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2 **Abstract**

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

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

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

10 **Results:** In Experiment 1, average time-trial power output was higher in the tramadol vs. placebo
11 condition (tramadol: 220 watts vs. placebo: 209 watts; $p < 0.01$). In Experiment 2, no differences between
12 conditions were observed in the average power output (tramadol: 234 watts vs. placebo: 230 watts; $p >$
13 0.05). No behavioural differences were found between conditions in the Oddball task. Crucially, the time
14 frequency analysis in Experiment 2 revealed an overall lower target-locked power in the beta-band ($p <$
15 0.01), and higher alpha suppression ($p < 0.01$) in the tramadol vs. placebo condition. At baseline, EEG
16 power spectrum was higher under tramadol than under placebo in Experiment 1 while the reverse was
17 true for Experiment 2.

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

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

22 **Keywords**

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

24 **Introduction**

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

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

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

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

64 **Methods**

65 The study involved a randomized, double blind, placebo controlled trial. The trial was approved
66 by the Spanish Agency of Medicines and Medical Devices (AEMPS), EudraCT number: 2015-
67 005056-96, and the Ethical Committee of Clinical Research in Granada. All experimental
68 procedures were designed to comply with the Declaration of Helsinki and Good Clinical
69 Practice (GCP). The randomization process, the audit and verification of compliance of GCP
70 rules were performed by an external clinical research organization (CRO; Delos Clinical,
71 Seville, Spain). The sample sizes were based on power calculations using G*Power Software ¹³
72 and assuming a 0.8 power and an alpha error of 0.05. Only cyclists and triathletes with a high-
73 medium level of physical fitness were included in the study. Exclusion criteria were the
74 presence of symptomatic cardiopathy, metabolic disorders such as obesity (BMI >30) or

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

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

101 On arrival at the laboratory for visit 2 and 3 (supplementary material Fig 1 for protocol
102 schematic), participants completed a Profile of Mood States Questionnaire (POMS), and a
103 visual analogue scale (VAS) concerning perceived activation, mental and physical fatigue. After
104 completing the questionnaires, participants consumed either tramadol or placebo as outlined
105 below. The experimental sessions were completed at the same time of the day (± 1 h). The time-
106 trial commenced 120 min following ingestion of the tramadol or placebo capsule (*see*
107 *experimental manipulation below*). Before the beginning of the time-trial, the participant's EEG
108 was recorded as a baseline measure and throughout the session. Next, participants performed a
109 10 min warm-up at 100 watts, followed immediately by a 20-min cycling time-trial on a cycle
110 ergometer (SRM, Julich, Germany). Participants adjusted saddle and handle bar height and
111 length, and it was kept for all sessions. The time-trial was conducted in a dimly-illuminated,
112 sound-attenuated faraday cage. Convective cooling was provided by one fan (2.5 m/s wind
113 speed) located 100cm from the ergometer. Participants were instructed to maintain the highest
114 average power possible during the time-trial and were freely able to change gearing and cadence
115 throughout. Participants were aware of the elapsed time, but did not have feedback on
116 performance (wattage and heart rate) during, or after the time-trial. Heart rate was measured
117 continuously throughout the protocol (V800, Polar Electro, Finland). Immediately after the
118 time-trial participants were asked to rate their average perceived exertion during the preceding
119 exercise. Then, participants completed 10 min cool-down (60 watts), following which another
120 EEG recording was taken. Finally, the POMS and VAS were completed again.

121 As we did not find any effect of gender in Experiment 1, and given the difficulty of finding a
122 large enough samples of females, we only recruited and tested males and in Experiment 2. One
123 participant only completed visit 1, and data from another was removed due to data acquisition
124 issues, meaning that the final sample for Experiment 2 was $n = 28$: age 25 (5) years, weight 73.2
125 (7.7) Kg; VO_{2max} : 54.1 (5.7) ml/min/kg. The procedure of the Experiment 2 was the same as that
126 of Experiment 1, except for the following: participants completed an oddball sustained attention
127 task during the 20-min time-trial with the purpose of assessing sustained attention during

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

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

144 We used the Spanish adapted version of the POMS ¹⁶, which has been used extensively for the
145 assessment of mood in the sport and exercise environments. This questionnaire has 58 items and
146 the factor structure representing six dimensions of the mood construct: Tension, Depression,
147 Anger, Vigour, Fatigue and Confusion. Participants answered the items on a 5-point Likert scale
148 (0 = not at all, 1 = a little, 2 = moderately, 3 = quite a bit, 4 = extremely). Raw scores were
149 transformed following the standard point table ¹⁶.

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

154 question “how would you rate the overall mental load for this experimental session?”.

155 Immediately after the time-trial participants were asked to rate their average perceived
156 exhaustion (RPE) using the 6-20 Borg scale.

157 An oddball task was designed to measure sustained attention by the random presentation of a
158 sequence of visual stimuli on a computer screen situated at 100 cm from the participants’ head
159 and at their eye level. A total of 600 stimuli were presented, consisting of frequent blue circle,
160 rare blue circle, and rare red square with probabilities of .80, .10, and .10, respectively via use
161 of computer software (E-Prime, Psychology Software Tools, Pittsburgh, PA, USA). The task
162 lasted approximately 18 minutes, starting two minutes after the beginning of the time-trial. The
163 small blue circle ($1.15^\circ \times 1.15^\circ$) was considered as the standard stimulus. The blue circle that
164 was slightly larger than the standard circle ($1.30^\circ \times 1.30^\circ$) was defined as target 1. Finally, the
165 rare red square ($2.00^\circ \times 2.00^\circ$) was defined as target 2. Each trial begun with the presentation of
166 a blank screen in a black background for 1200 ms. Then, the stimulus was presented in the
167 centre of the screen in a random time interval (between 0 and 800 ms) during 150 ms
168 (supplementary material Fig 2). Participants were required to respond to both target 1 and target
169 2 with their thumb finger of their dominant hand by pressing a button connected to the cycle
170 ergometer handlebar, and not to respond to the standard stimuli. Verbal and written instructions
171 were given to the participant prior to the start of the oddball sustained attention task.

172 Participants were instructed that the main goal of the task was to be as accurate as possible. A
173 brief familiarization of the task was included in the screening visit. For each stimulus, the RT
174 (in ms) and response accuracy (percentage of correct responses) were recorded.

175 At this point, we would like to note that, while the POMS used in the present study has shown a
176 high reliability (Cronbach alpha = 0.90)¹⁶, we do not have a measure of reliability for the
177 particular VAS and oddball tasks used in our study. The lack of reliability may suppose a
178 limitation in the present investigation, but, generally, oddball tasks show an interclass
179 correlation coefficient higher than 0.75¹⁷, and the VAS a Cronbach alpha higher than 0.90,
180 showing high test-retest reliability¹⁸. In any case, the purpose of these tasks were to compare

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

183 EEG data were recorded at 1000 Hz using a 62-channel actiCHamp System (Brain Products
184 GmbH, Munich, Germany) with active electrodes positioned according to the 10-20 EEG
185 International System and referenced to the Cz electrode. The cap was adapted to individual head
186 size, and each electrode was filled with Signa Electro-Gel (Parker Laboratories, Fairfield, NJ) to
187 optimize signal transduction. Participants were instructed to avoid body movements as much as
188 possible, and to keep their gaze on the centre of a computer screen during the measurement.
189 Electrode impedances were kept below 10 k Ω . EEG pre-processing was conducted using
190 custom Matlab scripts and the EEGLAB¹⁹ and Fieldtrip²⁰ Matlab toolboxes. EEG data were
191 resampled at 500 Hz, bandpass filtered offline from 1 and 40 Hz to remove signal drifts and line
192 noise, and re-referenced to a common average reference. Horizontal electrooculograms (EOG)
193 were recorded by bipolar external electrodes for the offline detection of ocular artefacts.
194 Independent component analysis was used to detect and remove EEG components reflecting eye
195 blinks²¹.

196 Electrodes presenting abnormal power spectrum were identified via visual inspection and
197 replaced by spherical interpolation. Processed EEG data from each protocol time period
198 (baseline-pre, warm-up, time-trial, cool-down, baseline-post) were subsequently segmented to
199 1-s epochs. The spectral decomposition of each epoch was computed using Fast Fourier
200 Transformation (FFT) applying a symmetric Hamming window and the obtained power values
201 were averaged across protocol time periods.

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

206 frequency. Power values were normalized with respect to a -50 ms to 0 ms pre-stimulus
207 baseline and transformed into the decibel scale ($10 \cdot \log_{10}$ of the signal).

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

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

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

240 **Results**

241 *In Experiment 1*, the average power output during the 20-min time-trial (Fig 3A) was
242 higher under tramadol condition than under placebo condition (220 W [95%CI = 203 – 240 W]
243 vs. 209 W [95%CI = 192 – 228W] for tramadol and placebo, respectively; $p < 0.01$). An
244 additional analysis revealed that the effect of tramadol did not depend on participants' gender: t
245 = 1.107, $p = 0.83$ (critical t -score: ± 2.177).

246 The average heart rate demonstrated a significant difference between conditions (166 beats.min⁻¹
247 [95%CI = 162 – 170 beats.min⁻¹] vs. 162 beats.min⁻¹ [95%CI = 153– 166 beats.min⁻¹] for
248 tramadol and placebo, respectively; $p < 0.01$).

249 The POMS data demonstrated a significant interaction between condition and time point for
250 fatigue ($p = 0.03$), confusion ($p < 0.01$) and tension ($p < 0.01$). Under the tramadol condition,
251 participants showed a higher fatigue ($M = 52$, 95%CI = 48 - 55 vs. $M = 47$, 95%CI = 44 - 49); p
252 < 0.01) and confusion ($M = 41$, 95%CI = 38 - 44 vs. $M = 38$, 95%CI = 36 - 39); $p < 0.01$) after
253 the time-trial, while no difference where found before the time-trial for fatigue ($M = 42$, 95%CI
254 = 39 - 45 vs. $M = 40$, 95%CI = 38 - 42), $p > 0.05$) and for confusion ($M = 39$, 95%CI = 37 - 40
255 vs. $M = 38$, 95%CI = 37 - 39); $p < 0.05$). There were trends for lower tension before ($M = 35$,
256 95%CI = 33 - 37 vs. $M = 38$, 95%CI = 35 - 39) tramadol vs. placebo, respectively; $p = 0.06$),
257 but not after the time-trial ($M = 35$, 95%CI = 32 - 36 vs. $M = 34$, 95%CI = 32 - 35), for tramadol

258 and placebo, respectively; $p > 0.05$). The anger index showed a main effect for time point ($p <$
259 0.01) with higher values before the time-trial ($M = 38$, $95\%CI = 37 - 40$ vs. $M = 39$, $95\%CI =$
260 $36 - 39$), but no effect for condition or interaction between the factors. No other POMS factors
261 (depression and vigour) demonstrated significant changes with condition or time point ($ps >$
262 0.05).

263 Post time-trial RPE did not demonstrate any significant differences between conditions ($p >$
264 0.05). VAS Mental Load demonstrated a trend for an interaction between condition and
265 measure. Specifically, after the time-trial participants had trend for a higher mental fatigue in
266 the tramadol vs. placebo condition ($M = 34$, $95\%CI = 24 - 44$ vs. $M = 22$, $95\%CI = 16 - 28$),
267 respectively; $p = 0.056$). The main effect for time point was not significant ($p > 0.05$).

268 The Activation and Fatigue indexes demonstrated a significant main effect of time point
269 (Activation: $M = 51$, $95\%CI = 44 - 57$ vs. $M = 60$, $95\%CI = 54 - 66$; $p < 0.01$. Fatigue: $M = 21$,
270 $95\%CI = 13 - 28$ vs. $M = 45$, $95\%CI = 36 - 52$; $p < 0.01$). Before vs. after the time-trial
271 respectively), while the main effect for condition and the interaction between both factors did
272 not reach significance ($p > 0.05$). Finally, the cognitive load of the session was not significantly
273 different between conditions ($p > 0.05$).

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

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

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

289 The main effect of condition did not reach statistical significance for the heart rate: tramadol
290 176 beats.min⁻¹ [95% CI = 172 – 179] vs. placebo (175 beats.min⁻¹ [95% CI = 170 – 179]; $p >$
291 0.05.

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

296 There were no differences in post time-trial RPE between conditions ($p > 0.05$). An overall
297 main effect of time point was found for fatigue and mental load in the VAS. Specifically, a
298 higher mental fatigue ($M = 31$, 95% CI = 21 - 40 vs. $M = 54$, 95% CI = 47 - 60; $p < 0.01$) and
299 higher mental load ($M = 25$, 95% CI = 17 – 32 vs. $M = 44$, 95% CI = 36 - 52; $p < 0.01$) were
300 found after the time-trial. None of the others items in the analysis reached significance ($p >$
301 0.05).

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

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

309 statistically significant: one cluster (17 electrodes) in the 3-6 Hz band ($p = 0.01$), and one cluster
310 (5 electrodes) in the 26–33 Hz band ($p = 0.02$). The analysis revealed an overall increase in the
311 power of frequencies in the placebo condition in comparison to the tramadol condition (Table
312 1).

313 The main effect of condition during the time-trial was not significant ($p > 0.05$). There was a
314 significant main effect of condition ($p < 0.05$) for the cool-down period. Two clusters were
315 statistically significant: one cluster (29 electrodes), in the (1–9 Hz) delta and theta band ($p <$
316 0.01); and one global cluster (41 electrodes) in (12–36 Hz) beta band ($p < 0.01$). Similar to other
317 period, the analysis revealed an overall increase in the power of frequencies in the placebo
318 condition. In the baseline-post period we found a main effect of condition. One cluster was
319 found (29 electrodes) and was statistically significant ($p = 0.01$) in the theta band. Similar to
320 other periods, the analysis showed an increase in the power for placebo compared to tramadol.

321 The event-related spectral perturbation (stimulus-locked) analysis in Experiment 2 showed a
322 main effect of condition in the alpha band for target 1, with a cluster (15 electrodes) between
323 118-224 ms after the onset of the target 1 ($p < 0.01$; see Fig 4 A- B). Alpha frequency band
324 exhibited a lower spectral power in the tramadol condition with regard to the placebo condition.
325 The Lower Beta band analysis showed a significant cluster (24 electrodes) between 136-230 ms
326 after the onset of target 1 ($p < 0.01$). Lower Beta frequency band exhibited a higher spectral
327 power in placebo condition with regard to the tramadol condition (see Fig 4C). The analysis of
328 the other frequency bands for target 1, target 2 and standard trials yielded no significant effects
329 ($p > 0.05$).

330 **Discussion**

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

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

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

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

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

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

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

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

387 **Conclusion**

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

398 **Practical applications**

399 Tramadol may improve cycling time-trial performance.
400 Tramadol influences information processing related to sustained attention at the brain level,
401 although it was not translated into an impaired behavioural performance.
402 Anti-doping authorities may reconsider tramadol's status

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504 **Table and figures captions**

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

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

522 **Supplementary materials**

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

524 **Fig 1** Experimental session’s schematic protocol

525 **Fig 2** Schematic representation of the oddball sustained attention task