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The true size of placebo analgesia:

Concordant neural and behavioural measures of placebo analgesia during experimental acute pain

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33 **Abstract**

34 ‘Placebo analgesia’ refers to the reduction of pain following the administration of an inactive
35 treatment. While most clinical trials compare a drug treatment against a placebo to determine the
36 efficacy of the analgesic, most experimental studies of placebo analgesia do not include a real
37 analgesic condition. A direct comparison of placebo against a real analgesic can inform us about the
38 true size of the placebo effect. To this end, we aimed to provide a robust estimate of placebo
39 analgesia by contrasting the effect of pain relief expectation from an inert cream (vaseline) against a
40 real topical analgesic agent (lidocaine) applied on two different limbs and their respective control
41 conditions. Pain reports and electroencephalography (EEG) responses triggered by laser nociceptive
42 stimulation were collected. Forty typical healthy adults were enrolled in a double-blind randomized
43 within-subject study where a standard placebo induction script of verbal suggestions in a sham
44 medical setting was used to enhance the expectation on treatment outcome. In line with the earliest
45 studies of placebo analgesia, majority (30 of 40) of participants was placebo responders, i.e. they
46 reported lower pain to the placebo treatment. Placebo responders reported low pain and displayed
47 low laser evoked potentials (LEPs) amplitude for both the analgesic and placebo treatment limbs
48 compared to the respective control limbs. Placebo analgesia correlated positively with the amplitude
49 of the LEPs, thus establishing convergent validity of the findings. This study provides a robust
50 estimate of the neural and behavioural measures of placebo analgesia, in comparison to a real
51 analgesic. These estimates can help inform the quantitative criteria for similar neural and
52 behavioural measures in assessing the effectiveness of a real drug in placebo controlled trials.

53

54 **Key words:** Electroencephalography, laser evoked potentials, lidocaine, nociception, pain, placebo
55 analgesia, vaseline.

56

57 **1. Introduction**

58 Placebo effects lead an individual to display/feel an experiential improvement following the
59 administration of an inert treatment with no actual therapeutic properties. In other words, factors
60 differing from the purported treatment can cause a beneficial physical response. This has been
61 observed in several clinical conditions and diseases, particularly in clinical pain (Tuttle et al., 2015).
62 While the phenomenon is well recognized, the magnitude of placebo effects, the influence of the
63 context, and their temporal course are less known (see Benedetti, 2008 for a general review).

64 Despite a robust body of evidence over the last four decades starting from (Levine, Gordon, &
65 Fields, 1978), there remain important concerns on the robustness and reliability of placebo,
66 especially in the clinical settings. Meta-analytic studies have indicated the presence of potential
67 confounds (e.g. regression to the mean; Artus, van der Windt, Jordan, & Hay, 2010; Hrobjartsson,
68 Kaptchuk, & Miller, 2011) that led to overestimation of very small to null placebo effects
69 (Hrobjartsson & Gotzsche, 2001, 2004, 2010; Hrobjartsson et al., 2011). Notwithstanding
70 considerable individual variability in the magnitude of placebo analgesia (Wager, Atlas, Leotti, &
71 Rilling, 2011), several studies indicate that placebo analgesia is a reliable and consistent
72 phenomenon (Atlas & Wager, 2014; Finniss, Kaptchuk, Miller, & Benedetti, 2010; Price et al., 1999;
73 Vase et al., 2015). Interestingly, clinical trials for analgesics and experimental studies of placebo
74 pose a methodological contrast. While clinical trials for analgesics routinely compare them against a
75 placebo to estimate the magnitude of the analgesic effect, most experimental studies of placebo
76 analgesia do not use a real analgesic treatment to estimate the size of the placebo effect (e.g. Price et
77 al., 1999, but see Vase, Robinson, Verne, & Price, 2005 for an exception). Here we address this
78 methodological difference by directly comparing the magnitude of placebo analgesia against that of
79 a known analgesic.

80 Laser thermal stimulation provides a targeted way to selectively stimulate nociceptive free
81 nerve endings in the skin. In particular, solid state lasers (as the one used in the current study) offers

82 a reduced risk of superficial burns than the CO₂ laser, due to its shorter wavelength (1.34 μm). In
83 addition, solid state lasers allow a better afferent-volley synchronization which results in enhanced
84 amplitudes and shorter latencies of cortical responses (Perchet et al., 2008). To date, recording of
85 electroencephalographic activity during laser thermal stimulation (Laser Evoked Potentials, LEP)
86 provides the most reliable and selective neurophysiological method of assessing the function of
87 nociceptive pathways (Garcia-Larrea, 2012). However, there is still relatively little research using
88 laser thermal stimulation to study placebo analgesia.

89

90 Using LEP, here we aimed to provide a robust estimation of placebo analgesia by contrasting
91 the effect of pain relief expectation from an inert cream (vaseline) against a real topical analgesic
92 agent (lidocaine) and their respective control conditions in a large sample of healthy volunteers
93 (n=40). We collected pain reports and EEG responses triggered by laser nociceptive stimulation in a
94 double-blind randomized within-subject design whereby healthy volunteers underwent a standard
95 placebo induction script of verbal suggestions in a sham medical setting meant to enhance the
96 expectation on treatment outcome. Verbal induction of expectations about the outcome can not only
97 lead to formation of conscious expectations, but also bring online effects of unconscious learning,
98 two processes that can lead to placebo analgesia (e.g. Benedetti et al., 2003; Pecina, Stohler, &
99 Zubieta, 2014).

100

101 **2. Material and Methods**

102 **2.1 Subjects**

103 EEG data were collected from 40 healthy volunteers. We excluded one participant from data
104 analysis as she questioned about covert experimental aims possibly concerning the investigation of
105 placebo in the debriefing phase. The remaining 39 participants (21 females) were aged 24.9±4.5
106 (mean±SD). All had normal or corrected-to-normal vision and were naïve as to the purpose of the

107 experiment. None of the participants had a history of neurological or psychiatric illnesses or
108 conditions that could potentially interfere with pain sensitivity (e.g. drug intake or skin diseases).
109 Participants gave written informed consent and were debriefed about the actual aim of the study at
110 the end of the experiment. The participants could therefore decide to withdraw their consent about
111 data usage if they wished so. All experimental procedures were approved by the Fondazione Santa
112 Lucia ethics committee and were in accordance with the standards of the Declaration of Helsinki.
113 No participant had short or medium term symptoms (e.g. Inflammation) associated with the
114 compounds used in this study.

115

116 ***2.2 Nociceptive stimulation***

117 Radiant-heat stimuli were generated by an infrared neodymium yttrium aluminium perovskite
118 (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, ElEn, Florence, Italy).
119 Laser pulses selectively and directly activate the A δ and C-fiber nociceptive terminals located in the
120 superficial layers of the skin (Cruccu et al., 2003). Laser pulses were directed at the dorsum of both
121 left and right hand and foot, on a squared area (5x5 cm) defined prior to the beginning of the
122 experimental session and highlighted using a He-Ne guide laser. The laser pulse (3 ms duration)
123 was transmitted via an optic fibre and its diameter was set at approximately 5 mm (28 mm²) by
124 focusing lenses. After each stimulus, the laser beam target was shifted by approximately 1 cm in a
125 random direction, to avoid nociceptor fatigue or sensitization.

126 Before the recording session, a familiarization and calibration procedure was carried out to check
127 the quality of the sensation associated with radiant heat stimuli. In this procedure, the energy of the
128 laser stimulus was individually adjusted using the method of limits (laser step size: 0.25 J),
129 separately for each of the four stimulated territories (left hand, right hand, left foot, right foot).
130 During this procedure subjects were asked to report the quality and the intensity of the sensation
131 elicited by each laser pulse using a numerical rating scale (NRS, ranging from 0=no sensation, to

132 8=unbearable pain). The energy of laser stimulation needed to achieve a rating of 6 (corresponding
133 to ‘moderate pain’) was chosen as experimental energy value. We checked that this value
134 corresponded to a rating of about 60 on visual analogue scale (VAS) ranging from 0 (not painful) to
135 100 (extremely painful). Once nociceptive intensity was calibrated, participants underwent a brief
136 familiarization block of 10 stimuli. Importantly, there was no difference in the average energy used
137 to obtain a moderate sensation of pain for both feet and hands: right and left hand, 2.27 ± 0.34 J;
138 right and left foot, 2.33 ± 0.32 J. According to the parameters mentioned above, laser pulses elicited a
139 clear pinprick/burning brief sensation of acute pain related to the activation of A δ and C fibres.

140

141 **2.3 EEG recording**

142 The electroencephalogram (EEG) was recorded using 54 tin scalp electrodes placed according to
143 the International 10-20 system, referenced against the nose and grounded at AFz.
144 Electro-oculographic (EOG) signals were simultaneously recorded using surface electrodes.
145 Electrode impedance was kept below 5 K Ω . The EEG signal was amplified and digitized at a
146 sampling rate of 1,000 Hz.

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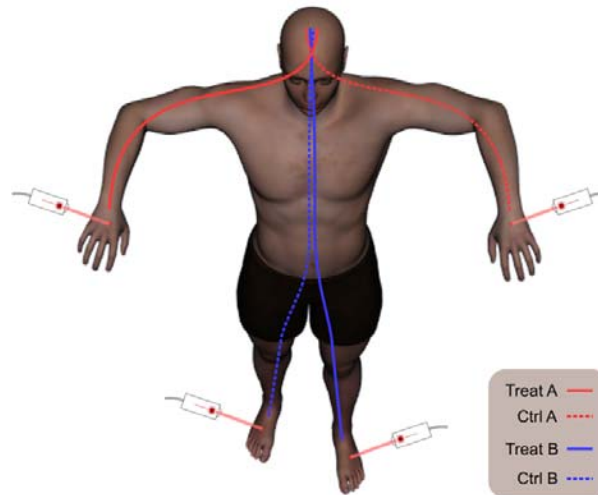
148 **2.4 Experimental design**

149 Upon arrival participants were welcomed in a temperature-controlled room by two experimenters
150 (EV, BC) dressed in white coats. They introduced the participants to the study using the same set of
151 sentences (see Appendix), and informed them about the whole procedure. In brief, participants were
152 told that two analgesics (named *Varicaine* and *Exacaine*) were being evaluated for their efficacy. In
153 reality, one of these was an inert cream (vaseline, labelled as cream A and called *Varicaine*), while
154 the other was a topical analgesic (5% lidocaine, labelled as cream B and called *Exacaine*).

155 Participants then underwent the EEG cap montage. The analgesic cream was applied on the
156 dorsal surface of one of four limbs (hand/foot, coded as Treat B). An identical site in the

157 contralateral limb was used as its control (no cream, control site, coded as Ctrl B). Same procedure
158 was adopted for the inert cream on the other pair of limbs (coded as Treat A and Ctrl A respectively).
159 The conditions were counterbalanced in a double-blind fashion across participants (Fig. 1).

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168 **Fig 1.** Participants were told that two analgesics were being evaluated for their efficacy. They were unaware that
169 one of these was an inert cream (vaseline, labelled as cream A and called Varicaine), while the other was an
170 actual topical analgesic (lidocaine, labelled as cream B and called Exacaine). Subjective pain thresholds for
171 moderate pain (a rating of 6 out of 10) was established for each participant before the application of creams. The
172 analgesic cream was applied on the dorsal surface of one of four limbs (hand/foot, coded here as Treat B). An
173 identical site in the contralateral limb was used as its control (no cream, control site, coded here as Ctrl B). The
174 same procedure was adopted for the inert cream on the other pair of limbs (coded here as Treat A and Ctrl A
175 respectively). The conditions were counterbalanced in a double-blind fashion across participants. Each block
176 lasted between 10 and 15 min, and an interval of 5 min separated the two blocks. In each block we delivered 30
177 laser pulses, using an inter-stimulus interval ranging between 5 and 15 s. At the end of each train of 10 stimuli,
178 participants were asked to rate the intensity of the painful sensation elicited by the laser stimuli using a visual
179 analogue scale ranging from 0 (not painful) to 100 (extremely painful).
180

181 Creams were spread and left acting on the skin for a mean duration of 13:52 min (SD=2.52 min).
182 After careful rubbing of the creams off the administration sites, all four limbs were stimulated using
183 a Nd:YAP laser at an energy level corresponding to subjective threshold for moderate pain (i.e.
184 NRS=6). Participants were asked to focus their attention on the painful stimuli while closing their
185 eyes and relax their muscles. Laser-evoked EEG responses were obtained following the stimulation
186 of the dorsum of the right and left hand and foot in four separate blocks, on the same day. Each
187 block lasted between 10 and 15 min, and an interval of 5 min separated the two blocks. In each
188 block we delivered 30 laser pulses, using an inter-stimulus interval (ISI) ranging between 5 and 15s.

189 At the end of each train of 10 stimuli, participants were asked to rate the pain intensity and
190 unpleasantness of the painful sensation elicited by the laser stimuli using a visual analogue scale
191 (VAS) ranging from 0 (no sensation, no unpleasant at all) to 100 (intolerable intensity/intolerable
192 unpleasantness).

193 At the end of the experiment, participants went through a structured debriefing interview in
194 which we asked their opinion on the experimental aims (e.g. "What do you think was the study
195 objective?" and "Did you notice any difference in the efficacy of the two creams?") and were
196 debriefed regarding the deception.

197

198 ***2.5 Data analysis***

199 ***2.5.1 General statistical approach***

200 Dependent variables were analyzed with repeated-measures Analysis of Variance (ANOVA) with
201 factors 'expectation' (treatment, no treatment) and treatment 'label' (A – placebo, B – analgesic).
202 Further, we run an additional ANOVA only on placebo responders, i.e. individuals who reported
203 significant lower pain unpleasantness during placebo vs. no treatment (n= 30). The choice of pain
204 unpleasantness as the variable of interest was supported by the evidence that the major feature of
205 the multidimensional pain experience is its affective quality rather than its intensity (Merskey,
206 Bogduk, & Pain, 1994).

207 Statistical analyses were performed using Statistica[®] 8.0 (StatSoft Inc., Tulsa, Oklahoma,
208 USA). Variability is reported as standard error of mean (SEM) unless reported otherwise. The level
209 of significance was set at $p < 0.05$. We reported Cohen's d and partial eta squared ($p\eta^2$) as measures
210 of effect size. Tukey HSD tests were used to perform post-hoc pairwise comparisons.

211

212 ***2.5.2 Laser evoked potentials***

213 EEG data were processed with EEGLAB (v.12; Delorme & Makeig, 2004 and Letswave 5,

214 <http://nocions.webnode.com/>). Single participant data were merged in a unique experimental
215 session file and down-sampled to 250 Hz. Sinusoidal artifacts (50-100 Hz) were then removed
216 using CleanLine, an EEGLAB plugin which enabled us to selectively delete power line frequency
217 contribution from the recorded signal (<http://www.nitrc.org/projects/cleanline>). Further, signal was
218 DC removed and band-pass filtered from 1 to 30 Hz (filter order: 4). Data were then segmented into
219 epochs using a time window ranging from 1 s before to 2 s after the stimulus (total epoch duration:
220 3 s) and baseline corrected using the mean of the entire epoch (Groppe, Urbach, & Kutas, 2011).
221 Epoched data were merged and further processed using independent component analysis (ICA;
222 Vigário, 1997) to subtract EOG and muscle-related artifacts, aided by the semi-automatic approach
223 offered by Adjust (Mognon, Jovicich, Bruzzone, & Buiatti, 2011), an EEGLAB plugin which
224 identifies artifactual independent components using an automatic algorithm that combines
225 stereotyped artifact-specific spatial and temporal features. After ICA and an additional baseline
226 correction (-500 to 0 ms), we re-referenced data to a common average reference (Lehmann &
227 Skrandies, 1980) and segmented in four average waveforms time-locked to the stimulus onset, one
228 for each experimental condition (Ctrl A; Treat A; Ctrl B; Treat B). Single-subject average
229 waveforms were subsequently averaged to obtain group-level average waveforms. Group-level
230 scalp topographies were computed by spline interpolation. Scalp topographies were plotted at the
231 peak latency of the N2 and P2 LEP waves, measured at the vertex (Cz electrode). The N2 wave was
232 defined as the most negative deflection after stimulus onset. The P2 wave was defined as the most
233 positive deflection after stimulus onset. We used group-level median peak values to identify the
234 temporal window to extract the minimum (N2, 180-280 ms) and the maximum (P2, 280-480 ms)
235 amplitudes for each participant. These two waves seem to result from sources in bilateral
236 operculo-insular and anterior cingulate cortices (Garcia-Larrea, Frot, & Valeriani, 2003). They are
237 significantly modulated by both top-down and bottom-up attentional factors (reviewed in Legrain et
238 al., 2012).

239

240 **2.5.3 Correlation between pain ratings and N2-P2 amplitudes**

241 Placebo and analgesia response magnitude was calculated as a ratio of the average ratings, N2-P2
 242 peak-to-peak amplitude, for the placebo control limb divided by that for the placebo treatment limb
 243 (Ctrl/Treat). In other words, the greater the value of this ratio the greater the analgesic effect.

244

245 **3 Results**

246 **3.1 Psychophysics**

247 All participants described the sensation elicited by the laser stimuli as clearly painful and pricking.
 248 The average ratings (mean±SD) of the pain unpleasantness for each experimental condition as well
 249 as the effect sizes are reported in Table 1.

250

251 **Table 1.** Mean (±SD) of pain ratings (unpleasantness)(top) in the full sample. Cohen's *d* as for both
 252 types of ratings as well as for the ratio Ctrl/Treat (bottom). A refers to the inert cream, and B refers
 253 to the real analgesic. Ctrl A refers to the no-treatment contralateral limb control for the inert cream;
 254 Ctrl B refers to the no-treatment contralateral limb control for the real analgesic.

255

	Pain rating (unpleasantness)			
	Treat A	Ctrl A	Treat B	Ctrl B
RATINGS	62.27 (±18.14)	70.27 (±15.76)	56.81 (±17.73)	60.94 (±18.13)
	Pain rating (unpleasantness)			
	Treat A vs. Ctrl A	Treat B vs. Ctrl B	Ctrl A/Treat A vs. Ctrl B/Treat B	
EFFECT SIZE	-0.51	-0.21	-0.07	

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258 **3.1.2 Effects of expectation and treatment label**

259 The ANOVA performed on the unpleasantness ratings revealed main effects of both 'expectation'
 260 ($F_{38}=19.62$; $P<0.001$; $\eta^2=0.34$) and treatment 'label' ($F_{38}=6.70$; $P=0.01$; $\eta^2=0.15$), but no
 261 significant interaction between the two factors ($F_{38}=2.21$; $P=0.14$; $\eta^2=0.05$). This pattern of results

262 indicates that participants felt less pain unpleasantness when expecting treatment compared to no
263 treatment and felt less pain unpleasantness during the analgesic-related (Ctrl B and Treat B) vs.
264 placebo-related (Ctrl A and Treat A) stimulation (Fig. 2, A). The analysis on responders (Fig. 2, B)
265 revealed no main effect of this ‘label’, suggesting that individuals responding better to the placebo
266 treatment had no different unpleasantness depending on the type of cream used and its related
267 control stimulation ($F_{29}=2.40$; $P=0.13$; $\eta^2=0.08$) but rather showed lower pain unpleasantness
268 when treatment was expected ($F_{29}=36.80$; $P<0.001$; $\eta^2=0.56$) and with both ‘expectation’ and
269 ‘treatment label’ ($F_{29}=7.83$; $P=0.009$; $\eta^2=0.21$). These interactions reflect (i) a larger reduction of
270 pain unpleasantness in responders when expecting the Treat A (i.e. *Varicaine*) compared to Ctrl A
271 (58.56 vs. 71.21; $P<0.001$), (ii) a greater pain unpleasantness in responders during the Ctrl A against
272 Treat B and Ctrl B (71.21 vs. 57.51 and 61.71; $P_s<0.001$).

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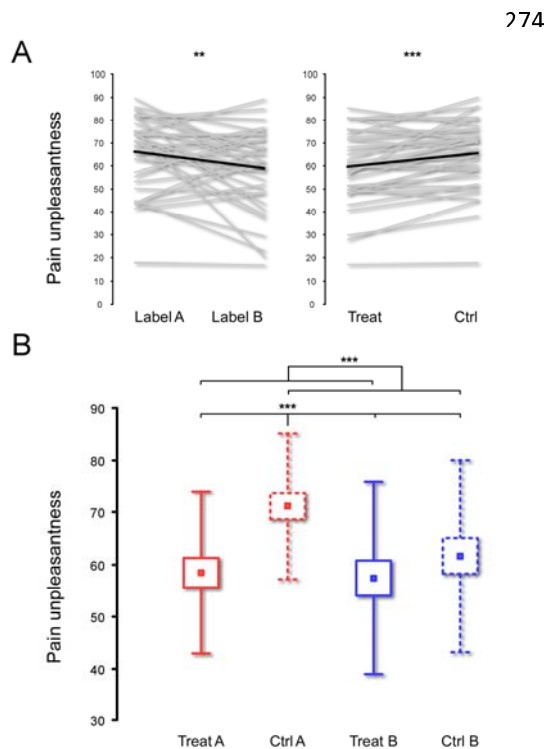


Fig 2. Panel A shows single subject average ratings of pain unpleasantness for the two levels (Label A, Label B) of factor treatment “label” (left) and the two levels (Treat, Ctrl) of the factor treatment “expectation” (right). Grand-average is shown with bold black line. Individuals reported lower pain unpleasantness during both placebo and analgesia treatment than in the respective control conditions ($***p<0.001$). They also reported lower pain unpleasantness during both actual analgesia and its control condition than during placebo and its control condition ($***p<0.001$). Panel B shows results only for placebo responders. Box-plots show (mean \pm SE \pm SD) of pain intensity ratings. The pattern observed in the full sample was enhanced in this subgroup ($***p<0.01$).

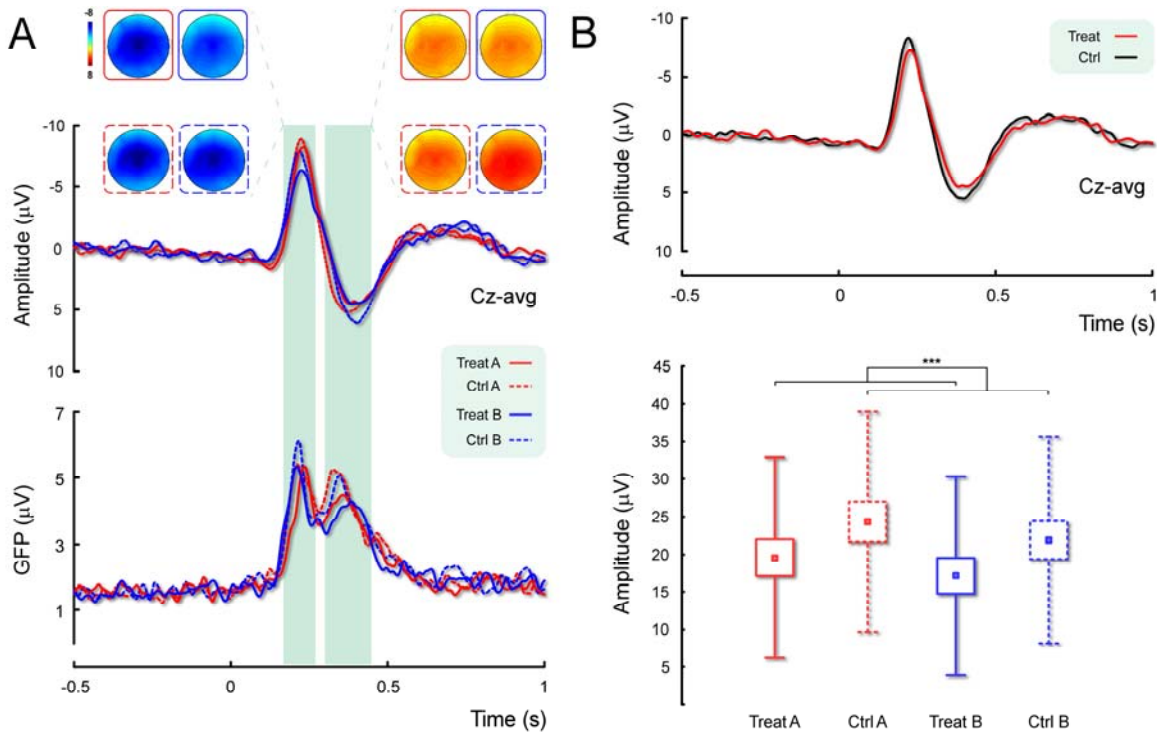
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301 3.2 Laser evoked potentials

302 Fig. 3 (A) displays the grand average waveforms and global field power (GFP) of LEPs.
303 Nociceptive stimuli delivered in the four conditions elicited maximal N2 and P2 waves at the
304 electrode Cz with topographies maximally expressed over the scalp vertex (Fig. 3, A, top).
305



306

307 **Fig 3.** Panel A shows group-level average LEPs and scalp topographies of peak amplitudes (top) within the N2
308 and P2 latency range (180-280 and 280-480 ms post-stimulus respectively) as well as global field power (GFP;
309 bottom) in the four conditions (Placebo-related in red, analgesia-related in blue; treatment in solid and control
310 conditions in dashed lines). Note the greater amplitudes elicited by the stimulation of the no-treatment (control)
311 limbs. Panel B clarifies this pattern by showing the main effect of treatment expectation on the vertex LEPs in the
312 full sample (top). Box-plots (mean \pm SE \pm SD) show N2-P2 peak-to-peak amplitude in placebo responders in the
313 four conditions (bottom). Note the amplitude reduction in Treat A and B compared to Ctrl A and Ctrl B
314 respectively.
315

316 3.2.1 Effects of expectation and treatment label on N2-P2

317 The ANOVA performed on the peak-to-peak amplitude of the main vertex potentials N2-P2
318 extracted at the Cz electrode revealed a main effect of 'expectation' ($F_{38}=11.54$; $P=0.002$; $\eta^2=0.23$)
319 but no effect of treatment 'label' ($F_{38}=0.69$; $P=0.41$; $\eta^2=0.02$) or interaction between the two

320 factors ($F_{38}=0.98$; $P=0.33$; $\eta^2=0.02$). This pattern of results indicates that participants displayed
321 lower vertex potentials amplitude when expecting treatment compared to no treatment (Fig. 3, B).
322 Peak-to-peak amplitudes in responders (Fig. 3, B, bottom) revealed no main effect of treatment
323 ‘label’, suggesting that individuals responding better to the placebo treatment had no different
324 N2-P2 LEP amplitude depending on the type of cream used and its related control stimulation
325 ($F_{29}=0.79$; $P=0.38$; $\eta^2=0.03$) but rather showed lower N2-P2 amplitude when treatment was
326 expected ($F_{29}=24.26$; $P<0.001$; $\eta^2=0.45$). However, there was no interaction between the two
327 factors ($F_{29}<0.001$; $P=0.99$; $\eta^2<0.001$).

328

329 **3.3 Correlation of pain ratings with LEPs**

330 The magnitude of the placebo response was calculated as the ratio of unpleasantness ratings of the
331 control limb divided by that of the treatment limb (Fig. 4). This magnitude was positively correlated
332 with the N2-P2 response, calculated similarly (i.e. N2-P2 response of the control limb divided by
333 that of the treatment limb) ($r_{38}=0.50$; $P=0.001$).

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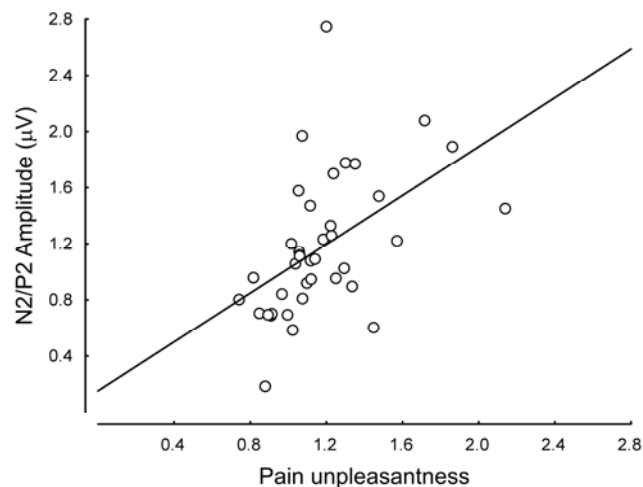
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343 **Fig 4.** Scatterplot representing the relationship between ratings of pain unpleasantness (x-axis) and the amplitude
344 of the N2/P2 LEPs (y-axis) with its linear fit. Both measures were calculated as a ratio of the control limb divided
345 by treatment limb, thus providing an index of the placebo effect. Note that the reduction of pain unpleasantness
346 associated with placebo is linked to a decrease of N2/P2 peak-to-peak amplitude.

347

348

349 **4 Discussion**

350 In this study, we estimated the magnitude of placebo analgesia against a real analgesic, using
351 self-report and laser-evoked potential measures. Our results show that healthy volunteers felt less
352 pain and displayed lower magnitude of EEG responses when receiving a purported analgesic
353 treatment (regardless of whether this was a sham or actual analgesic compound) compared to the
354 stimulation of non-treated skin territory (Figs 2 and 3). Magnitude of the placebo response
355 computed from pain unpleasantness ratings was positively correlated with that computed from the
356 neural response to placebo (Fig. 4). This study presents one of the few concordant behavioural and
357 neural estimates of the placebo analgesia effect, using a true analgesic and a sham treatment and
358 expand current knowledge about placebo analgesia and its neural correlates (Geuter, Koban, &
359 Wager, 2017; Wager & Atlas, 2015 for reviews). Despite the high number of placebo responders
360 ($n=30$ according to our identification criterion), the true effect size for the placebo effect was small
361 (Table 1, $d=-0.07$). This is consistent with previous research (Price, Finniss, & Benedetti, 2008).
362 Across all participants, our data demonstrate a small difference between placebo and analgesia
363 treatment in self-reported pain unpleasantness (Fig. 2). This difference is in the expected direction
364 and is explained by greater analgesia after the administration of the real analgesic (lidocaine) than
365 the placebo treatment (vaseline). Interestingly within placebo responders, the treatment effect size
366 (i.e. treatment vs. control) was larger for placebo than lidocaine for pain unpleasantness ($d=-0.53$ vs.
367 -0.21). This unexpected pattern may have been driven by the greater pain unpleasantness rating in
368 the placebo control condition, compared to the analgesic control condition (Fig. 2 B). This
369 difference is unlikely to be explained by response bias and social desirability (Hrobjartsson et al.,
370 2011), as participants were on the assumption that both creams were analgesics.

371 The current design allows us to parse the magnitude of placebo analgesia by not only comparing the
372 inert cream against an actual analgesic but also accounting for the variability associated with the
373 stimulation of mirror body territories which were not treated with the inert cream or actual analgesic

374 (Fig. 1), in a sample (n=39) larger than the majority of similar previous studies. Our results indicate
375 a small non-significant difference between placebo and the actual analgesic condition as reflected
376 by ratings of pain unpleasantness of pain (Fig. 2). Interestingly, the control conditions revealed a
377 trend similar to the treatment conditions (namely analgesia lower than placebo). This was accounted
378 for by greater pain unpleasantness during the placebo-control condition compared to all the other
379 conditions (Fig. 2 B). The N2-P2 LEPs confirmed that the most important factor explaining
380 variability of these neural responses was the expectation of being treated with an analgesic cream,
381 regardless of whether this cream was a real analgesic or just vaseline (Fig. 4).

382 These findings provide further evidence in support of the response expectancy theory (Kirsch,
383 1997; Koyama, McHaffie, Laurienti, & Coghill, 2005; Montgomery & Kirsch, 1997). Akin to other
384 studies we provided our volunteers with positive expectation about the treatment and did not
385 implement a conditioning procedure (De Pascalis, Chiaradia, & Carotenuto, 2002; Paul Enck,
386 Bingel, Schedlowski, & Rief, 2013; Pollo et al., 2001). On the contrary, we implemented a
387 well-established script of verbal suggestion within a ritual context (see appendix) that led the
388 majority of healthy volunteers to believe in the experience of a reduction of pain following
389 administration of an inert cream, particularly a decrease in the affective component of their
390 sensation. Interestingly, we observed a greater difference between placebo treatment and control
391 (namely, a greater reduction of pain) than between analgesic treatment and control (Table 1).
392 Individuals who showed a greater self-reported placebo effect as measured with the pain
393 unpleasantness ratings also demonstrated a greater modulation of the N2-P2 amplitude for placebo
394 treatment (Fig. 4). This robust positive relationship between the behavioral and the neural marker
395 provides an index of convergent validity for the reported results.

396 An alternative interpretation of the current results can also be based on a “nocebo” effect
397 associated with the control (i.e. no treatment) conditions. Such an interpretation would suggest that

398 individuals who experienced a lower placebo effect had greater negative expectation from the pain
399 stimulation on the control limb, and this correlated with the extent of the N2-P2 modulation. Other
400 authors have similarly speculated that the placebo and nocebo conditions may be used by
401 experimental volunteers as reference perceptual criterion against which compare the sensations
402 experienced during the “neutral” control condition (Freeman et al., 2015). Future studies may
403 address not only the role of implicit and explicit positive expectations in triggering and maintaining
404 placebo analgesia but also the role of co-occurring implicit contextual negative expectations that
405 may arise from the stimulation of non-treated body parts. This observation leads us to two important
406 caveats. First, the significance of these findings, and more generally of those obtained in the context
407 of laboratory experiments on healthy volunteers, should not be generalized to the understanding of
408 placebo responses in pain patients. In fact, a lack of correlation between placebo analgesia in
409 experimental pain and clinical pain has been reported (Muller et al., 2016). Second, the
410 interpretation of placebo effects is context-dependent and importantly relies on individuals’
411 interpretation of the treatment context (Enck & Klosterhalfen, 2013; Whalley, Hyland, & Kirsch,
412 2008). Consequently, different experimental designs can affect participant’s interpretation to a
413 different extent and contribute to differences in the magnitude of the placebo effect.

414 Notwithstanding these caveats, our experimental design allowed us to precisely test the size of
415 the placebo effect by calibrating it against a true analgesic. The experimental design allowed a
416 head-to-head comparison between the analgesic and the placebo, due to the presence of both a real
417 analgesic compound and of a non-treated skin territory on a body area exactly contralateral to the
418 experimentally treated one. Unfortunately however, this design does not allow us to examine the
419 earliest response to nociceptive stimuli, as measured through the N1 component (Valentini et al.,
420 2012) as upper and lower limbs are associated with different arrival time in the somatosensory
421 cortices, and thus with different latencies of the evoked brain signals. Hence we focused on the
422 magnitude of the N2 and P2 potentials for the current study. It is noteworthy that the majority of

423 previous studies report a reduction of the N2 and P2 potentials during placebo analgesia (Colloca et
424 al., 2008; Martini, Lee, Valentini, & Iannetti, 2015; Wager, Matre, & Casey, 2006; Watson,
425 El-Deredy, Vogt, & Jones, 2007).

426 In conclusion, our findings provide an ecologically valid estimate of the placebo analgesia
427 effect by comparing a placebo treatment directly against that of a real analgesic. We show that
428 verbal suggestions alone are sufficient to establish a moderate placebo effect and that
429 unpleasantness of pain is the most sensitive measure of the placebo analgesia. We also show that the
430 EEG measures of placebo analgesia are strongly correlated with the magnitude of the placebo
431 analgesia computed from pain unpleasantness ratings. Future studies should examine individual
432 differences in the behavioural and neural measures of placebo analgesia.

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438 **Appendix**

439 *Induction script*

440 "Thanks for coming. You are volunteering for the final phase of a clinical evaluation of two new
441 analgesics, *Exacaine* and *Varicaine* (these are the commercial labels and the active component
442 cannot be disclosed). The active components are completely harmless and have no side effects in
443 humans. You will participate in a study in which we will be testing the efficacy of a new analgesic
444 technique on the experience of pain and on brain activity. During the experiment we will deliver
445 thermal (laser) stimuli which can induce pricking and hot sensations. These sensations may be
446 interpreted as painful depending on your very personal estimate. Importantly, we will use only one
447 stimulus energy during the experiment, which will correspond to what you will judge as a moderate
448 sensation of pain. We will spread one cream on one limb and the other cream on another limb. It
449 will take about 10 minutes to come into action. Afterwards we will rub it off from your skin and
450 start with the stimulation protocol".

451

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