory receipt engage dissociable neural systems[8]. The first cluster (Fp1, Fp2, F5, and F6) was applied to the anticipatory window preceding taste onset, when participants were visually exposed to the colour of the solution. This cluster was selected to capture reward-related neural activity, as previous research has demonstrated that reward feedback stimuli elicit increases in beta power over these frontal channels, with effects maximally expressed at F6 and sourced to the right DLPFC [30]. Within a placebo framework, this anticipatory phase corresponds to expectancy formation, wherein visual cues such as colour

generate predictions about the upcoming sensory experience and activate reward-related prefrontal circuitry prior to any chemosensory input, consistent with evidence that anticipation of a placebo effect is associated with activation of orbitofrontal and dorsolateral prefrontal cortices [14]. The second cluster (Fp1, F3, and F5) was applied to the gustatory window during taste stimulation. This cluster was selected to index decision-making processes, given that F3 overlies the left DLPFC and F5 captures left-lateralised lateral frontal activity associated with analytical reasoning and approach motivation [38]. Frontal TBR derived from left frontal electrodes has been linked to flexible, goal-directed decision-making, with higher TBR associated with riskier and less adaptive choices [39, 40]. During taste stimulation, this left-lateralised prefrontal network may reflect the integration of incoming gustatory information with prior colour-based expectations, consistent with evidence that expectancy-outcome comparisons in the DLPFC underlie belief updating and the formation of placebo responses [41].

Experiment 3 demonstrated that the sweetness-incongruent green solution improved 4-km time-trial performance by approximately 2.8%, despite containing no carbohydrate and producing no condition-specific differences in blood glucose or lactate. Sweetness perception during exercise did not differ between conditions, indicating that the performance effect was not mediated by conscious differences in taste intensity. Moreover, post-exercise questionnaires indicated that participants did not explicitly believe the treatment improved performance, suggesting that implicit rather than conscious expectations contributed to the observed ergogenic effect. Surprisingly, performance benefits were only apparent in the incongruent green condition. Several, explanations may account for this pattern: (i) perceptual incongruence may increase attentional engagement or conflict monitoring [42, 43]; (ii) green may carry culturally embedded associations with freshness or hydration [2, 44]; (iii) athletes may develop associative priors regarding coloured or citrus-flavoured sports beverages as ergogenic cues through repeated exposure and learned expectations within training and competition environments, consistent with placebo and expectancy-based mechanisms [45, 46]. In addition, conditioning processes may differentially modulate these effects across colours. Repeated prior exposure to highly familiar colours such as red or pink, commonly used in carbohydrate-based sports beverages, may elicit strong anticipatory expectations that attenuate novelty-related or prediction-error responses, potentially neutralising placebo-related performance effects. In contrast, less familiar colour–flavour pairings, such as green, may generate greater expectancy violation upon tasting, reflected in altered prefrontal activity and facilitating downstream performance benefits. Experimental evidence indicates that conditioning can amplify or modulate placebo responses in exercise contexts, supporting the role of learned associations in shaping performance outcomes [47]. These interpretations remain speculative and should be investigated in future research.

Across the three experiments, colour operated as a contextual cue capable of shaping sweetness perception (Experiment 1), modulating prefrontal neural dynamics (Experiment 2), and influencing exercise performance via expectancy-related placebo mechanisms (Experiment 3). These converging behavioural, neural, and performance outcomes support the interpretation that colour can meaningfully influence multisensory perception and behaviour through predictive processes. At the same time, these

findings should be interpreted cautiously since EEG does not provide causal evidence, and changes in TBR should be understood as neural correlates of expectancy-driven modulation rather than direct markers of sensory processing. Moreover, TBR represents an indirect index of prefrontal engagement, and EEG's limited spatial resolution precludes precise localisation of the underlying neural generators. Future studies employing neuroimaging techniques with higher spatial resolution, such as functional magnetic resonance imaging (fMRI), may help to better characterise the neural substrates underlying these effects.

Additional limitations warrant consideration. Colour-based cues were not fully dissociated from verbal expectations, as all solutions were presented as carbohydrate-containing beverages, which may have induced performance-related expectancies despite participants not explicitly endorsing treatment efficacy. Explicit and implicit expectations were not independently manipulated, limiting inference regarding their relative contributions. Furthermore, the findings may currently be confined to cycling performance, as this was the exercise model employed in the present study. Finally, colour–flavour correspondences are known to vary across cultures, highlighting the need for replication in more diverse populations.

In conclusion, colour cues shaped sweetness perception, modulated prefrontal neural dynamics during anticipatory and consummatory phases of taste processing, and elicited placebo-like effects on cycling performance. These findings highlight colour as a potent contextual cue capable of influencing multisensory perception and behaviour in the absence of metabolic input, suggesting that visual properties of sports drinks may represent a simple yet underexplored strategy to shape athletes' perceptual and performance responses.

Methods

Ethical Approval and General Procedures

All procedures were approved by the Ethics Committee of the University of São Paulo (CAAE: 51939621.3.0000.0068) and preregistered on the Open Science Framework (https://osf.io/dkm5g/; https://osf.io/4dfjw/). Participants were naïve to the study hypothesis: all were informed only that they would evaluate different commercially available sports-drink mouth rinses. Each experiment (Fig. 1) used a separate informed consent form, and no participant was permitted to enrol in more than one experiment. Participants provided written informed consent prior to participation.

Participants were recruited through institutional mailing lists, online announcements (social media), and word-of-mouth advertising. General inclusion criteria for participants were: age 18–45 y, normal or corrected-to-normal vision, no known gustatory or olfactory impairment, non-smokers, free from acute illness on the testing day. Cyclists were eligible if they had ≥1 year of consistent cycling training ≥60 $km \cdot week^{-1}$ [48], and had not used creatine or β-alanine in the previous 6 months due to their long

washout[49, 50]. Exclusion criteria included any visual, gustatory, olfactory, or auditory dysfunction, allergy to artificial colourants or non-caloric sweeteners, COVID-19 diagnosis in the previous 12 months.

All experiments used the same standardised solution formulation: 25 mL of distilled water sweetened with sucralose (0.024%) [10], coloured with food-grade dyes (Arcos®, Brazil) according to preset RGB/Hex values (Supplementary Table 1), or presented without colour (colourless). Solutions were stored at ~4°C and provided in translucent cups. Participants rinsed for 10 s and expectorated. Session order was randomised using randomizer.org and counterbalanced via a Latin-square design (http://www.hamsterandwheel.com).

Experiment 1

Part A – Sensory stimulus

Sample size was determined based on a heuristic guideline [51] which suggests that ~20 participants per condition generally provides adequate precision for behavioural studies. This target was reinforced by the large effect sizes observed in a similar experiment on colour–sweetness [22], indicating robust perceptual shifts induced by colour cues. Accordingly, we aimed to recruit **≥30 participants per subgroup** (cyclists and non-cyclists). Forty healthy individuals (age 24 ± 23 y; 20 men and 20 women) and 32 cyclists (age 37 ± 32 y; 16 men and 16 women) participated in this study.

Participants were presented with distilled water coloured with distinct pigmentations: red, purple, blue, yellow, green, brown, and pink (Supplementary Table 1). The seven coloured cups were set against a white partition screen and cabin. Participants were asked to subjectively rank the colours visually by associating them with perceived sweetness, from first (“most sweet-associated” or congruent) to seventh (“least sweet-associated” or incongruent). At no point did participants taste the solutions. Based on the results of Experiment 1 Part A, solution colours congruent and incongruent with sweetness were selected for use in all subsequent experiments.

Statistical Analyses

Sweetness scores were summarized and visualized as counts and percentages. Ordinal regression models were used to assess which colours were more likely to show the highest (*i.e.*, 7) and lowest (*i.e.*, 1) scores which could then be classified as the congruent and incongruent colours for subsequent studies. All models were fitted in R using the *MASS* package [52], considering scores as the outcome variable of interest, and colour of the solution as the predictor, with an interaction between colour and sex added to investigate whether scores were different between sexes. A type III analysis of variance (ANOVA) table was used to assess significance of model predictors. The *marginal effects* package [53] was used to produce predictions of the probabilities of scores in each colour across sexes. An alpha of 0.05 was previously established for these and all analyses.

Part B – Taste evaluation

Thirty-two healthy adults (17 men, 15 women; 23 ± 2 y) and fifteen trained cyclists (7 men, 8 women; 36 ± 3 y) participated in this study. Participants were asked to judge the intensity of sweetness of a solution whose colour was congruent (CS) or incongruent (IS) with sweetness (from Part A) compared to a colourless control solution. Participants first rinsed their mouth with 25 mL water for 10 s before mouth rinsing with the colourless control solution for 10 s. Following a 1-min wait, one of the CS or IS solutions was mouth rinsed for 10 s. Perceived sweetness, acceptability, and purchase intention was recorded. The same procedure was then followed with the remaining CS or IS solution. All solutions were expectorated into a cup. A Visual Analog Scale (VAS) [22] was used to measured perceived sweetness. The VAS consisted of a 10 cm horizontal line with three vertical markers, one on the extreme left (i.e., 0 cm) labelled “less sweet”, one in the middle (i.e., 5 cm) labelled “equally sweet” and one on the extreme right labelled “sweeter”. Participants indicated their perceived sweetness by drawing a vertical line on this scale. The acceptance of the solutions was evaluated using a 9-point structured hedonic scale, where 1 represented “disliked extremely” and 9 represented “liked extremely” [54]. Purchase intention was assessed using a five-point structured scale, with 1 indicating “certainly would not buy” and 5 indicating “certainly would buy” [54].

Statistical Analyses

Perceived sweetness was summarized using medians and interquartile range, while acceptability and purchase intention were summarized using counts. Statistical analysis consisted of a linear mixed model to assess whether solution colour influenced perceived solution sweetness. Additional fixed factors for sex (male, female) and training status (cyclist, non-cyclist) were also added to assess the impact of these variables, and individuals were considered as random factors. Exploratory models were done to assess whether there was a significant interaction between solution colour, sex and athlete status. Ordinal logistic regression models were used for acceptability and purchase intention. Models were fit in R using *lmerTest* [55]and *MASS* [52], and predictions made using *marginal effects* [53].

Experiment 2

The sample size was determined *a priori* using G*Power (v.3.1, University of Düsseldorf, Germany) [56], based on a mixed ANOVA design with two independent groups (cyclists and healthy participants) and three repeated measures (colour conditions), with α = 0.05, power (1-β) = 0.80, correlation among repeated measures of 0.90, and a medium effect size (f = 0.25) derived from previous EEG studies on colour and taste perception [57, 58]. The analysis indicated a minimum of 14 participants. Electrical brain activity of 300 observation units was measured from 10 cyclists (7 men, 3 women; 33 ± 5 y) and 10 healthy participants (5 men, 5 women; 26 ± 4 y) using electroencephalography (EEG). Each participant completed 15 mouth rinses: 5 with a red (congruent; CS) solution, 5 with a green (incongruent; IS) solution, and 5 with a colourless (control) solution. The order of conditions was randomized using a random number generator via www.random.org to minimize order effects in this within-subject crossover design.

All testing was conducted in a single session within a neutral, noise-free environment to minimize external influences. Participants were instructed to avoid menthol, spicy foods, and coffee on the day of testing. Participants were familiarised with the EEG setup, the Visual Analogue Scale (VAS), and the standardised handling procedures (lifting the cup slowly, positioning the solution on the tongue, minimising facial/oral movement). Following this, participants performed the experimental protocol, consisting of 5 mouth rinses with each solution lasting approximately 1 min each, totalling 15 min. **In the setup, participants were seated on a white chair at a white table. The coloured solution was placed on the table in front of the participant. A resting-state EEG (eyes open) was recorded for 3 min before starting the protocol.** Each measurement was conducted following a verbal cue from the researcher. Upon the first cue, the participant independently picked up the cup from the table and brought it to their mouth. Upon a second verbal cue, the participant retained the solution in their mouth, without swallowing, for 10 seconds. The solution was held still on the tongue, with participants instructed to avoid any tongue or oral movements to minimise EEG artefacts and ensure consistent sensory exposure across all trials. Upon a third verbal cue, the participant expectorated the solution into a designated container provided by the researcher.Throughout the procedure, participants maintained a steady and relaxed posture with their mouths closed to minimize movements that could disrupt the sensory evaluation via EEG. Detailed instructions were provided for **participants not to move their tongue or other parts of their mouth to avoid disturbing the solution. A custom-built trigger sensor was attached to the solution cup, which was positioned near the participant's chin. This setup allowed for the precise recording of the timing when the solution made contact with the tongue and when it was removed (Supplementary Fig. 1)**. After expectorating each solution, participants provided a sweetness-perception rating on a VAS [22] adapted using reference standards (dipyrone® = minimal sweetness; cotton candy = maximal sweetness).

Cortical electrical activity was recorded using 16 active electrodes positioned according to the international 10–20 system [59] and connected to a 24-bit BrainVision **V-Amp amplifier (Brain Products GmbH) with a sampling rate of 512 Hz. Impedances were maintained below 10 kΩ. Online acquisition filters included a 1–50 Hz band-pass and a 60 Hz notch filter. The reference electrode was positioned at Fpz. Signal processing was performed using the EEGLab toolbox in MATLAB® R2022b [60] for Windows. Pre-processing included downsampling to 250 Hz,** which exceeds twice the maximum frequency of interest (50 Hz) in accordance with the Nyquist theorem, **high-pass and low-pass filtering (0.5–50 Hz), and independent component analysis (ICA) to remove artifacts associated with ocular, muscular, and movement artefacts.** Cleaned EEG data were epoched into 1-s windows time-locked to approach and withdrawal triggers. Spectral power was computed using Welch's method [61] (Hamming window, 50% overlap) for theta (4–8 Hz) and beta (13–30 Hz) frequency bands, and the theta/beta ratio (TBR) was calculated. Prefrontal electrodes included channels Fp1, Fp2, F3, F5, F6 **(Supplementary Fig. 2)**. **Data analysis focused on the 1-s intervals prior to solution contact (approach) and 1-s interval during solution contact (taste). Previous EEG studies investigating expectancy, placebo, and gustatory processing have consistently analyzed a pre-stimulus interval of up to 1 s to capture anticipatory and predictive neural activity, particularly in prefrontal regions [62]. Gustatory and crossmodal taste-related**

EEG responses have been shown to extend beyond early sensory components, with relevant neural information present up to approximately 1 s following stimulus onset [63, 64].

Statistical Analyses

Spectral biomarker data were summarised as mean ± SD or median interquartile range (IQR), with normality assessed using Kolmogorov–Smirnov tests. Conditions (CS vs. IS vs. colourless), trigger (withdrawal), prefrontal channel groups (Fp1, FP2, F5, F6) and (Fp1, F3, F5) and participant groups (cyclists vs. healthy adults) were evaluated using Kruskal–Wallis tests with Bonferroni-adjusted post-hoc comparisons. Sweetness-perception VAS ratings were analysed using a linear mixed-effects model with condition as a fixed factor and participant as a random intercept, following the same model structure described for Experiment 1. Statistical significance was defined as $p < 0.05$.

Experiment 3

An *a priori* power analysis was performed in G*Power (v.3.1, University of Düsseldorf, Germany) [56], using the ANOVA test with intra-factor repeated measures with α=0.05 and β=0.8, correlation among repeated measures of 0.9 and an effect size of 0.25 obtained from a previous study [10], indicating that thirty-two participants were required. Thirty-five participants initially enrolled in the study, but five dropped out for reasons unrelated to the study leaving data sets for 29 trained cyclists (18 males and 11 females; age 37 ± 7 y, $\dot{V}O_{2peak}$ 49 ± 11ml·kg^{-1}min^{-1}, body mass 75 ± 14 kg, height 1.69 ± 0.09 m) who completed the study. Participants were required to have at least 1 y of experience in cycling training ≥60 km per week, with a $\dot{V}O_{2peak}$ of ≥45 ml·kg^{-1} for men and ≥37 ml·kg^{-1} for women [48, 65]. Female participants were required to be using monophasic to avoid confounding effects of cyclical variations in endogenous oestrogen and progesterone, which are known to influence sensory perception, cognitive processing, affective responses, and cortical excitability [66, 67]. Participants with a history of anabolic steroid use, smoking, chronic-degenerative diseases, visual dysfunctions in colour distinction, gustatory or olfactory dysfunctions, or those who had contracted COVID-19 within the past year were excluded from the study.

The study comprised of six laboratory visits. The first session compromised an incremental cycling test to exhaustion to determine $\dot{V}O_{2peak}$, followed by a familiarization to the 4-km cycling time-trial. The incremental cycling test was performed on an electromagnetically braked ergometer (Lode Excalibur, Lode, The Netherlands). The initial workload was 100 W for men and 50 W for women and increased by 25 W in 3-min stages until exhaustion [68]. Breath-by breath gas measurements were continuously recorded using a calibrated gas analyser (K5, Cosmed, Italy). The highest $\dot{V}O_2$ value averaged over a 30-s period during the test was defined as $\dot{V}O_{2peak}$. Participants also performed an Ishihara test on the first visit to ensure the ability to assess colour vision. On the second visit, participants performed another familiarisation to the 4-km cycling time-trial.

There were four main intervention trials performed in a randomized, crossover, counterbalanced and double-blind design. Participants were blinded to the true aims of the study and were informed that they

would be testing the effects of various commercially available carbohydrate mouth rinse products on exercise performance. Upon arrival of each visit, participants watched a pre-recorded video presentation entitled, “Benefits of carbohydrate mouth rinse” (video available at https://osf.io/uq2e5). The use of the video presentation was to generate belief in the carbohydrate mouth rinse in a standardised manner, meanwhile, none of the mouth rinses contained any carbohydrate and differed only in colour. Following the instructional video, participants received an artificially sweetened, non-caloric placebo mouth rinse during three main trials, using the colour solution that were either congruent (red, CS) or incongruent (green, IS) with sweetness, or a colourless solution. Solutions were mouth rinsed for 10 s before being expectorated back into the contained cup. There was also a control visit where no mouth rinse was administered. Participants performed the mouth rinse twice during each main trial, once before the warm-up and once immediately prior to initiating the 4-km cycling time-trial. A Visual Analog Scale (VAS) was used to measured perceived sweetness. The VAS consisted of a 10 cm horizontal line with three vertical markers mail, one on the extreme left (*i.e.*, 0 cm) labelled “less sweet”, one in the middle (*i.e.*, 5 cm) labelled “equally sweet” and one on the extreme right labelled “sweeter”. Participants indicated their perceived sweetness by drawing a vertical line on this scale [22]. Each solution was stored at approximately 4°C before being decanted into a clear plastic cup on the day of the experimental trial. The researcher involved in data collection was blinded to the solutions; at the moments of mouth rinse administration, the researcher left the room, and another researcher not involved in data collection administered the mouth rinse. Participants were randomly assigned to a session order. A balanced number of session orders was created using a Latin Square model (Mason, 2003) (https://hamsterandwheel.com/grids/index2d.php) and participants were randomized using an online tool (https://www.randomizer.org/) and assigned to one of these orders based upon their order of entry into the study.

The 4-km cycling time-trial was performed on a medium-size bicycle (Caloi, Brazil) attached to a Cyclus 2 ergometer (RBM elektronik-automation GmbH, Leipzig, Germany). Participants first undertook a 10 min warm-up at a load of 125 W for men and 100 W, before 1-min rest during which they mouth rinsed a solution (or not in control) prior to performing the time-trial. Participants were instructed to complete the exercise in the shortest time possible and received no verbal encouragement during the test. Time-to-completion was considered as the primary outcome measure. Heart rate was continuously monitored using the Polar H7 (Polar, USA). At every 400 m interval, athletes were prompted to indicate their ratings of perceived exertion (RPE) using the 15-point Borg scale [69]. Participants were asked to complete a post-exercise questionnaire adapted from previous placebo research in sport [70] to indicate whether they believed their performance had been influenced by the treatment. Responses were recorded using a 5-point Likert scale, where 1 corresponded to “not at all,” 2 to “little,” 3 to “moderately,” 4 to “very much,” and 5 to “extremely.” Fingertip blood samples were collected pre-exercise and 5-min post-exercise and immediately analysed for blood lactate and glucose concentrations (Stat Profile Prime Plus, Nova Biomedical, USA). To minimize the influence of diet on performance, cyclists were requested to maintain the same diet prior to all main trials, and this was confirmed by the analysis of 24-h food recalls prior to each test. The 24-h dietary recall consisted of listing the foods and beverages consumed during the 24 h

before the assessment. Nutritional supplements were also recorded. Energy and macronutrient intake were analyzed with specialised software (Dietbox, Sao Paulo, Brazil).

Statistical Analyses

Continuous variables were summarized as means ± one standard deviation. Scores in the sweetness perception scale were summarized as counts, median and IQR. Differences in time-to-completion across the experimental visits were evaluated by a linear mixed model, with the independent variable of interest being visit (Control, Colourless, CS, IS), and including a random factor for participant. An interaction between group and sex was added to investigate potential differences in the effect between sexes. An exploratory model including the post-exercise performance questionnaire score as a fixed factor was done to assess whether belief in improved performance correlated with improved performance. Differences in sweetness perception, blood glucose and lactate, and RPE, were evaluated by a linear mixed model, with a fixed effect of condition for sweetness perception; an interaction between condition and timepoint being included for the blood related variables; and an interaction between condition and distance included for the RPE model. When the included interaction was not significant, the more parsimonious model with only the simple effects and interaction models were compared using Akaike information criterion (AIC). The model with lowest value (*i.e.,* best fit) was considered as the final model. A type III ANOVA table was used to assess significance of model predictors. Pairwise comparisons and predictions were made when pertinent, utilizing Tukey's adjustment for multiple comparisons, and are reported as mean difference, 95% confidence intervals and p-values. Models were fit in R using *lmerTest* and *MASS*.

Declarations

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Figures

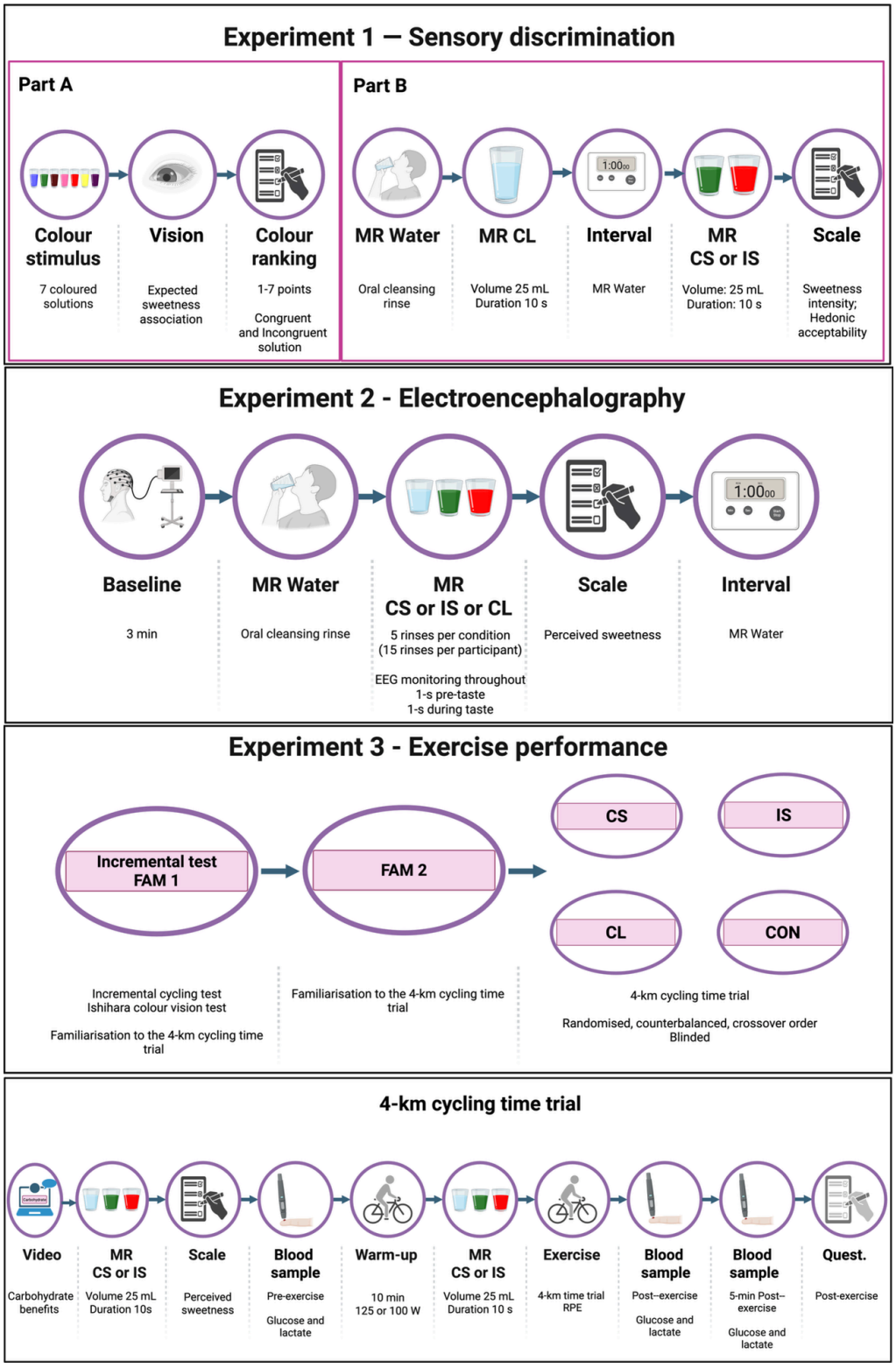


Figure 1

Overview of the experimental design. The study comprised three experiments examining how colour influences sweetness perception, prefrontal neural activity and exercise performance. Experiment 1 included a sensory stimulus protocol with two components: Part A, a visual colour-ranking task in which participants ordered seven coloured solutions according to their expected sweetness association, and Part B, a taste-evaluation in which the coloured solutions congruent (red) and incongruent (green) with

sweetness (from part A) were compared to a colourless solution and rated for sweetness intensity, hedonic acceptability, and purchase intention. Experiment 2 evaluated prefrontal electroencephalographic activity during controlled solution tasting in trained cyclists and healthy individuals. A custom trigger system marked the approach and withdrawal events, allowing precise temporal alignment of behavioural and neural signals across congruent (red), incongruent (green), and colourless conditions. Experiment 3 recruited trained cyclists in a randomized, counterbalanced, crossover fashion. Participants performed a 4-km time trial following mouth rinsing of one of three artificially sweetened, non-caloric, placebo solutions, one congruent with sweetness (red), one incongruent with sweetness (green) and one without colour. A control visit was also performed in which no mouth rinsing was performed. Participants were informed that the solutions were different carbohydrate sports drinks. Blood lactate and glucose and ratings of perceived exertion were measured. MR = Mouth rinse; CS = Congruent solution; IS = Incongruent solution; CL = Colourless solution; CON = Control; Quest = Questionnaire. Created in https://BioRender.com

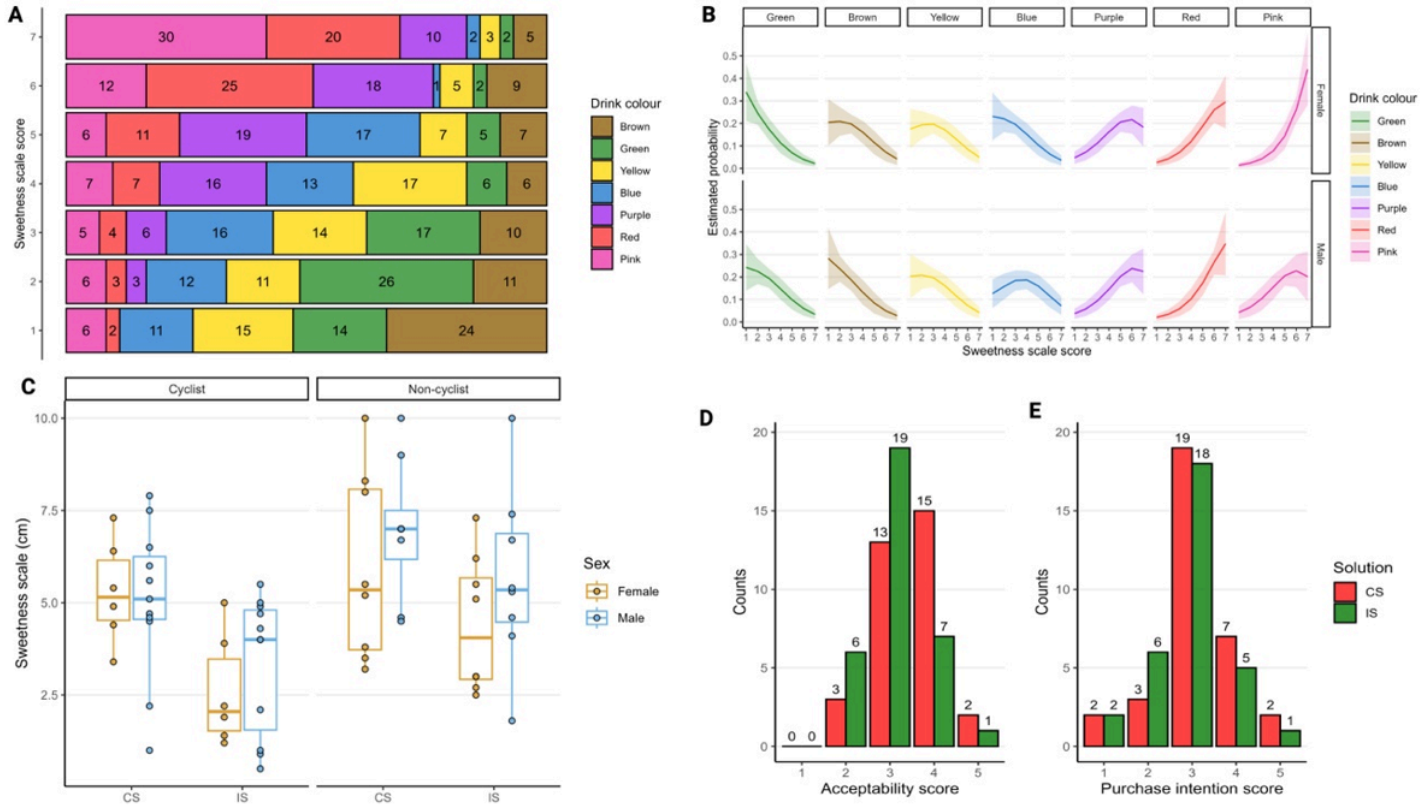


Figure 2

Colour–sweetness associations and taste-perception outcomes (Experiment 1). (A) Distribution of sweetness-association rankings for the seven coloured solutions. Bars represent the number of times each colour was ranked from 1 (least sweet-associated) to 7 (most sweet-associated). (B) Model-estimated probabilities of sweetness-ranking scores by colour, stratified by sex. Solid lines show predicted probabilities and shaded bands represent 95% confidence intervals. Experiment 2. (C) Sweetness-intensity ratings (VAS, cm) for congruent (CS) and incongruent (IS) solutions in cyclists (left)

and non-cyclists (right). Boxplots display the median (central line), interquartile range, and individual data points in female (yellow) and male (blue) participants. (D-E) Distribution of hedonic acceptability (D) and purchase-intention (E) scores for CS and IS solutions. Created in https://BioRender.com

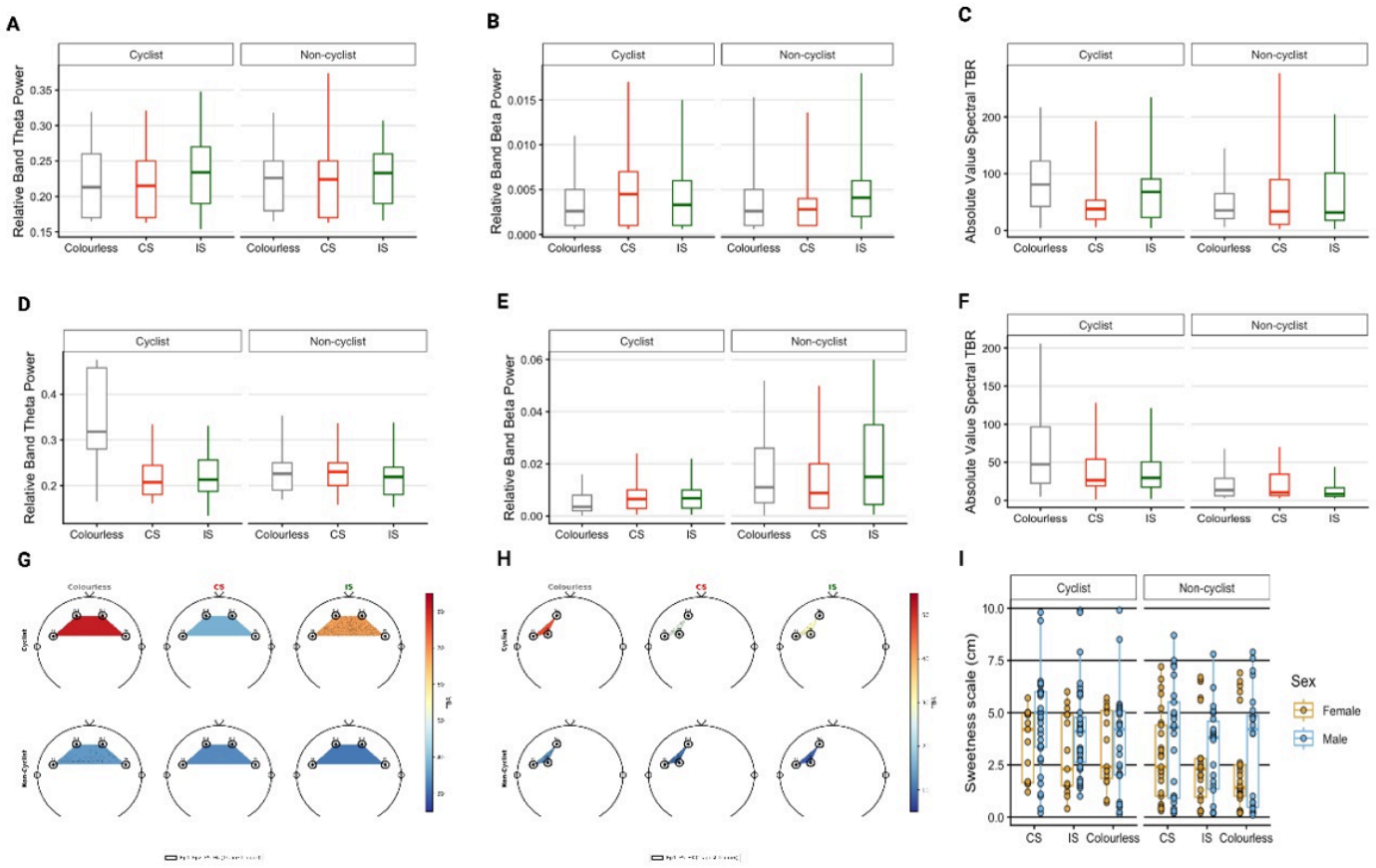


Figure 3

Prefrontal EEG modulation and sweetness perception across colour conditions (Experiment 2). Boxplots show medians and interquartile ranges (IQR). (A) Relative theta band power from the Fp1–Fp2–F5–F6 electrode cluster measured 1 s before the withdrawal trigger for congruent (CS), incongruent (IS), and colourless (CL) solutions in cyclists and non-cyclists. (B) Relative beta band power from the same cluster and time window. (C) Theta–beta ratio (TBR) from the Fp1–Fp2–F5–F6 cluster at 1 s before withdrawal. Cyclists showed significantly lower TBR in the CS condition relative to CL ($p = 0.026$), whereas no condition effects were observed in non-cyclists. (D) Relative theta band power from the Fp1–F5–F3 electrode cluster measured 1 s after the withdrawal trigger. (E) Relative beta band power from the same cluster and time window. (F) TBR from the Fp1–F5–F3 cluster at 1 s after withdrawal. Cyclists displayed lower TBR for both CS ($p = 0.047$) and IS ($p = 0.049$) relative to CL; no significant differences emerged in non-cyclists. Scalp topography of prefrontal activity (G) at −1-s pre-trigger (approach), with the Fp1–Fp2–F5–F6 cluster highlighted and (H) at +1-s post-trigger (taste), highlighting the Fp1–F5–F3. (I) Sweetness-intensity ratings (VAS, cm) for congruent (CS) and incongruent (IS) solutions in cyclists (left) and non-cyclists (right). Boxplots display the median (central line), interquartile

range, and individual data points in female (yellow) and male (blue) participants. Created in https://BioRender.com

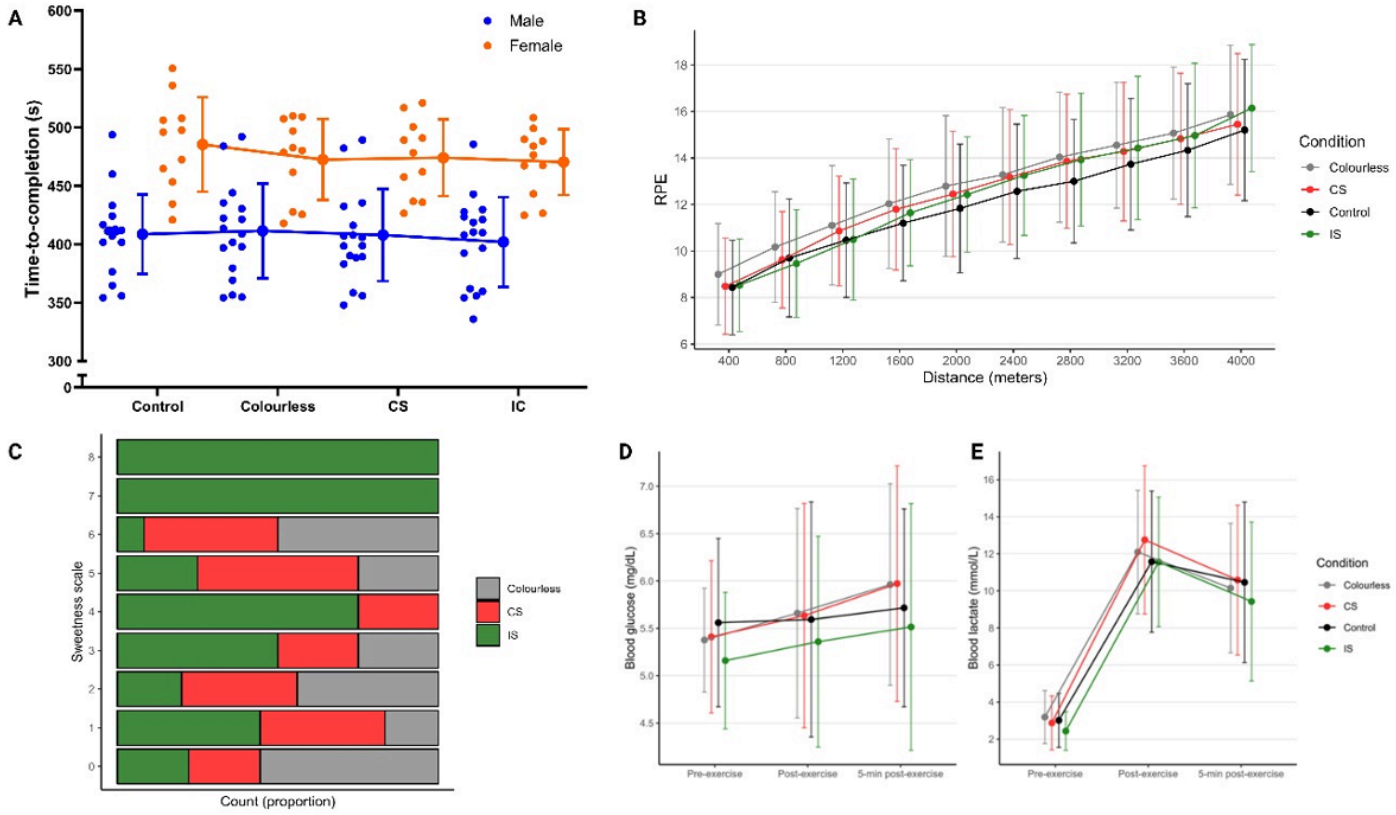


Figure 4

Performance, perceptual, and physiological responses to coloured mouth rinses (Experiment 3). (A) Time-to-completion for the 4-km cycling time trial across conditions, stratified by sex. Large circles and error bars indicate mean ± SD; small circles represent individual participants (jittered for visibility). (B) Ratings of perceived exertion (RPE) across the 4-km trial (mean ± SD) for each condition. (C) Sweetness-perception ratings following each mouth-rinse condition. (D–E) Blood glucose (D) and blood lactate (E) concentrations measured at pre-test, post-test, and 5-min post-exercise (mean ± SD). CS = congruent solution; IS = incongruent solution; CL = colourless solution. Created in https://BioRender.com

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