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Abstract

Objectives: To investigate the effect of tramadol on performance during a 20-min cycling time-trial (Experiment 1), and to test whether sustained attention would be impaired during cycling after tramadol intake (Experiment 2).

Design: randomized, double-blind, placebo controlled trial.

Methods: In Experiment 1, participants completed a cycling time-trial, 120-min after they ingested either tramadol or placebo. In Experiment 2, participants performed a visual Oddball task during the time-trial. Electroencephalography measures (EEG) were recorded throughout the session.

Results: In Experiment 1, average time-trial power output was higher in the tramadol vs. placebo condition (tramadol: 220 watts vs. placebo: 209 watts; $p < 0.01$). In Experiment 2, no differences between conditions were observed in the average power output (tramadol: 234 watts vs. placebo: 230 watts; $p > 0.05$). No behavioural differences were found between conditions in the Oddball task. Crucially, the time frequency analysis in Experiment 2 revealed an overall lower target-locked power in the beta-band ($p < 0.01$), and higher alpha suppression ($p < 0.01$) in the tramadol vs. placebo condition. At baseline, EEG power spectrum was higher under tramadol than under placebo in Experiment 1 while the reverse was true for Experiment 2.

Conclusions: Tramadol improved cycling power output in Experiment 1, but not in Experiment 2, which may be due to the simultaneous performance of a cognitive task. Interestingly enough, the EEG data in Experiment 2 pointed to an impact of tramadol on stimulus processing related to sustained attention.

Trial registration: EudraCT number: 2015-005056-96.

Keywords

Doping in Sport; Opioid Analgesic; Athletes; EEG; Exercise, Brain

Introduction

There is an increasing tendency to treat minor sporting injuries with the use of analgesic drugs in order that an athlete is able to continue training and competing. One of these "trending" analgesics is tramadol that is an opioid agonist and is used in the treatment of moderate to severe pain. Tramadol has a dual mechanism of action, being both an μ-opioid receptor agonist, and a serotonin and norepinephrine reuptake inhibitor [1]. Activation of the μ-opioid receptor agonist can cause analgesia and sedation. Likewise, by inhibiting serotonin and norepinephrine reuptake, tramadol seems to reduce pain perception [1]. Given the negative association between pain and exercise capacity, the prophylactic use of analgesic medication (also known as "painkillers") is relatively common to reduce pain in order to enhance sport performance [2]. Similar to other painkillers [3], it is therefore possible that tramadol could improve exercise performance via its effect on effort, pain perception, or mood. However, little is known about the effect of tramadol in sporting performance, with the literature being limited to non-athletic populations [4]. Of the limited research to date, results are conflicting with some suggesting beneficial effects of reduced pain perception and improved effort based exercise performance [4], some reporting uncertain effects on cognitive function [5], and some proposing a negative effect on cognitive function and chemosomatosensory evoked potentials [6].

Informal reports from professional World-Tour cyclists and staff suggest that there may be some abuse of tramadol for potential performance enhancement reasons [7]. Indeed, results of a recent study involving young elite cyclist suggested that they identified tramadol as a potential doping agent [8]. Despite a significant media interest surrounding tramadol [9], little is known of its ergogenic effect in cycling. Currently, tramadol is not included on the list of banned substances by the World Anti-doping Agency (WADA) but, it is placed on WADA's monitoring program from 2012 to 2017 to detect potential patterns of abuse [10]. According to the WADA monitoring program, 71 to 82 percent of the tramadol use between 2012 and 2015 in globally monitored sports occurred in cycling [11]. Of particular concern is the drowsiness reported following

tramadol administration, which could lead to reduced perception, attention and vigilance causing possible falls in the pro-cycling peloton [12].

In this study, we aimed to test the potential ergogenic effect of tramadol during cycling, and whether it reduces sustained attention (i.e. the ability to keep focused on a particular task over the time). Sustained attention was investigated at the behavioural and brain level, by asking participants to perform a cognitive task while performing the cycling exercise and by recording electroencephalography (EEG). Specifically, we tested the hypothesis that acute oral administration of tramadol would improve 20-min cycling time-trial performance (Experiment 1). We hypothesised that information processing and behavioural responses in a sustained attention task would be influenced by tramadol during the 20-min time-trial (Experiment 2). Given the aforementioned effect of painkillers on perceptual variables [3], we also investigated subjective measures of the participants' mood, perceived effort and mental fatigue. We hypothesised that tramadol would affect mood at rest and reduce perceived effort and fatigue during the 20 min TT.

Methods

The study involved a randomized, double blind, placebo controlled trial. The trial was approved by the Spanish Agency of Medicines and Medical Devices (AEMPS), EudraCT number: 2015-005056-96, and the Ethical Committee of Clinical Research in Granada. All experimental procedures were designed to comply with the Declaration of Helsinki and Good Clinical Practice (GCP). The randomization process, the audit and verification of compliance of GCP rules were performed by an external clinical research organization (CRO; Delos Clinical, Seville, Spain). The sample sizes were based on power calculations using G*Power Software [13] and assuming a 0.8 power and an alpha error of 0.05. Only cyclists and triathletes with a high-medium level of physical fitness were included in the study. Exclusion criteria were the presence of symptomatic cardiopathy, metabolic disorders such as obesity (BMI >30) or

diabetes, chronic obstructive pulmonary disease, epilepsy, therapy with ß-blockers and medications that would alter cardiovascular function, hormonal therapy (and estrogen-progestogen contraception for females participants) and smoking. Participants were asked to refrain from drinking alcohol (48 h abstinence) and caffeine (24 h abstinence), to keep their pre-exercise meal the same, and not to perform any exhaustive exercise in the 48 h before each experimental visit.

In Experiment 1, we recruited 30 cyclists, 20 males and 10 females. Two participants could not complete Experiment 1: one male due to nausea and drowsiness after tramadol ingestion (approximately 90min), and one female due to an ankle injury not related with the experiment. The final sample was 28 participants, 19 males and 9 females, (mean (SD) age 25.6 (5.9) years, weight 69.07 (10.3) Kg; $VO_{2max:}$ 49.17 (7.29 ml/min/kg) for Experiment 1. For male participants, tramadol dose corresponded to 1.35 mg/BM, with a dose of 1.77 mg/BM given to females**.** Participants visited the research laboratory on three separate occasions at the Mind, Brain and Behaviour Research Centre of the University of Granada, firstly for an assessment of their cardiorespiratory fitness, with two further visits for the experimental manipulation. At initial visit participants performed a maximal incremental exercise test to establish their maximal oxygen uptake following a standard laboratory protocol [14]. During the test, participants' VO_2 was measured on a breath-by-breath basis using an online gas analyser (JAEGER MasterScreen; CareFusion GmbH, Germany). After completing the maximal incremental test, participants performed a 10-minutes time-trial in order to familiarised with protocol. The shorter duration of the familiarization test (with respect to the proper experimental time-trial) might be seen as a limitation of our study. However, two reasons motivated our choice: 1) our participants were experienced cyclists used to performing this type of (sustained) physical effort, and given their expertise, the purpose was that of familiarize them with the laboratory setting testing procedure, 2), we were mindful that the 10' test was performed after the maximal incremental exercise test from which participants were already fatigued.

On arrival at the laboratory for visit 2 and 3 (supplementary material Fig 1 for protocol schematic), participants completed a Profile of Mood States Questionnaire (POMS), and a visual analogue scale (VAS) concerning perceived activation, mental and physical fatigue. After completing the questionnaires, participants consumed either tramadol or placebo as outlined below. The experimental sessions were completed at the same time of the day (±1 h). The time-trial commenced 120 min following ingestion of the tramadol or placebo capsule (see experimental manipulation below). Before the beginning of the time-trial, the participant's EEG was recorded as a baseline measure and throughout the session. Next, participants performed a 10 min warm-up at 100 watts, followed immediately by a 20-min cycling time-trial on a cycle ergometer (SRM, Julich, Germany). Participants adjusted saddle and handle bar height and length, and it was kept for all sessions. The time-trial was conducted in a dimly-illuminated, sound-attenuated faraday cage. Convective cooling was provided by one fan (2.5 m/s wind speed) located 100cm from the ergometer. Participants were instructed to maintain the highest average power possible during the time-trial and were freely able to change gearing and cadence throughout. Participants were aware of the elapsed time, but did not have feedback on performance (wattage and heart rate) during, or after the time-trial. Heart rate was measured continuously throughout the protocol (V800, Polar Electro, Finland). Immediately after the time-trial participants were asked to rate their average perceived exertion during the preceding exercise. Then, participants completed 10 min cool-down (60 watts), following which another EEG recording was taken. Finally, the POMS and VAS were completed again.

As we did not find any effect of gender in Experiment 1, and given the difficulty of finding a large enough samples of females, we only recruited and tested males and in Experiment 2**.** One participant only completed visit 1, and data from another was removed due to data acquisition issues, meaning that the final sample for Experiment 2 was n = 28: age 25 (5) years, weight 73.2 (7.7) Kg; $VO_{2max:}$ 54.1 (5.7) ml/min/kg. The procedure of the Experiment 2 was the same as that of Experiment 1, except for the following: participants completed an oddball sustained attention task during the 20-min time-trial with the purpose of assessing sustained attention during

exercise. Participants completed a visual three-stimulus oddball paradigm based on that used by Sawaki and Katayama [15] while performing the 20-min time-trial.

Participants consumed either a single oral dose of 100 mg of tramadol or placebo (microcrystalline cellulose) with water. The tramadol dose used in this study has been demonstrated to have effect on μ-opioid receptor (compared with placebo), with a mean time to maximum plasma concentration of 156 min (range: 87-208 min; [1]). Importantly, Bastami et al. [1], showed good tolerability to adverse events with this dose. The Hospital Pharmacology Section of the University of Granada prepared the tramadol and placebo oral doses. Tramadol and placebo were made following the good manufacturing practice (GMP) audited and approved by Spanish authority (AEMPS). The randomization was performed on a 1:1 balanced allocation where a code was assigned for tramadol and placebo to each patient in different visit order. Only a pharmacist who was not involved in the experimental work of this study knew the participant randomization. Tramadol and placebo were made in dark red hard gelatine capsules, which prevented the possibility to see the contents. Each capsule was packed in a monodose blister with the patient code and visit number in the information label. No less than 7 days were allowed between experimental sessions to allow for washout time and recovery.

We used the Spanish adapted version of the POMS [16], which has been used extensively for the assessment of mood in the sport and exercise environments. This questionnaire has 58 items and the factor structure representing six dimensions of the mood construct: Tension, Depression, Anger, Vigour, Fatigue and Confusion. Participants answered the items on a 5-point Likert scale (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely). Raw scores were transformed following the standard point table [16].

The VAS to rate participants' perceived activation, perceived mental and physical fatigue, ranging from 0 (low) to 100 (high) in response to the following questions: (a) "What is your activation level now?" (b) "What is your physical fatigue level now?" and (c) "What is your mental fatigue level now? At the end of the session, participants also rated the following

question “how would you rate the overall mental load for this experimental session?”. Immediately after the time-trial participants were asked to rate their average perceived exhaustion (RPE) using the 6-20 Borg scale.

An oddball task was designed to measure sustained attention by the random presentation of a sequence of visual stimuli on a computer screen situated at 100 cm from the participants’ head and at their eye level**.** A total of 600 stimuli were presented, consisting of frequent blue circle, rare blue circle, and rare red square with probabilities of .80, .10, and .10, respectively via use of computer software (E-Prime, Psychology Software Tools, Pittsburgh, PA, USA). The task lasted approximately 18 minutes, starting two minutes after the beginning of the time-trial. The small blue circle (1.15º x 1.15º) was considered as the standard stimulus. The blue circle that was slightly larger than the standard circle (1.30º x 1.30º) was defined as target 1. Finally, the rare red square (2.00º x 2.00º) was defined as target 2. Each trial begun with the presentation of a blank screen in a black background for 1200 ms. Then, the stimulus was presented in the centre of the screen in a random time interval (between 0 and 800 ms) during 150 ms (supplementary material Fig 2). Participants were required to respond to both target 1 and target 2 with their thumb finger of their dominant hand by pressing a button connected to the cycle ergometer handlebar, and not to respond to the standard stimuli. Verbal and written instructions were given to the participant prior to the start of the oddball sustained attention task. Participants were instructed that the main goal of the task was to be as accurate as possible. A brief familiarization of the task was included in the screening visit. For each stimulus, the RT (in ms) and response accuracy (percentage of correct responses) were recorded.

At this point, we would like to note that, while the POMS used in the present study has shown a high reliability (Cronbach alpha = 0.90) [16], we do not have a measure of reliability for the particular VAS and oddball tasks used in our study. The lack of reliability may suppose a limitation in the present investigation, but, generally, oddball tasks show an interclass correlation coefficient higher than 0.75 [17], and the VAS a Cronbach alpha higher than 0.90, showing high test-retest reliability [18,]. In any case, the purpose of these tasks were to compare

performance in within-participants experimental conditions and not to compare participants' scores with a normalized scale.

EEG data were recorded at 1000 Hz using a 62-channel actiCHamp System (Brain Products GmbH, Munich, Germany) with active electrodes positioned according to the 10-20 EEG International System and referenced to the Cz electrode. The cap was adapted to individual head size, and each electrode was filled with Signa Electro-Gel (Parker Laboratories, Fairfield, NJ) to optimize signal transduction. Participants were instructed to avoid body movements as much as possible, and to keep their gaze on the centre of a computer screen during the measurement. Electrode impedances were kept below 10 kΩ. EEG pre-processing was conducted using custom Matlab scripts and the EEGLAB [19] and Fieldtrip [20] Matlab toolboxes. EEG data were resampled at 500 Hz, bandpass filtered offline from 1 and 40 Hz to remove signal drifts and line noise, and re-referenced to a common average reference. Horizontal electrooculograms (EOG) were recorded by bipolar external electrodes for the offline detection of ocular artefacts. Independent component analysis was used to detect and remove EEG components reflecting eye blinks [21].

Electrodes presenting abnormal power spectrum were identified via visual inspection and replaced by spherical interpolation. Processed EEG data from each protocol time period (baseline-pre, warm-up, time-trial, cool-down, baseline-post) were subsequently segmented to 1-s epochs. The spectral decomposition of each epoch was computed using Fast Fourier Transformation (FFT) applying a symmetric Hamming window and the obtained power values were averaged across protocol time periods.

Task-evoked spectral EEG activity was assessed by computing event-related spectral perturbations in epochs extending from –500 ms to 500 ms time-locked to stimulus onset for frequencies between 4 Hz and 40 Hz. Spectral decomposition was performed using sinusoidal wavelets with 3 cycles at the lowest frequency and increasing by a factor of 0.8 with increasing

frequency. Power values were normalized with respect to a −50 ms to 0 ms pre-stimulus baseline and transformed into the decibel scale (10*log10 of the signal).

All analyses were completed using statistical non-parametric permutation tests with a Monte Carlo approach [22]. These tests do not make any assumption of the underlying data distribution, are unbiased, and as efficient and powerful as parametric statistics [23]. When statistical significance ($p < 0.05$) was found, values were corrected by the false discovery rate method. Subsequently, the effect of experimental condition (tramadol vs. placebo) on: Experiment 1 - cycling time trial power output and heart rate; and Experiment 2 - cycling time trial power output, heart rate, and RT and accuracy in the oddball task were analysed using a within-subject design condition (tramadol, placebo). Data from POMS, and VAS, were analysed using a condition (tramadol, placebo) and time point (pre, post) within-subject design.

EEG spectral power main effects of condition (tramadol, placebo) were separately tested for significance at each protocol time period. In the absence of strong a priori hypotheses, we used a stepwise, cluster-based, non-parametric permutation test [24] without prior assumptions on any frequency range or area of interest. The algorithm performed a t-test for dependent samples on all individual electrodes x frequencies pairs and clustered samples with positive and negative t-values that exceeded a threshold based on spatial and spectral adjacency. These comparisons were performed for each frequency bin of 1Hz and for each electrode without a priori assumptions on the frequency range or region of interest. Cluster-level statistics were then calculated by taking the sum of the t-values within each cluster. The trials from the two datasets (tramadol, placebo) were randomly shuffled and the maximum cluster-level statistic for these new shuffled datasets was calculated. The above procedure was repeated 5000 times to estimate the distribution of maximal cluster-level statistics obtained by chance. The two-tailed Monte-Carlo p-value was determined by the proportion of random partitions that resulted in a larger test statistic than the original. A p-value of the original cluster statistic smaller than the critical Monte-Carlo p-value indicated significant differences between the two datasets.

In Experiment 2, event-related spectral perturbation main effects of condition (tramadol, placebo) for each stimulus of the odd-ball task (target 1, target 2 and standard) were also analysed by applying the cluster-based permutation test. In order to reduce the possibility that the type II error rate was inflated by multiple comparisons correction, we set a priori criteria of collapsing data into four frequency bands: Theta (4–8 Hz), Alpha (8–14 Hz), lower Beta (14–20 Hz) and upper Beta 1 (20–40 Hz). To avoid an overlap with behavioural responses, we also limited the time windows of interest to the first 300 ms and 500 ms after the stimuli onset (based on average behavioural response times) for target and standard trials, respectively.

Results

In Experiment 1, the average power output during the 20-min time-trial (Fig 3A) was higher under tramadol condition than under placebo condition (220 W [95%CI = 203 – 240 W] vs. 209 W [95%CI = 192 – 228W] for tramadol and placebo, respectively; $p < 0.01$). An additional analysis revealed that the effect of tramadol did not depend on participants' gender: $t = 1.107$, $p = 0.83$ (critical t-score: ± 2.177).

The average heart rate demonstrated a significant difference between conditions (166 beats.min^{-1} [95%CI = 162 – 170 beats.min^{-1}] vs. 162 beats.min^{-1} [95%CI = 153– 166 beats.min^{-1}] for tramadol and placebo, respectively; $p < 0.01$).

The POMS data demonstrated a significant interaction between condition and time point for fatigue ($p = 0.03$), confusion ($p < 0.01$) and tension ($p < 0.01$). Under the tramadol condition, participants showed a higher fatigue (M = 52, 95%CI = 48 - 55 vs. M = 47, 95%CI = 44 - 49); $p < 0.01$) and confusion (M = 41, 95%CI =38 - 44 vs. M = 38, 95%CI = 36 - 39); $p < 0.01$) after the time-trial, while no difference where found before the time-trial for fatigue (M = 42, 95%CI = 39 - 45 vs. M = 40, 95%CI = 38 - 42), $p > 0.05$) and for confusion (M = 39, 95%CI = 37 - 40 vs. M = 38, 95%CI = 37 - 39); $p < 0.05$). There were trends for lower tension before (M = 35, 95%CI = 33 - 37 vs. M = 38, 95%CI = 35 - 39) tramadol vs. placebo, respectively; $p = 0.06$), but not after the time-trial (M = 35, 95%CI =32 - 36 vs. M = 34, 95%CI = 32 - 35), for tramadol

and placebo, respectively; $p > 0.05$). The anger index showed a main effect for time point ($p < 0.01$) with higher values before the time-trial (M = 38, 95%CI = 37 – 40 vs. M = 39, 95%CI = 36 – 39), but no effect for condition or interaction between the factors. No other POMS factors (depression and vigour) demonstrated significant changes with condition or time point (ps > 0.05).

Post time-trial RPE did not demonstrate any significant differences between conditions ($p > 0.05$). VAS Mental Load demonstrated a trend for an interaction between condition and measure. Specifically, after the time-trial participants had trend for a higher mental fatigue in the tramadol vs. placebo condition (M = 34, 95%CI = 24 - 44 vs. M = 22, 95%CI =16 - 28), respectively; $p = 0.056$). The main effect for time point was not significant ($p > 0.05$).

The Activation and Fatigue indexes demonstrated a significant main effect of time point (Activation: M = 51, 95%CI = 44 - 57 vs. M = 60, 95%CI = 54 - 66; $p < 0.01$. Fatigue: M = 21, 95%CI = 13- 28 vs. M = 45, 95%CI = 36 - 52; $p < 0.01$). Before vs. after the time-trial respectively), while the main effect for condition and the interaction between both factors did not reach significance ($p > 0.05$). Finally, the cognitive load of the session was not significantly different between conditions ($p > 0.05$).

The analysis of tonic spectral power showed a significant main effect of condition ($p < 0.01$) for the baseline period. One cluster (frequency-localization) was found and was statistically significant: a global cluster (51 electrodes) in the beta band (13-40 Hz). The analysis revealed an overall increase in the power of frequencies in the tramadol condition with regard to the placebo (supplementary material Table 1).

There was a significant main effect of condition ($p = 0.01$) for the warm-up period. One cluster was statistically significant: a 33 electrodes cluster in the alpha band (7-10 Hz). The analysis revealed an overall increase in the power of frequencies in the tramadol condition in comparison to the placebo condition. There were no statistically significant terms in the analysis of the EEG data from the time-trial, cool-down and baseline-post phases ($p > 0.05$).

In Experiment 2, one participant only completed visit 1, and data from another was removed to due data acquisition issues meaning that the final sample was n = 28 for Experiment 2. The power output during the time trial was not significantly different between conditions see (Fig 3B): tramadol (234 W [95% CI = 218– 248 W]) vs. placebo (230 W [95%CI = 215– 246 W] $p > 0.05$).

The main effect of condition did not reach statistical significance for the heart rate: tramadol 176 beats.min^{-1} [95%CI = 172 – 179] vs. placebo (175 beats.min^{-1} [95%CI = 170 – 179]; $p > 0.05$.

Analysis of the POMS demonstrated a main effect of time point for the factor anger and fatigue ($p < 0.05$). Participants showed lower anger (M = 42, 95%CI = 40 - 44 vs. M = 40, 95%CI = 38 - 42) and higher fatigue (M = 44, 95%CI = 41 - 46 vs. M= 51 95%CI = 48 - 53), after the time-trial. None of the other POMS items reached statistical significance ($p > 0.05$).

There were no differences in post time-trial RPE between conditions ($p > 0.05$). An overall main effect of time point was found for fatigue and mental load in the VAS. Specifically, a higher mental fatigue (M = 31, 95%CI = 21 - 40 vs. M = 54, 95%CI = 47 - 60; $p < 0.01$) and higher mental load (M = 25, 95%CI = 17 – 32 vs. M = 44, 95%CI = 36 - 52; $p < 0.01$) were found after the time-trial. None of the others items in the analysis reached significance ($p > 0.05$).

In the Oddball task, there were no significant differences between conditions for the target1, target2 or standard stimuli, for RT and accuracy (all ps > 0.05).

The analysis of tonic spectral power revealed a significant main effect of condition ($p < 0.01$) for the baseline-pre period. One cluster (frequency-localization) was found and was statistically significant: a global cluster (41 electrodes) in alpha band. The analysis revealed an overall increase in the power of frequencies in the placebo condition with regard to tramadol. There was a significant main effect of condition ($p < 0.01$) in the warm-up period. Two clusters were

statistically significant: one cluster (17 electrodes) in the 3-6 Hz band ($p = 0.01$), and one cluster (5 electrodes) in the 26–33 Hz band ($p = 0.02$). The analysis revealed an overall increase in the power of frequencies in the placebo condition in comparison to the tramadol condition (Table 1).

The main effect of condition during the time-trial was not significant ($p>0.05$). There was a significant main effect of condition ($p < 0.05$) for the cool-down period. Two clusters were statistically significant: one cluster (29 electrodes), in the (1–9 Hz) delta and theta band ($p < 0.01$); and one global cluster (41 electrodes) in (12–36 Hz) beta band ($p < 0.01$). Similar to other period, the analysis revealed an overall increase in the power of frequencies in the placebo condition. In the baseline-post period we found a main effect of condition. One cluster was found (29 electrodes) and was statistically significant ($p = 0.01$) in the theta band. Similar to other periods, the analysis showed an increase in the power for placebo compared to tramadol.

The event-related spectral perturbation (stimulus-locked) analysis in Experiment 2 showed a main effect of condition in the alpha band for target 1, with a cluster (15 electrodes) between 118-224 ms after the onset of the target 1 ($p < 0.01$; see Fig 4 A- B). Alpha frequency band exhibited a lower spectral power in the tramadol condition with regard to the placebo condition. The Lower Beta band analysis showed a significant cluster (24 electrodes) between 136-230 ms after the onset of target 1 ($p < 0.01$). Lower Beta frequency band exhibited a higher spectral power in placebo condition with regard to the tramadol condition (see Fig 4C). The analysis of the other frequency bands for target 1, target 2 and standard trials yielded no significant effects ($p > 0.05$).

Discussion

To the best of our knowledge, this is the first study to investigate the effect of tramadol on cycling performance, sustained attention and brain dynamics in trained cyclists. Data from Experiment 1 revealed that tramadol improved 20-min cycling time-trial performance by ~5%.

In contrast, there was no difference in average power output between tramadol and placebo condition in Experiment 2. Although no effect of tramadol was found on behavioural performance in the sustained attention tasks, EEG time-frequency (stimulus-locked) analysis showed effects of tramadol on brain functioning related to stimulus processing.

Tramadol allowed participants to sustain a higher power and greater cardiorespiratory stress (higher heart rate) during the 20-min time-trial than in the placebo condition in Experiment 1. However, the RPE following the time-trial was similar between both substance conditions. Similar results have been reported with other analgesics. For instance, previous research has shown that paracetamol improves performance in a 10-mile cycling time trial compared to placebo in the absence of a reduction in perceived pain or perceived exertion [3]. In another study, Foster et al. [25], also found that paracetamol improved performance compared to placebo. However, in this study testing repeated sprint ability, the authors concluded that paracetamol increased the level of performance due to an increase in participant's normal pain threshold. In the present study, although no differences were found in perceived exertion, we cannot ensure that pain perception was modulated by tramadol, since we did not ask participants to rate it. Interestingly, in Experiment 2, the effect of tramadol on time-trial power output was not significant. The reason for these divergent results between Experiment 1 and 2 is uncertain (see below for further discussion on this issue).

We only found an effect of tramadol on the POMS items for confusion and fatigue in Experiment 1. In both cases, participants showed a higher score after the time-trial with tramadol. However, it is uncertain whether tramadol caused higher fatigue and confusion, or whether these higher rating were a consequence of the greater physical effort achieved during the time-trial.

The oddball task was chosen to test the hypothesis of whether tramadol may impair cognitive function during cycling. This oddball task tests participant's ability to discriminate between a standard (frequent and irrelevant) and target (rare and infrequent) stimuli [15]. These continuous

discriminations between monotonous frequent information and relevant infrequent stimuli are characteristic of those encountered in a cycling peloton (e.g., avoiding a pothole in the road, or sudden breaking in front). Our hypothesis was that tramadol would impair attention level and participants would perform worse in the oddball task. However, RT and accuracy results did not show significant differences between conditions for any of the stimuli. Nevertheless, EEG data did reveal an interesting pattern of results in relation to (brain) event-related activity following tramadol ingestion.

Specifically, there was target-locked higher suppression of the alpha activity (i.e., lower activity) and overall reduced beta frequency after tramadol intake with respect to the placebo condition. A previous study using a similar visual oddball task [26] also found a greater alpha suppression for the odd target stimuli that was interpreted as a higher mental effort to detect infrequent targets. Hence, in our study, the higher alpha suppression under tramadol condition may be interpreted as the result of participants allocating more attentional resources than in the placebo condition (which enabled them to achieve similar behavioural performance). This higher mental effort may have affected the cyclists' physical performance during the time trial, which might explain the divergent results between Experiment 1 and 2 [27] in terms of power output. Hence, while the cognitive load induced by the oddball task might have interacted with substance intake, modulating the effect of tramadol on physical performance.

Due to the analgesics properties of tramadol, it might have been expected that EEG amplitude was greater in alpha and beta bands, since a decrement in attention has been reported after the overall increase in these bands [28]. However, our EEG results present conflicting findings, as opposite results were found in Experiment 1 and 2. In Experiment 1, we found a higher spectral (tonic) power in the tramadol condition (at baseline-pre and warm-up), while in Experiment 2, the power spectral was higher in the placebo condition (at baseline-pre, post, warm-up and cool-down). Indeed, the effect of opioids at the EEG level is not clear in the previous literature as some have reported an increase in these frequencies band [29], whilst others have found the opposite effect [6].

Conclusion

The results of Experiment 1 showed that tramadol improves performance in a 20-min cycling time-trial, although the failed replication in Experiment 2 points to an influence of a concurrent cognitive task on the potential manifestation of the tramadol effect at the physical performance level. Tramadol does not seem to impair (behavioural) cognitive performance in the ability to maintain attention during exercise, although it may influence information processing as highlighted by EEG time-frequency data. It appears then that the presence of tramadol on the WADA's monitoring program seems reasonable as far as performance enhancement is concerned. Even though the present findings have to be considered with caution (as this is the first empirical approach to this issue), they open interesting venues for future research on this relevant topic.

Practical applications

Tramadol may improve cycling time-trial performance.

Tramadol influences information processing related to sustained attention at the brain level, although it was not translated into an impaired behavioural performance.

Anti-doping authorities may reconsider tramadol's status

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Table and figures captions

Fig 3 Power output (watts) profile for Experiment 1 (A) and 2 (B). * $p < 0.05$

Fig 4 A Event-related spectral perturbations time-locked at Target 1 of the oddball sustained attention task. Grand averages are illustrated separately for each condition (tramadol, placebo). The enclosed areas denote significant clusters of channels and time with $p < 0.025$**. B** Main effect in event-related alpha frequency perturbations time-locked at target 1 of the oddball sustained attention task. Left panel: Non-Parametric paired t-test colormap comparing the relative power for the alpha frequency band across time (x-axis) and channels (y-axis); right panel: grand average spectral power curves showing the main effect between condition (tramadol, placebo) in the alpha frequency band. The "x" marks in the topographical map highlight the 15 electrode sites included in the significant cluster. Three electrode sites are not represented as they were present for less than 25% of the total duration of the cluster. The grey region denotes the latency range 118-224 ms) of the significant main effect between conditions. Red and blue shaded areas represent 95% confidence intervals. **C** Main effect of condition in event-related lower-beta frequency perturbations time-locked at target 1 of the oddball sustained attention task. Right panel shows grand average spectral power curves indicating the main effect of condition at the lower-beta frequency band. The topography depicts t-test distribution across surface localization, showing the 24 electrode sites included in the significant cluster. The grey region denotes the latency range (136-230 ms) of the significant main effect between conditions.

Supplementary materials

Table 1 Power spectral values (10^{+}log10 (V2/Hz)) for tramadol and placebo in Experiment 1 and 2

Fig 1 Experimental session's schematic protocol

Fig 2 Schematic representation of the oddball sustained attention task