Title:

Novel evidence on the effect of tramadol on self-paced high-intensity cycling

Authors:

Thomas Zandonai[a], Darías Holgado[a], Luis F. Ciria[a], Mikel Zabala[b], James Hopker[c], Tristán Bekinschtein[d], & Daniel Sanabria*[a]

[a] Mind, Brain and Behaviour Research Center, Department of Experimental Psychology, Faculty of Psychology, University of Granada, Campus de Cartuja s/n, 18071, Granada, Spain

[b] Department of Physical Education & Sport, University of Granada, Carretera de Alfacar s/n, 18071 Granada, Spain

[c] Endurance Research Group, School of Sport and Exercise Sciences, University of Kent, Medway ME4 4AG, UK

[d] Department of Psychology, University of Cambridge, Cambridge CB2 3EB, UK

Corresponding author:

* Daniel Sanabria

Mind, Brain and Behaviour Research Center, Department of Experimental Psychology, Faculty of Psychology, University of Granada, Campus de Cartuja s/n, 18071, Granada, Spain.

daniel@ugr.es

Authors' ORCID and Twitter

Thomas Zandonai (0000-0002-7606-9675), @thomaszando

Darías Holgado (0000-0003-3211-8006), @DariasHolgado

Luis F. Ciria (0000-0001-7067-5060), @Luis_Ciria

Mikel Zabala (0000-0002-8700-0382), @ZabalaMikel

James Hopker (0000-0002-4786-7037), @JamesHopker

Tristán Bekinschtein (0000-0001-5501-8628), @TrikBek

Daniel Sanabria (0000-0002-4164-7607), @SanabriaLucenaD

Abstract

The use of tramadol is a controversial topic in cycling. In order to provide novel evidence on this issue, we tested 29 participants in a pre-loaded cycling time trial (TT; a 20-min TT preceded by 40-min of constant work-rate at 60% of the VO_{2max}) after ingesting 100 mg of tramadol (vs placebo and paracetamol (1.5 g)). Participants performed the Psychomotor Vigilance Task (PVT) at rest and a Sustained Attention to Response Task (SART) during the 60 min of exercise. Oscillatory electroencephalography (EEG) activity was measured throughout the exercise. The results showed higher mean power output during the 20-min TT in the tramadol vs. paracetamol condition, but no reliable difference was reported between tramadol and placebo (nor paracetamol vs. placebo). Tramadol resulted in faster responses in the PVT and higher heart rate during exercise. The main effect of substance was reliable in the SART during the 40-min constant workload (no during the 20-min TT), with slower reaction time, but better accuracy for tramadol and paracetamol than for placebo. This study supports the increased behavioural and neural efficiency at rest for tramadol but not the proposed ergogenic or cognitive (harmful) effect of tramadol (vs. placebo) during self-paced high intensity cycling.

Keywords:

Analgesics; Opioids; Paracetamol; Sport performance; Sustained attention; Painkillers

Introduction

The debate about the use of tramadol in cycling has pervaded the sport's environment[1]. Athletes have been shown to take tramadol and other analgesics in an attempt to have relief from the pain and fatigue that are typical components of an endurance sport like cycling[2]. Indeed, there is a wealth of literature on the effectiveness of tramadol in therapy for musculoskeletal pain, its efficacy, safety, and tolerability[3–5]. The mechanism of action of tramadol is two-fold, as a m-opioid receptor agonist, and as a serotonin and norepinephrine reuptake inhibitor, enhancing inhibitory effects on pain transmission in the spinal cord[3,5]. In addition to the potential ergogenic effect due to its analgesic and stimulant properties, concerns have been raised in regard to side-effects like dizziness and somnolence[6] that could increase the likelihood of attentional lapses (impaired sustained attention) compromising the safety of the cycling peloton[7]. These issues led the WADA to include tramadol in its monitoring program of doping substances since 2012[8]. The Union Cycliste Internationale (UCI) has taken an even more extreme position, banning tramadol in competition from the 1st of March 2019[7]. However, these concerns are not supported by solid empirical evidence about the ergogenic, or potentially harmful (cognitive), effects of this substance.

To the best of our knowledge, only three randomized controlled trials (RCT) have investigated the potential ergogenic effect of tramadol on cycling performance[9,10]. The first RCT conducted on this matter[9] showed a ~5% performance (power output) improvement in a 20-min indoor cycling time trial (TT), a result that was not replicated in a further experiment reported in the same manuscript, nor in a more recent study by Bejder et al.[10] (who tested participants in a 15km TT preceded by 1h constant work-rate at 60% of peak power). Crucially, neither Holgado et al.[9] (Experiment 2) nor Bejder et al.[10] found any effect of tramadol at the cognitive (attention) level. However, Holgado et al.[9] (Experiment 2) did show reliable differences between tramadol and placebo conditions in event-related electroencephalographic (EEG) oscillatory activity (from the attentional task performed during the cycling TT) that hinted at a possible attentional effect of tramadol.

The scarce and mixed evidence described above motivated the present research, which aims to test the hypothesis that tramadol improves cycling (physical) performance at the expense of the ability to stay focused (indexed by both behavioural and EEG measures). Together with placebo, we included paracetamol as a further control condition. Paracetamol is another legal mild analgesic, popular among athletes[11], and previously shown to elicit ergogenic effects in cycling[11,12] (although as with tramadol, the evidence is still weak). The exact mechanism by which paracetamol achieves its pain-relieving effect is unclear, although research has suggested it may be due to the inhibition of the cyclooxygenase enzymes, potentiation of descending serotoninergic pathways, and modulation of opioid and cannabinoid CB1 receptors[13]. The dose of tramadol, paracetamol and a placebo were ingested prior to a pre-loaded TT, i.e., 40-min constant work-rate at 60% of peak power output followed by 20-min indoor TT. The purpose of the 40-min constant work-rate was to induce fatigue, maximizing the effect of the analgesics during the 20-min TT (see Bejder et al.,[10] for a similar procedure), an useful test for assessing performance in trained cyclists[14].

Materials and Methods

Study Design

The study was a randomized, double blind and placebo-controlled trial. All experimental procedures were designed to comply with the Declaration of Helsinki and Good Clinical Practice (GCP). The Spanish Agency of Medicines and Medical Devices (AEMPS) -EudraCT number 2018-000388-10-, and the Ethical Committee of Clinical Research of University of Granada approved the trial. The randomization process, the audit and verification of compliance of GCP rules was performed by Foundation for the Biosanitary Research of Eastern Andalusia (FIBAO) in collaboration with Adknoma Health Research S.L. company. The method and planned analyses of this study were pre-registered on the Open Science Framework (April 25, 2018 update January 01, 2020: https://osf.io/2f4vq/). All data were entered in a case report form and subsequently in a computerized and scripted database, stored at the Mind, Brain and Behaviour Research Center (CIMCYC, University of Granada).

110

111 ***Participants***

112 The calculation of the sample size was based on an expected medium effect size (η_p^2 =
113 0.16). An a priori power analysis (using G* Power Version 3.1)[15] recommended testing 28
114 participants to detect that effect with a statistical power of 0.8. We decided to test 30
115 participants to increase the statistical power and to account for possible drop out. Therefore,
116 we recruited 30 moderately trained male participants who were enrolled by local
117 advertisements. They were cyclists and triathletes with an age ranging from 18 to 40 years.
118 Exclusion criteria were the presence of symptomatic cardiopathy, metabolic disorders such as
119 obesity (BMI >30) or diabetes, chronic obstructive pulmonary disease, epilepsy, therapy with
120 ß-blockers and medications that would alter cardiovascular function, hormonal therapy and
121 smoking[16]. Moreover, the existence of allergy to tramadol and paracetamol or any excipients
122 was considered. Participants were excluded from recruitment if they reported high levels of
123 regular alcohol consumption, or use of recreational drugs (e.g., heroin, cocaine, etc.) for at
124 least one year.

125 One participant could not complete the study due to nausea, vomiting and dizziness after
126 tramadol ingestion (approximately 130 min after Time 0). The final sample included 29
127 participants. The participants' characteristics are displayed in Table 1.

128

129 ***Procedures***

130 Each participant visited the CIMCYC in four separate occasions. The first visit was
131 dedicated to a maximal incremental test and familiarization with cognitive task and the 20-min
132 TT. During the second, third, and fourth visits, a dose of tramadol and placebo, paracetamol
133 and placebo, or two doses of placebo were administered to participants before starting the
134 cycling exercise according to the randomization. No less than three days were allowed
135 between experimental sessions to allow time for washout[17] and all sessions were carried out
136 within two weeks.

137 During the first visit, all participants read and signed an informed consent form. Then,

138 descriptive anthropometric parameters of weight, height and body mass index, as well as
139 information about cycling experience (i.e., years of practice, competition, etc.) were obtained
140 from each participant. Participants then undertook a maximal incremental exercise test to
141 exhaustion.

142 The participants completed a 5 min warm-up at 90 Watts (W) on a cycle ergometer using
143 their preferred cadence (within the range of 60 – 90 pedal revolutions per minute). They were
144 asked to maintain this cadence throughout the rest of the protocol. The incremental exercise
145 test started at 100 W and then increased at a rate of 30 W min^{-1} until volitional exhaustion (or
146 when cadence fell > 10 rpm below the self-selected rate). Heart Rate (HR) and cycling
147 resistance (W) were continuously monitored, and expiratory ventilation (VE), oxygen (O_2)
148 consumption rate (VO_2), rate of CO_2 production (VCO_2), and respiratory exchange ratio (RER)
149 we recorded on a breath-by-breath basis. Participants were verbally encouraged throughout
150 to achieve their maximal performance. The test was considered maximal if one of the following
151 criteria was met: 1) final HR within 10% of predicted maximum (220-age); 2) a clear plateau
152 in oxygen uptake noticed; or 3) respiratory exchange ratio equal to, or above, 1.1[16].

153 Before leaving the laboratory, participants read a page with standardized written
154 instructions in order to familiarize with the 6-20 Borg scale[18].

155 At least 48h after the maximal incremental test, participants visited the laboratory for the
156 second session. Participants abstained from physical activity, alcohol and caffeine 24h before
157 the test. The same pre-exercise meal was kept before starting the experimental sessions.
158 Upon arrival, they completed a 5 min version of the Psychomotor Vigilance Task (PVT; see
159 details below). Immediately after, a single dose of oral tramadol or placebo (depending on the
160 randomization) was administered to participants (Time 0). Then, they rested in the laboratory.
161 After 90 min from Time 0, the participants ingested a single dose of paracetamol or placebo
162 (see Fig. 1, black columns; Time 90). The administration time was based on previous empirical
163 evidence[19–21] documenting the time-course plasma paracetamol concentration in order to
164 maximize its effect. As noted above, including a placebo dose at Time 90 in the tramadol and
165 placebo experimental sessions ensured that we controlled for the number of capsules

ingested by the participants, crucial to maintain the double-blind procedure. Once participants ingested the substances, they were prepared for EEG measurement in a dimly-illuminated, sound-attenuated Faraday cage. After 105 min from Time 0 participants performed a second 5 min PVT task. In order to record the resting EEG activity, participants were then encouraged to stay as relaxed as possible during 5 min with their eyes open. Next, participants warmed-up for 5 minutes on the cycle ergometer prior to performing a 40-min constant work-rate at 60% of their VO_{2max} (commenced 120 minutes after Time 0). During the constant work-rate bout, participants were required to simultaneously perform a cognitive task (SART, see details below). At the end of the 40 min exercise, participants were asked to provide a rating of their perceived exertion (RPE) using the 6-20 Borg scale[18].

Immediately after the submaximal cycling trial, participants performed a 20-min cycling TT in which they were asked to achieve the highest average power output possible. Participants continued responding to the SART task during the 20-min TT. Immediately following the 20-min TT participants were again asked to provide a rating of their perceived exertion using the Borg RPE scale[18]. At the end of the experimental visit, and after 24h, participants were contacted to ask about any adverse events (if yes: mild / moderate / serious).

The procedures for visits 3 and 4 were similar to that in visit 2 (each athlete began the test at visits 3 and 4 as the same time as in visit 2), except that participants ingested the other substances or a placebo, depending on the randomization.

Materials

An SRM indoor cycle ergometer (Jülich, Germany) was used for all cycling trials. A RS800CX Polar monitor (Polar Electro, Finland) was used to monitor and record (via a sensor band attached to the participants' chest) Heart Rate (HR) of the participants during the experiments. A Jaeger Master Screen gas analyzer (CareFusion GmbH, Germany) was used to collect gaseous exchange data during the maximal incremental test. A computer and the Psychtoolbox were used to control stimulus presentation, response collection, and to generate and send triggers indicating the onset of each period. Behavioural and EEG data pre-

processing, and analysis were conducted using a combination of custom Matlab scripts (Matlab 2014a, Mathworks Inc.), and the EEGLAB[23] and Fieldtrip[24] Matlab's toolboxes.

Tramadol and paracetamol doses

In this clinical trial, we administered a 100 mg oral dose of tramadol. According to an exhaustive review by Grond and Sablotzki[3] tramadol is rapidly absorbed with a bioavailability of about 70% after single doses and it is eliminated with a half-life of about 5.6 h[3,25]. Importantly, Bastami et al.[26] identified good tolerability to doses of 100 mg of tramadol, showing a mean time to maximum plasma concentration of 156 min (range: 87–208 min). In our previous study[9], we confirmed the same tolerability to adverse events.

Paracetamol is metabolized mainly in the liver via glucuronidation (50-60%), sulfation (25-30%) and oxidation (< 10%)[13]. This non-opioid analgesic has an excellent tolerance, for therapeutic doses and is a major reason for its recommendation and widespread approbation as an analgesic[27]. In this study participants took a capsule containing 1.5 g of paracetamol. This dose was based on previous empirical evidence on plasma paracetamol concentration to maximize the effect[27–29].

All oral doses were prepared at the Hospital "Virgen de las Nieves" pharmacology department (Granada, Spain). The doses were made following the good manufacturing practice (GMP) audit and approved by Spanish authorities (i.e., AEMPS). Only the pharmacist knew the content of the randomization list. Each capsule was packed in a monodose blister with the patient code and visit number on the information label. The placebo dose was composed of microcrystalline cellulose.

Cognitive tasks

Psychomotor Vigilance Task (PVT)

We used a modified version of the PVT proposed by Wilkinson and Houghton[30]. This task was developed to measure sustained attention by recording participants' reaction time (RT) to visual stimuli that occur at random inter-stimulus intervals. Each trial began with the

presentation of a blank screen in a black background for 2000 ms and subsequently, an empty red circle (i.e., cue stimulus, 6.68° Å~ 7.82° of visual angle at a viewing distance of 60 cm) appeared in a black background. Following a random time interval (between 2000 and 10000 ms), the circle was also filled with a red colour (i.e., target stimulus). The instruction given to participants was to respond as fast as they could, once they had detected the presentation of filled red circle, which was presented for 500 ms with a maximum time to respond of 1500 ms. RTs <100 ms were considered anticipations and we discarded from the analysis. Participants had to press the space bar on the keyboard with their dominant hand. The task involved a single block of 5 minutes.

Sustained Attention to Response Task (SART)

We used a modified version of the SART as documented by Robertson et al[31]. The task consisted of a sequential presentation of numbers ranging between 1 and 9. Participants were instructed to respond by pressing a button connected to the cycle-ergometer handlebar with the thumb of their dominant hand as quickly as possible upon the presentation of each number (Go trials), except for the number "3", which they had to ignore (NoGo trials). Stimuli appeared in white colour over a black background at the centre of the computer screen in one of five possible font sizes (48, 72, 94, 100 and 120 points, *Times New Roman*). Each trial started with the presentation of a white cross on a black background for 800 ms. Stimuli were presented at a random time interval (between 0 and 100 ms) for 150 ms. Participants had a 1100 ms time-window to respond to the stimuli. Stimuli were distributed in a quasi-random fashion to avoid the presentation of two consecutive NoGo trials. Participants completed the task during both the 40-min constant work-rate test and the 20-min TT. The data set was then divided in blocks of 10 min for analytical purposes to study the potential effect of time-on-task (induced fatigue), and the interaction with the substances. Participants were familiarized with the task during the first laboratory visit.

EEG recording analysis

Continuous EEG data were recorded at 1000 Hz using a 30-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 the participant's 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 the screen during the exercise. Electrode impedances were kept below 10 kΩ throughout the recording. To ensure an acceptable signal-to-noise ratio and to reduce the type I error rate possibility by *post hoc* exclusion of participants, we set an *a priori* criteria of 75% of artefact-free trials per subject and substance[32,33]. EEG data were resampled at 500 Hz, bandpass filtered offline from 1 and 40 Hz to remove signal drifts and line noise and to a common average reference. Horizontal electrooculograms were recorded by bipolar external electrodes for the offline detection of ocular artefacts. Independent component analysis was used to confirm and remove EEG components reflecting blinks and other eye movements[34]. Electrodes presenting abnormal power spectrum were identified via visual inspection and replaced by spherical interpolation.

Spectral power analysis

Pre-processed EEG data from each experimental period (baseline, warm-up, 40-min constant work-rate test, 20-min TT) were segmented into 1-s epochs. The spectral decomposition of each epoch was computed using Fast Fourier Transformation (FFT) applying a symmetric Hamming window (0.5 s). The obtained power values were averaged across experimental periods.

Time-frequency analysis

Task-evoked spectral EEG activity was assessed by computing event-related spectral perturbations in epochs extending from −100 ms to 300 ms time-locked to stimulus onset for frequencies between 4 Hz and 40 Hz. Spectral decomposition was performed using sinusoidal

wavelets with three cycles at the lowest frequency and increasing by a factor of 0.8 with increasing frequency. Power values were normalized with respect to a −300 ms to 0 ms pre-stimulus baseline and transformed into the decibel scale (10*log10 of the signal).

Statistical analysis

Baseline-corrected (Post–Pre/Post+Pre) RT data from the PVT were analyzed using a within-participants' ANOVA with the factor of substance (tramadol, paracetamol, placebo). The RT for Go trials on the SART, and false alarms (errors) for the NoGo trials were analyzed by a within-subjects ANOVA with the factors of substance (tramadol, paracetamol, placebo) and block (x 4 for the 40 min constant intensity exercise period and x 2 for the 20 min TT period).

Exercise performance data (power output and HR) were analyzed using a within-participants' ANOVA with the factors of substance (tramadol, paracetamol, placebo) and time-on-task (x 4 blocks of 10 min in the case of the 40 min constant intensity exercise period and x 2 blocks of 10 min for the 20 min TT period). A one-way within-subjects ANOVA was used to analyze the RPE data. ANOVAs were followed up by *post hoc* pairwise comparisons with Holm-Bonferroni.

A stepwise, cluster-based, non-parametric permutation test approach[35] without prior assumptions on any frequency range or brain area of interest, was used to examine the spectral power differences between substances (tramadol, paracetamol, placebo), separately at each period (baseline, warm-up, 40-min constant work-rate test and 20-min TT). We performed a *t*-test for dependent samples on all individual electrodes and frequency pairs (30 channels, 40 frequencies), clustering samples with *t*-values that exceeded a threshold ($p < 0.025$) based on spatial and spectral adjacency. This procedure was repeated 5,000 times to estimate the distribution of maximal cluster-level statistics obtained by chance. The proportion of random partitions that resulted in a larger test statistic than the original determined the two-tailed Monte Carlo p value (see Holgado et al.,[36] for a similar approach).

Event-related spectral perturbation main differences of substance (tramadol, paracetamol,

placebo) for each stimulus of the SART (Go, NoGo) were also analyzed 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 an *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 limited the time windows of interest to the first 300 ms after the stimuli onset (based on average behavioral response times) for Go trials.

The raw physical performance, EEG and behavioural data, as well as Matlab custom scripts are available at the OSF repository: https://osf.io/2f4vq/

Results

Modified PVT task

The analysis of the baseline-corrected RT data for the modified PVT revealed a main effect of substance, F (2,56) = 5.76, p = 0.005, η_p^2 = 0.17 [0.03 - 0.29]. *Post-hoc* comparisons showed that participants were faster in the tramadol condition: -0.003 95% CI [-0.0154 – 0.0097] in comparison to paracetamol: 0.013 95% CI [0.0051 – 0.0219], t(2) = 2.78, p = 0.026, Cohen's d = 0.51 [0.19 – 1.25]; and placebo: 0.017 95% CI [0.0100 – 0.0255] ms); t(2) = 2.82, p = 0.026, Cohen's d = 0.52 [0.20 – 1.27] (see Table 2).

Physical performance

The analysis of the average power output during the 20-min TT revealed a main effect of substance, F (2, 56) = 4.408, p = 0.017, η_p^2 = 0.13 [0.01 - 0.25] (see Fig. 2A). *Post-hoc* comparisons only revealed a reliable difference between tramadol (227 W, 95% CI [215.6 – 238.1]) and paracetamol (213 W 95% CI [99.4 – 227.3]), t (2) = 3.753, p =.002, Cohen's d = 0.69 [0.43 – 1.52]). Crucially, neither the difference between tramadol and placebo (221 W 95% CI [207.6 – 233.7]), $t(2)$ = 1.242, p =0.3, Cohen's d = 0.23 [-0.19 – 0.84] nor that between placebo and paracetamol were reliable (t(2) = 1.48, p = 0.3, Cohen's d = 0.27 [-0.13 – 0.9).

Neither the main effect of block: F (1, 28) = 2.02, p = 0.16, η_p^2 = 0.06 [0 – 0.23] nor the interaction between substance and block F (2, 56) = 2.71, p = 0.07, η_p^2 = 0.08 [0 – 0.19] reached statistical significance (see Fig. 2B).

Heart rate

The HR values collected during the 40-min constant work-rate test period evidence of a main effect of substance F (2,56) = 7.636), p = 0.001, η_p^2 = 0.21 [0.06 – 0.34]. *Post-hoc* comparisons revealed higher HR for tramadol (144 bpm, 95% CI [140 – 149]) than for paracetamol (139 bpm, 95% CI [135 – 135], t(2)= 3.65, p = 0.003, Cohen's d = 0.67 [0.41 – 1.49]) and placebo (139 bpm, 95% CI [134 – 144], t(2) = 3.06, p = 0.01, Cohen's d = 0.56 [0.26 – 1.35). A main effect of Block, F (3,84) = 38.139), p < 0.001, η_p^2 = 0.57 [0.44 – 0.64] was also found. HR was higher in blocks 2 t(3)= 8.68, p < 0.001, Cohen's d = 1.61 [1.60 – 2.29], 3 t(3) = 7.26, p < 0.001, Cohen's d = 1.35 [1.27 – 2.52] and 4 t(3)= 7.41, p < 0.001, Cohen's d = 1.37 [1.31 – 2.56] compared with block 1, and in block 4 compared with block 2; t(1)= 3.61, p = 0.007, Cohen's d = 0.62 [0.40 – 1.48]. Nonetheless, the interaction between substance and block was again not reliable F (6,168) = 1.47), p = 0.19, η_p^2 = 0.05 [0 - 0.07].

During the 20-min TT, HR values showed a main effect of substance, F (2,56) = 6.160, p = 0.004, η_p^2 = 0.18 [0.03 – 0.3]. *Post-hoc* comparisons yielded significant differences between tramadol and placebo (*t(2)* = -2.681; p = 0.024, Cohen's d = -0.49 [-1.23 - -0.16]) and between tramadol and paracetamol (t(2) = -3.809; p = 0.002, Cohen's d = -0.70 [-1.54 - -0.44]). Participants had higher HR values in the tramadol condition [162 bpm 95% CI (156.8 – 167.2)] than in the paracetamol [153 bpm 95%CI (146.2 – 159.4)] and placebo conditions [154 bpm 95% CI (146.4 – 161)]. There was also a main effect for block, F (1,28) = 25.817, p < 0.001, η_p^2 = 0.48 [0.23 – 062], with HR being higher in the second block: 158 95% CI (153.35 – 164.24 than in the first block: 153 95% CI (147.8 – 159.0) *t(1)* = -5.081; p = 0.001, Cohen's d = -0.94 [-1.91 - -0.75]). The interaction between substance and block was not reliable , F (2,56) = 2.45, p = 0.09, η_p^2 = 0.08 [0 – 0.18].

Subjective scales

The analysis of rating of perceived exertion showed reliable differences between the three substances after the 40-min constant work-rate, F (2, 56) = 6.96, p = 0.002, η_p^2 = 0.19 [0.05 – 0.32]. *Post-hoc* comparisons yielded reliable differences between tramadol and placebo *t(2)* = 3.35; p = 0.007, Cohen's d = 0.62 [0.33 – 1.41]) and between tramadol and paracetamol (t(2) = 3.05; p = 0.01, Cohen's d = 0.56 [0.26 – 1.33]). RPE values were lower in the tramadol condition [13, 95%CI (12.7 – 14.1)], than in the placebo condition [14, 95%CI (13.8 – 15.36)] and paracetamol condition [14, 95%CI (13.6 – 15.3)]. However, there were not any reliable differences in RPE between conditions for the 20-min TT, F (2, 56) = 0.85, p = 0.43, η_p^2 = 0.03 [0 – 0.1].

Sustained Attention to Response Task (SART)

The analysis of the false alarms (NoGo trials) in the SART for the 40-min constant work-rate test revealed a main effect of substance, F (2,50) = 4.25, p = 0.02, η_p^2 = 0.14 [0.13 - 0.27]. There were more false alarms in the placebo condition (0.57 95% CI (0.41 - 0.62) than in paracetamol (0.43 95% CI (0.33 - 0.54) and tramadol (0.45 95% CI (.34 - 56), although *post-hoc* comparisons did not yield reliable differences between substances t(2)= 2.42, p = 0.06, Cohen's d = 0.47 [0.11 – 1.25] and t(2) < 0.77, p = 0.44, Cohen's d = 0.15 [-0.53 – 0.57] respectively. Additionally, there was a main effect of block F (3,75) = 12.8, p < 0.001, η_p^2 = 0.33 [0.17 – 0.44]. *Post-hoc* comparisons showed that participants committed less false alarms in the first 10 minutes in comparison with 20 (t(3) = 3.39, p = 0.009, Cohen's d = 0.66 [0.36 – 1.54]), 30 (t(3) = 3.82, p = 0.004, Cohen's d = 0.75 [0.48 – 1.67]) and 40 minutes (t(3) = 4.72, p < 0.001, Cohen's d = 0.92 [0.71 – 1.94]). The interaction between substance and block was not reliable (F < 1).

The analysis of the RT to Go trials for the 40-min constant work-rate test revealed a main effect of substance, F (2,50) = 4.67, p = 0.01, η_p^2 = 0.15 [0.01 – 0.28]. Participants were faster in the placebo condition: 321 95% CI (296 - 347) ms; compared with the paracetamol: 354 95% CI (314 - 395); and tramadol: 342 95% CI (302 - 381) ms, although *post-hoc* comparisons

did not yield reliable differences between substances. t(2) = 2.53, p = 0.054, Cohen's d = 0.49 [0.13 – 1.28] for placebo vs. paracetamol and t(2) = 1.89, p = 0.14, Cohen's d = 0.37 [-0.03 – 1.09] for placebo vs. tramadol. Additionally, there was a main effect of block F (3,75) = 4.01, p = 0.01, η_p^2 = 0.13 [0.01 – 0.23]. *Post-hoc* comparisons showed faster RTs in the last 10 minutes compared with the first 10 (t(3) = 4.45, p = 0.02, Cohen's d = 0.6 [0.64 – 1.86]. The interaction between substance and block was not reliable F (6,1250) = 1.35, p = 0.23, η_p^2 = 0.05 [0.01 – 0.23].

The analysis of the false alarms (NoGo) in the SART for the 20-min TT did not show a reliable main effect of substance or block (F < 1), or interaction between substance and block F (2,48) = 1.81 p = 0.17, η_p^2 = 0.07 [0 – 0.18]. Similarly, there was no effect of substance F (2,48) = 1.89, p = 0.16, η_p^2 = 0.07 [0 – 0.18] or block F (1,24) = 2.11, p = 0.15, η_p^2 = 0.08 [0 – 0.27] or interaction between substance and block F (2,48) =2.49, p = 0.09, η_p^2 = 0.09 [0 – 0.21 for the RT (to Go trials).

EEG data

Spectral power analysis

The analysis of tonic spectral power revealed reliable differences between substances (p < 0.001, η_p^2 = 0.81 [0.71 – 0.90) for the baseline period, in the frequency range of 21-40 Hz (23 electrodes), showing more power for tramadol than for placebo and paracetamol. The tonic spectral power analysis of the other periods (i.e., warm-up, 40-min constant work-rate test or the 20-min TT) yielded no reliable differences.

Time-frequency analysis

The time frequency analysis during the SART did not reveal any reliable differences between substances (tramadol, paracetamol, placebo) for any of the stimuli (Go, NoGo), either in the 40-min constant work-rate test or the 20-min TT (all clusters p ≥ 0.05; see Fig. 4).

Adverse events

Three participants reported adverse symptoms (nausea, dizziness and vomiting) at the end of the tramadol experimental session. All manifested symptoms were moderate and disappeared within the next 24 hours.

Discussion

Tramadol has long been in the spotlight of the doping controversy in cycling. The current study aimed to test the potential ergogenic and cognitive (harmful) effects of this substance compared with placebo and paracetamol conditions. The main findings of the study suggests that 100 mg of tramadol did not induce changes in physical performance during a 20-min TT after 40 min of cycling exercise at 60% of VO_{2max}. This result is consistent with that of Holgado et al.'s[9] Experiment 2 and Bejder et al.[10] but at odds with the findings of Holgado et al.'s[9] Experiment 1. These failed replications could be suggestive of a false positive from Holgado et al.'s[9] Experiment 1, or be due to the inclusion of a cognitive task during the TT both in Holgado et al.'s[9] Experiment 2, and in the present study that might have somehow reduced the effect of tramadol. Nevertheless, Bejder et al.[10] did not include a cognitive task during their 15 km TT and still failed to report an effect of tramadol on physical (and cognitive) performance. Apart from the presence or not of a cognitive task during the cycling effort, the other potentially relevant difference between studies was the inclusion of female participants in Holgado et al.'s[9] Experiment 1 (other factors like the nutrition status, time of test day and exercise demands -time trial- were similar in the studies conducted in our laboratory; note that Bedjer et al.[10] also used a TT as the exercise test). However, the data analyses of that experiment revealed that the effect of tramadol did not depend on participants' gender (p = 0.83[9]), hence it would seem unlikely that this factor could explain the presence of the effect in Holgado et al.'s Experiment 1 in contrast to the other three studies.

Tramadol did, however, exert an effect on physiological responses recorded during exercise. Similar to Bejder et al.'s study[10] (4 bpm in the TT), tramadol induced higher HR than both placebo and paracetamol during the 40 min at 60% of VO_{2max} and the 20-min TT. A

445 reliable difference between tramadol and placebo was also found in Holgado et al.'s[9]
446 Experiment 1 (4 bpm). This outcome could be accounted for by tramadol's action as both a
447 serotonin and norepinephrine reuptake inhibitor, which can lead to cardiac effects[37,38].
448 However, the 8 bpm difference reported in the present study could be negligible in practical
449 terms, as it was not followed by changes in performance. In addition, the lack of a reliable
450 difference in Holgado et al.'s[9] Experiment 2 hinders any explanation of the tramadol effect on
451 HR.

452 RPE was also higher in the tramadol condition, but only during the 40-min constant work-
453 rate task. Whatever the explanation for the HR and RPE results, they were not followed by a
454 change in physical performance in the TT. Indeed, differences were reported only between
455 tramadol and paracetamol conditions (227 vs 213 W, respectively; p =.002), with paracetamol
456 showing even lower values than placebo, in contrast to previous studies [28,39,40], although that
457 difference was not statistically reliable (213 vs 221 W, respectively; p = 0.3).

458 At the cognitive level, our results suggest that tramadol did not impair the ability to stay
459 focused during a high-intensity effort. Nevertheless, the accuracy and RT results yielded a
460 statistically reliable effect of substance during the 40-min constant work-rate, although the lack
461 of reliable pairwise comparisons between the three substances hinders any explanation. In
462 any case, the reduced number of false alarms and larger RTs in the tramadol condition (vs.
463 placebo) could be interpreted as a sign of enhanced cognitive control, i.e., better ability to
464 inhibit undesired responses at the expense of being slower[41]. Moreover, tramadol induced the
465 best PVT (baseline-corrected) performance at rest, and no substance effects were shown in
466 the SART during the 20-min TT. These results, together with the overall increase of oscillatory
467 brain activity after substance intake and prior to exercise, do not seem to support the notion
468 that tramadol impairs the ability to stay focused. Instead, these effects at baseline could be
469 due to the stimulant effect of the substance[5].

470 The absence of evidence is not evidence of the absence of an effect, and therefore our
471 null findings could be accounted for by various factors (apart from the obvious lack of a true
472 effect) including: i) 100 mg of tramadol might have not been enough to exert any effect in

performance (compared with placebo). Moreover, as with other previous research, the dose was not individualized (e.g., as a function of body weight), which might have included between-participants variability because of a (potential) dose-response dependency of the tramadol effects on physical and cognitive performance; ii) all studies to date have only tested the effects of an acute dose of tramadol during exercise. However, the question remains as to whether a multi-day administration of tramadol (vs. placebo) might effectively induce ergogenic and (potential harmful) cognitive effects; iii) related to this, tramadol could provide a further benefit after days of prolonged and intense physical workloads as encountered during a multi-stage cycling tour; iv) tramadol induces a "true" but fairly small effect and so all studies on this matter to date could have been underpowered to detect it.

The present results suggest that tramadol does not have any ergogenic effect or impair the ability to stay focused during a maximal cycling TT effort. Why do pro and amateur cyclists appear to be taking it to improve performance then? A true effect under any (or more than one) of the circumstances discussed in the paragraph above and/or a most than likely placebo effect (see Kayser, 2020, for discussion on this issue)[42] could certainly explain the use (and potential abuse) of this substance. Given the relevance of this matter to sports in general, and cycling in particular, the typical final "further research is needed" clause in scientific papers seems more than appropriate here.

Acknowledgements

This project has been carried out with the support of the World Anti-doping Agency (WADA) (REF: 17C09DL).

Competing interest

The authors declare that they have no competing interests.

References

1. USADA. United State Anti-doping. Tramadol: why some athletes and anti-doping experts want it banned. 2017. Available at: https://www.usada.org/spirit-of-sport/education/tramadol-why-some-athletes-and-anti- doping-experts-want-it-banned/ Accessed 20 June 2020.
2. Holgado D, Hopker J, Sanabria D et al. Analgesics and sport ferformance: beyond the pain-modulating effects. *PM&R*. 2018; 10(1):72-82.
3. Grond S, Sablotzki A. Clinical pharmacology of tramadol. *Clin Pharmacokinet* 2004; 43:879-923.
4. Mattia C, Coluzzi F. Tramadol. Focus on musculoskeletal and neuropathic pain. *Minerva Anestesiol* 2005; 71:565-584.
5. Miotto K, Cho AK, Khalil MA et al. Trends in tramadol: pharmacology, metabolism, and misuse. *Anesth Analg* 2017; 124:44-51.
6. Vazzana M, Andreani T, Fangueiro J et al. Tramadol hydrochloride: Pharmacokinetics, pharmacodynamics, adverse side effects, co-administration of drugs and new drug delivery systems. *Biomed Pharmacother* 2015; 70:234-238.
7. The Union Cycliste Internationale. Tramadol ban: all you need to know. 2019. Available at: https://www.uci.org/inside-uci/press-releases/tramadol-ban-all-you-need-to-know. Accessed 20 June 2020.
8. The World Anti-Doping Agency. The 2012 Monitoring Program. 2012. Available at: https://www.wada-ama.org/sites/default/files/resources/files/WADA_Monitoring_Program_2012_EN.pdf Accessed 20 June 2020.
9. Holgado D, Zandonai T, Zabala M et al. Tramadol effects on physical performance and sustained attention during a 20-min indoor cycling time-trial: a randomised controlled trial. *J Sci Med Sport* 2018; 21:654–660.
10. Bejder J, Andersen AB, Bonne TC et al. Tramadol does not improve performance or impair motor function in trained cyclists. *Med Sci Sport Exerc* 2019; 52:1169-1175.
11. Lundberg TR, Howatson G. Analgesic and anti-inflammatory drugs in sports:

implications for exercise performance and training adaptations. *Scand J Med Sci Sports* 2018; 28:2252-2262.

12. Esh CJ, Mauger AR, Palfreeman RA et al. Acetaminophen (paracetamol): Use beyond pain management and dose variability. *Front Physiol*. 2017;22;8:1092.

13. Jozwiak-Bebenista M, Nowak JZ. Paracetamol: mechanism of action, applications and safety concern. *Acta Pol Pharm* 2014; 71:11-23.

14. MacInnis MJ, Thomas ACQ, Phillips SM. The reliability of 4-minute and 20-minute time trials and their relationships to functional threshold power in trained cyclists. *Int J Sports Physiol Perform*. 2019;14:38-45.

15. Faul F, Erdfelder E, Buchner A et al. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 2009; 41:1149-1160.

16. Ferguson B. ACSM's Guidelines for Exercise Testing and Prescription 9th Ed. 2014. *J Can Chiropr Assoc* 2014; 58:328.

17. The European Medicine Agency. Guideline on the investigation of bioequivalence. 2010. Avaliable at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf Accessed 20 June 2020.

18. Borg GAV. Psychophysical bases of perceived exertion. *Med Sci Sport Exerc* 1982; 14:377-381.

19. Portole A, Puerro M, Terleira A et al. A new high-absorption-rate paracetamol 500-mg formulation: a comparative bioavailability study in healthy volunteers. *Curr Ther Res Clin Exp* 2003; 64:401-411.

20. Aminoshariae A, Khan A. Acetaminophen: old drug, new issues. *J Endod* 2015; 41:588-593.

21. Tanner T, Aspley S, Munn A et al. The pharmacokinetic profile of a novel fixed-dose combination tablet of ibuprofen and paracetamol. *BMC Clin Pharmacol* 2010; 10:10. https://doi.org/10.1186/1472-6904-10-10

22. Bossi AH, Lima P, Lima JP et al. Laboratory predictors of uphill cycling performance

in trained cyclists. *J Sports Sci* 2016; 35:1364-1371.

23. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods* 2004; 134:9-21.

24. Oostenveld R, Fries P, Maris E et al. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci* 2011; 1:156869.

25. Curry SC, Watts DJ, Katz KD et al. The effect of single-dose tramadol on oxycodone clearance. *J Emerg Med* 2007; 33:407-411.

26. Bastami S, Haage P, Kronstrand R et al. Pharmacogenetic aspects of tramadol pharmacokinetics and pharmacodynamics after a single oral dose. *Forensic Sci Int* 2014; 238:125-132.

27. Graham GG, Davies MJ, Day RO et al. The modern pharmacology of paracetamol: Therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology* 2013; 21:201-232.

28. Foster J, Taylor L, Chrismas BCR et al. The influence of acetaminophen on repeated sprint cycling performance. *Eur J Appl Physiol* 2014; 114:41-48.

29. Mauger AR, Hopker JG. The effect of acetaminophen ingestion on cortico-spinal excitability. *Can J Physiol Pharmacol* 2013; 91:187-189.

30. Wilkinson RT, Houghton D. Field test of arousal: a portable reaction timer with data storage. *Hum Factors* 1982; 24:487-493.

31. Robertson IH, Manly T, Andrade J et al. “Oops!”: Performance correlates of everyday attentional failures in traumatic brain injured and normal subjects. *Neuropsychologia* 1997; 35:747-758.

32. Lopez-Calderon J, Luck SJ. ERPLAB: an open-source toolbox for the analysis of event-related potentials. *Front Hum Neurosci* 2014; 8:213.

33. Luck S. *An Introduction to Event- Related Potential Technique*. Cambrigde Mass: MIT Press; 2005.

585 34. Hoffmann S, Falkenstein M. The correction of eye blink artefacts in the EEG: a
586 comparison of two prominent methods. *PLoS One*. 2008; 3:e3004.
587 35. Maris E, Oostenveld R. Nonparametric statistical testing of EEG- and MEG-data. *J*
588 *Neurosci Methods* 2007; 164:177-190.
589 36. Holgado D, Zandonai T, Ciria LF et al. Transcranial direct current stimulation (tDCS)
590 over the left prefrontal cortex does not affect time-trial self-paced cycling performance:
591 evidence from oscillatory brain activity and power output. *PLoS One*. 2019;
592 14:e0210873.
593 37. Behzadi M, Joukar S, Beik A. Opioids and cardiac arrhythmia: A literature review.
594 *Med Princ Pract* 2018; 27:401-414.
595 38. Chen A, Ashburn MA. Cardiac effects of opioid therapy. *Pain Med* 2015; 16:S27-S31.
596 39. Mauger AR, Jones AM, Williams CA. Influence of acetaminophen on performance
597 during time trial cycling. *J Appl Physiol* 2010; 108:98-104.
598 40. Morgan PT, Vanhatalo A, Bowtell JL et al. Acetaminophen ingestion improves muscle
599 activation and performance during a 3-min all-out cycling test. *Appl Physiol Nutr*
600 *Metab* 2019; 44:434-442.
601 41. Aron AR. The neural basis of inhibition in cognitive control. *Neuroscientist* 2007;
602 13:214-228.
603 42. Kayser B. Why are placebos not on WADA's prohibited list? *Perform Enhanc Heal*
604 2020; 100163. doi:10.1016/j.peh.2020.100163
605
606

607 Table 1. Characteristics (mean ± SD) of the participants in the study.

Age (years)	26 ± 7
Weight (kg)	68.8 ± 7.5
Height (cm)	175.3 ± 5.2
Body mass index (kg/m^2)	22.3 ± 2.2
VO_{2max} (ml/min/kg)	52.7 ± 6.3
Maximal power output (W)	346 ± 29
Power 60% of VO_{2max} (W)	191 ± 16

608

609

610 Table 2. Mean ± Standard Deviation for the PVT data.

Substance	Pre	Post	Baseline-corrected
TRA	278.2 ± 36.5	275.8 ± 28.3	-0.003 ± 0.033
PAR	271.1 ± 27.0	278.3 ± 24.9	0.013 ± 0.021
PLA	268.9 ± 26.4	278.6 ± 27.2	0.017 ± 0.020

611

612 PAR, paracetamol; PLA, placebo; TRA, tramadol. Data are expressed in ms.

613

614

615 **Figure legends**

616

617 Fig. 1. Experimental protocol in Day 2, 3 and 4.

618 Note: Time (min): PVT: Psychomotor Vigilance Task (white columns). Black columns
619 represent substances administration phase. Grey columns represent the EEG baselines,
620 exercise and cognitive performance test (SART) and the RPE (6-20 Borg scale)
621 measurement.

622

623 Fig. 2. Power output in the 20-min TT as a function of substance (panel A), and as a function
624 of substance and block (panel B (block 1, 0-10 min; block 2, 10-20 min).
625 Panel A: TRA, tramadol; PAR, paracetamol; PLA, placebo. Panel B: Tramadol, red square;
626 Paracetamol, black square; Placebo, blue square. Values are means and error bars indicate
627 the standard deviation.

628

629 Fig. 3. Average EEG power spectrum across all channels for paracetamol (black line), placebo
630 (blue line) and tramadol (red line) substance at baseline, warm-up, 40-min constant work-rate
631 test and 20-min TT period. Reliable differences between substances are marked by grey area,
632 showing the higher spectral power for tramadol compared with placebo and paracetamol at
633 baseline.

634

635 Fig. 4. Event-related spectral perturbation during the SART. Event-locked spectral power
636 averaged across all electrodes for each substance. Each panel illustrates time-frequency
637 power across time (x-axes) and frequency (y-axes) for the Go and NoGo stimuli (blue:
638 decreases; red: increases). Dashed vertical line represents stimulus onset.

639

640

641