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Combining a Breath-Synchronized Olfactometer and Brain Stimulation to Study the Effect of Odor on Corticospinal Excitability and Effective Connectivity

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1 **TITLE:**

2 Combining a Breath-Synchronized Olfactometer and Brain Stimulation to Study the Effect of Odor
3 on Corticospinal Excitability and Effective Connectivity

4
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30
31 **KEYWORDS:**

32 olfaction, odor hedonics, primary motor cortex, dorsolateral prefrontal cortex, connectivity,
33 brain stimulation.

34
35 **SUMMARY:**

36 This paper describes using a breath-synchronized olfactometer to trigger single- and dual-coil
37 transcranial magnetic stimulation during odorant presentation synchronized to human nasal
38 breathing. This combination allows us to objectively investigate how pleasant and unpleasant
39 odors impact corticospinal excitability and brain-effective connectivity in a given individual.

40
41 **ABSTRACT:**

42 It is widely accepted that olfactory stimulation elicits motor behaviors, such as approaching
43 pleasant odorants and avoiding unpleasant ones, in animals and humans. Recently, studies using
44 electroencephalography and transcranial magnetic stimulation (TMS) have demonstrated a
45 strong link between processing in the olfactory system and activity in the motor cortex in

46 humans. To better understand the interactions between the olfactory and the motor systems
47 and to overcome some of the previous methodological limitations, we developed a new method
48 combining an olfactometer that synchronizes the random order presentation of odorants with
49 different hedonic values and the TMS (single- and dual-coil) triggering with nasal breathing
50 phases. This method allows probing the modulations of corticospinal excitability and effective
51 ipsilateral connectivity between the dorsolateral prefrontal cortex and the primary that could
52 occur during pleasant and unpleasant odor perception. The application of this method will allow
53 for objectively discriminating the pleasantness value of an odorant in a given participant,
54 indicating the biological impact of the odorant on brain effective connectivity and excitability. In
55 addition, this could pave the way for clinical investigations in patients with neurologic or
56 neuropsychiatric conditions that could present odor hedonic alterations and maladaptive
57 approach-avoidance behavior.

58

59 **INTRODUCTION:**

60 It is widely accepted that olfactory stimulation elicits automatic reactions and motor behaviors.
61 For example, in humans, the existence of an avoidance motor response (leaning away from the
62 odor source) occurring 500 ms after negative odor onset has been recently demonstrated¹. By
63 recording freely moving human participants exploring odors emanating from flasks, Chalencón
64 et al. (2022) showed that motor behaviors (i.e., speed of approach to the nose and withdrawal
65 of the flask containing the odorant) are closely linked to odor hedonics². Moreover, a close link
66 between processing in the olfactory system and activity in the motor cortex has been recently
67 demonstrated in humans by using electroencephalography¹. Specifically, approximately 350 ms
68 after the onset of negative odors, a specific mu rhythm desynchronization, known to reflect
69 action preparation processes, was observed over and within the primary motor cortex (M1),
70 shortly followed by a behavioral backward movement¹. Strengthening the idea of a relationship
71 between the olfactory and motor systems, another recent study showed that exposure to a
72 pleasant odorant increased corticospinal excitability compared to a no-odor condition³. In this
73 study, single-pulse transcranial magnetic stimulation (spTMS) was applied to M1 to evoke a
74 motor-evoked potential (MEP) in a target hand muscle, recorded peripherally with
75 electromyography (EMG) during odor perception. The exposure to pleasant odorant was
76 passively provided by paper strips sodden with pure bergamot essential oil and placed on a metal
77 holder under the nose³. In this context, it remains unclear whether the facilitation of the
78 corticospinal excitability is due to the pleasant odorant stimulation or to unspecific behavioral
79 effects such as sniffing and teeth clenching^{4,5}. Furthermore, it is still unknown how an unpleasant
80 odorant modulates M1 excitability probed by TMS.

81

82 In summary, this highlights the need to develop a method that offers the following advantages
83 over existing techniques used in previous studies^{3,6}: (1) randomizing the presentation of different
84 odor conditions (pleasant/unpleasant/no-odor) within the same experimental phase, (2)
85 precisely synchronizing odorant presentation and TMS timing according to the human nasal
86 breathing phases (inspiration and expiration) when studying the motor system.

87

88 TMS can also be used as a tool to investigate cortico-cortical interactions, also called effective
89 connectivity, between multiple cortical areas and M1 with a high temporal resolution⁷⁻¹². Here,

90 we use a dual-site TMS (dsTMS) paradigm, in which a first-conditioning stimulation (CS) activates
91 a target cortical area, and a second-test stimulation (TS) is applied over M1 using another coil to
92 evoke an MEP. The effect of the CS is evaluated by normalizing the amplitude of the conditioned
93 MEP (dsTMS condition) to the amplitude of the unconditioned MEP (spTMS condition)¹³. Then,
94 negative ratio values denote suppressive cortico-cortical interactions, while positive ratio values
95 denote facilitatory cortico-cortical interactions between the two stimulated areas. The dsTMS
96 paradigm thus provides a unique opportunity to identify the nature (i.e., facilitatory or
97 suppressive), the strength, and the modulations of the effective connectivity between the
98 preactivated area and M1. Importantly, cortico-cortical interactions reflect a complex balance of
99 facilitation and suppression that may be modulated in different timing and mental states or
100 tasks^{7,14}.

101
102 To our knowledge, the relatively new dsTMS paradigm has never been used to investigate
103 cortico-cortical interactions during odor perception with different hedonic values. However,
104 neuroimaging studies have shown that exposure to pleasant and unpleasant odorants induces
105 connectivity changes in areas involved in emotion, decision-making, and action control, including
106 the supplementary motor area, the anterior cingulate cortex, and the dorsolateral prefrontal
107 cortex (DLPFC)^{15,16}. Indeed, the DLPFC is a key node mediating emotional control, sensory
108 processing, and higher-level aspects of motor control, such as preparatory processes¹⁷⁻¹⁹. In
109 addition, both human and animal studies have provided evidence that the DLPFC has diverse
110 neuronal projections to M1^{17,18,20-22}. Depending on the context, these DLPFC projections can
111 either facilitate or inhibit M1 activity^{7,19,20}. Thus, it seems possible that the effective connectivity
112 between DLPFC and M1 is modulated during odor presentation and that pleasant and unpleasant
113 odorants recruit separated cortical networks, leading to a differential effect on DLPFC-M1
114 connectivity.

115
116 Here, we propose a new method suitable for the methodologically rigorous study of the
117 modulations of corticospinal excitability and effective connectivity that might occur during the
118 perception of pleasant and unpleasant odors, all delivered in synchrony with human nasal
119 breathing.

120

121 **PROTOCOL:**

122

123 All experimental procedures described in the following sections have been approved by an Ethics
124 Committee (CPP Ile de France VII, Paris, France, protocol number 2022-A01967-36) in accordance
125 with the Declaration of Helsinki. All participants provided written informed consent before study
126 enrollment.

127

128 **1. Participant recruitment**

129

130 **1.1. Inclusion/exclusion criteria.**

131

132 **1.1.1. Include adult (> 18 years) participants. Screen all participants for any contraindications to**
133 **TMS according to international expert guidelines are excluded²³.**

134

135 1.1.2. Exclude participants with implanted medical devices (e.g., cochlear implant, cardiac
136 pacemaker, etc.), a personal or family history of seizure, headache, brain trauma, and
137 neuroactive medication. Exclude participants considered “anosmic” according to the European
138 Test of Olfactory Capabilities²⁴.

139

140 1.2. Handedness: Check for right-handedness as assessed by the Edinburgh Handedness
141 Inventory questionnaire²⁵.

142

143 NOTE. It is highly recommended to recruit only right-handed participants in studies assessing
144 corticospinal excitability and effective connectivity in the motor system^{26,27}.

145

146 1.3. Information and informed consent: Give all participants basic information about the study
147 objectives, procedures, and risks approved by the Ethics Committee and ask them to sign written
148 informed consent.

149

150 **2. Experimental procedure**

151

152 2.1. Patient installation: Ask the participant to sit on a comfortable chair (dental chair type)
153 with both hands relaxed and pronated. Position the participant’s head on a chin rest to minimize
154 head movement during stimulation.

155

156 2.2. Electromyography recordings

157

158 2.2.1. Prepare the participant’s skin before electrode application using an exfoliant scrub to
159 lightly abrade the areas and clean the areas using alcohol pads where electrodes will be applied.

160

161 2.2.2. Apply two silver/silver chloride disposable recording electrodes with a belly-tendon
162 montage of the first dorsal interosseous (FDI) muscle. Add the ground electrode to the styloid
163 process of the ulna (**Figure 1**).

164

165 2.2.3. Connect the electrodes to the amplifier with cables and the data acquisition system.

166

167 2.2.4. Record the EMG signal using an analog-to-digital (AD) conversion system. Amplify and
168 filter EMG signals (gain = 1000) using a bandwidth frequency between 10 Hz and 1 kHz. Digitize
169 at a sampling rate of 2,000 Hz and store each EMG file for offline analysis.

170

171 2.2.5. Check the quality of the signal displayed on the computer screen connected to the data
172 acquisition system.

173

174 2.3. TMS coil_{M1} position.

175

176 2.3.1. Connect this coil to the A stimulator (**Figure 1**).

177

178 2.3.2. Place a tight-fitting cap over the participant's head. Use a tape measure to perform
179 nasion-inion, tragus-tragus, and head-circumference measurements based on standard cranial
180 landmarks. Identify and mark with a pen the scalp vertex at the intersection of the mid-sagittal
181 (nasion-inion) and interaural (tragus-tragus) lines²⁸.

182
183 2.3.3. Place tangentially to the scalp the first small figure-of-eight coil (inner diameter: 40 mm)
184 over the presumed hand area of the left M1 (coil_{M1}), which is 5 cm lateral from the vertex, with
185 the handle pointing backward and laterally at a 45° angle to the midsagittal line, resulting in a
186 posterior-anterior current flow (monophasic current waveform). This orientation corresponds to
187 a maximum induced current flowing within M1 within M1²⁹.

188
189 2.3.4. Ensure that the placement of the coil_{M1} is optimal, in accordance with the most recent
190 international recommendations³⁰. Start by delivering a few single pulses at 30% of the maximum
191 stimulator output (%MSO) and check that the stimulation produces an MEP as recorded by the
192 EMG system and displayed on the computer screen connected to the data acquisition system.

193
194 2.3.4.1. If there are no visible responses, gradually increase the stimulation intensity (5
195 %MSO increments) until MEPs are observed. Then, test four spots around the first site by
196 delivering multiple pulses. Determine the mean peak-to-peak MEP amplitude for each site.

197
198 2.3.4.2. Select the location where the average peak-to-peak MEP amplitude is the highest.
199 This is the so-called hotspot location for the participant³⁰. Mark the coil_{M1} location on the cap to
200 ensure proper coil placement throughout the experiment.

201
202 2.4. Resting motor threshold (rMT) and TMS intensities

203
204 2.4.1. Determine the resting motor threshold (rMT) defined as the TMS intensity that produces
205 a 50% probability of eliciting an MEP^{23,30}.

206
207 2.4.1.1. Use the available online freeware (TMS Motor Threshold Assessment Tool, MTAT
208 2.1), which is based on a maximum-likelihood parameter estimation using a sequential testing
209 strategy²⁹. The stimulation sequence always starts with the intensity set at 37 %MSO.

210
211 2.4.1.2. Let one experimenter hold the coil_{M1} while another indicates whether the MEP
212 amplitude is > 0.05 mV. The predictive algorithm then determines the next stimulation intensity
213 to be delivered and is stopped after 20 stimulations, which provides sufficient accuracy for the
214 rMT estimation according to previous studies³¹⁻³⁴.

215
216 2.4.2. Set the %MSO for the conditioning and the test pulse stimulation. Use the previously
217 determined rMT value of the participant.

218
219 NOTE: Here, the intensity for the first conditioning stimulation (coil_{DLPFC}) was set to 110% of the
220 rMT^{19,20}. The intensity of the test stimulation (coil_{M1}) was set at 120% of the rMT, an intensity
221 that differs slightly from previous studies that used a TS intensity that evoked a MEP of ~1 mV in

222 all participants^{19,20}. This fixed peak-to-peak intensity occurs at very different points on the input-
223 output recruitment curves due to the high inter-subject variability in motor output³⁵. Therefore,
224 the stimulation intensity could be optimized using 120% RMT intensity across individuals.

225

226 2.5. TMS coil_{DLPFC} positioning

227

228 2.5.1. Connect this coil to the B stimulator (**Figure 1**).

229

230 2.5.2. Use the recently updated scalp heuristic to locate the region of the scalp corresponding
231 to the left DLPFC^{36,37} to estimate the position of the second small figure-of-eight coil (internal
232 diameter: 40 mm) over the DLPFC (coil_{DLPFC}). Download the online Excel Spreadsheet Calculation
233 Tool³⁶ and enter the nasion-inion and tragus-tragus distances and the head circumference in
234 centimeters as inputs. Report the X_{LA} and the Y_{LA} distances directly on the participant's head.

235

236 2.5.3. Place tangentially to the scalp the coil_{DLPFC} over the presumed left DLPFC location, with
237 the handle pointing downward and laterally at a -45° angle to the mid-sagittal line. Mark the
238 coil_{DLPFC} placement on the cap to ensure proper coil placement throughout the experiment.

239

240 NOTE: This scalp-based targeting method for both coil_{M1} and coil_{DLPFC} locations is not optimal. In
241 fact, it is known to be less accurate than the neuronavigation method used to target the brain
242 areas of interest based on individual T1 anatomical magnetic resonance imaging (MRI)³⁸.

243

244 2.6. Delay between the conditioning and test pulses: Set this delay to 10 ms on the pulse
245 generator device.

246

247 NOTE: Here, the delay is fixed at 10 ms based on previous studies showing an inhibitory influence
248 from the left DLPFC to the left M1 at this interval^{19,20}. This inhibitory effect observed at 10 ms is
249 likely due to the activation of the basal ganglia via the DLPFC projections to the pre-SMA, thereby
250 exerting an indirect influence on M1³⁹. The delay can be adjusted in the code according to the
251 user's needs. For example, a longer interstimulation interval (i.e., 25 ms) could be used to
252 investigate polysynaptic indirect cortico-subcortical-cortical circuits connecting DLPFC to M1¹⁹.
253 Furthermore, differential facilitatory/inhibitory influences have been demonstrated using dual-
254 site ppTMS between multiple cortical areas, with intervals ranging from 1 ms to 150 ms^{40,41}. Thus,
255 the fact that the interval can be adjusted opens the way to a wide range of possibilities for future
256 research studies.

257

258 2.7. Olfactometer settings

259

260 2.7.1. Select odorants with pleasant and unpleasant hedonic values. Dilute in advance the
261 odorants individually in mineral oil to create iso-intense perception.

262

263 NOTE: Here, the selection and concentration of odorants (i.e., isoamyl acetate and butyric acid
264 diluted to 0.6% and 0.11% vol/vol concentrations, respectively) were based on previous studies
265 by our group using the same olfactometer setup and odorants^{42,43}. A pilot study confirms that

266 the positive and the negative odors did not differ in terms of intensity but were opposite in
267 hedonic value. In the control condition (i.e., no odorant), only airflow is delivered to the
268 participant.

269

270 2.7.2. Write the code to deliver the odorants. For each trial, indicate the total duration of the
271 trial, the odorant to be delivered, the flow rate of the odorant controller (in milliliters per
272 minute), the flow rate of the carrier air regulator (in milliliters per minute), and the flow rate of
273 the suction regulator.

274

275 NOTE: The order of the odorant delivered can be randomized between positive, negative, and
276 no-odor. Here, each trial has a duration of 12 s. The order of odor delivered was pseudo-
277 randomized. In addition, based on a pilot experiment, the flow rate of the odor controller was
278 set to 200 mL/min, the flow rate of the carrier air regulator at 500 mL/min, and the flow rate of
279 the suction regulator at 100 mL/min.

280

281 2.7.3. Position the nasal cannula near the participant's nostrils to measure nasal breathing.
282 Instruct the participant to breathe normally through the nose.

283

284 2.7.4. Turn on the portable air compressor, the olfactometer case, and the PC containing the
285 software. Check all the cable connections (**Figure 1**).

286

287 NOTE: The olfactometer used in the current study has been described in detail in a previous
288 publication⁴⁴ but has been modified here to allow TMS triggering with variable delays after
289 inspiration onset detection. Briefly, the device is composed of several modules, including 1) an
290 air source and air treatment system coming from a portable air compressor, 2) a stimulation
291 system including electronic and pneumatic devices, 3) a homemade mixing head coupled to a
292 delivery system that allows the diffusion of odorants in the participant's nose, 4) a respiratory
293 sensory system that triggers the olfactometer according to the nasal respiration measurement
294 with a nasal cannula and 5) a software control system⁴⁴.

295

[Place **Figure 1** here]

296

297
298 2.7.5. Calibration: Proceed to the calibration phase (about 20 s.), which allows for calibrating
299 the participant's respiratory signal and adjusting the detection thresholds of the expiratory and
300 inspiratory phases. In this software, the expiratory phase is positive, and the inspiratory phase is
301 negative.

302

303 2.7.6. Odor hedonic and intensity ratings: Deliver the two odorants in a randomized order and
304 ask participants to rate the hedonic value and intensity of each odorant on visual analog scales
305 ranging from 1 "not at all pleasant" to 9 "extremely pleasant" and from 1 "not at all intense" to
306 "extremely intense."

307

308 2.8. Combining olfactometer and TMS: Set the delay between the detection of the inspiration
309 phase and the trigger for sending the TMS at 600 ms.

310

311 NOTE: The setting of the delay is important and has to be determined according to the
312 literature and the needs of the user. In this protocol, the delay was set at 600 ms, which has
313 been shown to be the maximum conscious perceptual representation of odors⁴⁵. For single-
314 pulse TMS condition, this trigger immediately activates the A stimulator, and a pulse is
315 delivered by the coil positioned on the left M1 to evoke an unconditioned MEP. For dual-coil
316 TMS condition, this trigger is sent to two different devices (via two coaxial cables connected by
317 a T-connection): the first one immediately activates the B stimulator and a conditioning pulse is
318 delivered by the coil positioned on the left DLPFC; the second one is received by a pulse
319 generator which makes it possible to induce a fixed delay before activating the A stimulator,
320 thus delivering a test-stimulation through the coil positioned on the left M1 to evoke a
321 conditioned MEP (**Figure 1**).

322

323 **3. Measurements**

324

325 3.1. Run the custom-made coding script in the olfactometer software (see 2.7.2) to deliver all
326 combinations of spTMS and dsTMS with pleasant and unpleasant odors and no-odors occurring
327 in a random order.

328

329 NOTE: Here, 20 trials were recorded for each condition (120 trials in total). The experiment was
330 divided into 6 blocks of 20 trials each. The number of trials for each condition can be changed
331 according to the user's needs.

332

333 **4. Data analyses**

334

335 4.1. For each participant, condition, and trial, extract the peak-to-peak MEP amplitude. This
336 can be done using one of the open-source Toolboxes available online^{46,47}.

337

338 4.2. Normalize the data by calculating an MEP ratio expressing MEPs elicited by the test
339 stimulation in dsTMS trials relative to MEPs elicited by the test stimulation in spTMS trials¹². Do
340 this separately for each participant and for each odor condition (i.e., no-odor, positive odor, and
341 negative odor). After this procedure, interpret the results as follows: MEP ratios above 1 indicate
342 a facilitatory influence of the DLPFC on M1, whereas MEP ratios below 1 indicate an inhibitory
343 influence of the DLPFC on M1.

344

345 **REPRESENTATIVE RESULTS:**

346 The representative data presented here reflect recordings from participants after completing the
347 step-by-step protocol above to provide a preliminary insight into what we might expect.

348

349 **Figure 2** shows an example of a representative participant's respiratory signals recorded with the
350 olfactometer software. The expiratory and inspiratory phases are well detected when the
351 thresholds are crossed. The odorant is triggered immediately after the expiration phase threshold
352 and diffuses for 5 s. The TMS pulse is triggered with a delay (600 ms) after the inspiration phase
353 threshold.

354 This result demonstrates that the method developed here can precisely synchronize odorant
355 diffusion and TMS timing according to human nasal breathing phases.

356
357
358

[Place **Figure 2** here]

359 **Figure 3** illustrates the results obtained from EMG data recordings from the right FDI muscle
360 (MEP recordings), according to the conditions (spTMS and dsTMS) and the odor hedonic values
361 (no-odorant, positive odorant and negative odorants) for a representative participant. The peak-
362 to-peak amplitude of the MEPs evoked by spTMS (**Figure 3A**) and by dsTMS (**Figure 3B**) varied
363 according to the hedonic value of the odorant. When the results are normalized (**Figure 3C**), all
364 MEP ratios are below 1, indicating a suppressive effect of the left DLPFC on the left M1. This
365 result demonstrates that the method developed here allows the investigations of modulations
366 of corticospinal excitability and effective connectivity that occur during pleasant and unpleasant
367 odor perception, all delivered in a synchronized manner with human nasal breathing. These
368 results are preliminary and deserve further investigation to conclude on the specific effects of
369 odors hedonic values on corticospinal excitability and effective connectivity.

370
371
372

[Place **Figure 3** here]

373 **FIGURE AND TABLE LEGENDS:**

374 **Figure 1: Experimental setup.** The bold lines represent pneumatic connections. An air
375 compressor is connected to the olfactometer to generate different air flows. A regulator controls
376 the pressure, and the input air flow is directed to 3 channels (through 3 mass regulators): one for
377 the air conveyor (blue line), one for the aspiration system (brown line) to clean and help control
378 the stimulation time and the last one for the odorants⁴⁴. Two U-shaped tubes contain the
379 odorants (green: pleasant; red: unpleasant) in which they are conditioned under pressure in the
380 saturated steam state, ensuring an odorized air flow with stable intensity over time. The mixing
381 head is used to mix the clean and odorized air streams. The airflow (odorized or pure) is delivered
382 to the nostrils through two tubes (gray lines) attached to a nasal cannula, which is also used to
383 record nasal breathing (purple line). Based on the respiratory signal, as soon as the inhalation
384 phase is detected, for the spTMS condition a trigger is sent to a pulse generator device used to
385 set a delay (here: 10 ms), then to a TMS stimulator A connected to Coil_{M1} applied over the left
386 M1 hand muscle representation, while the TMS stimulator B is turned off. For the dsTMS
387 condition, a trigger is immediately sent to the TMS stimulator B connected to the Coil_{DLPFC} applied
388 over the left DLPFC, and the pulse generator device is used to set a delay (here: 10 ms) before
389 triggering the TMS stimulator A connected to the Coil_{M1}. The respiratory signal and MEP
390 amplitude acquired by the EMG system are recorded by software installed on a PC.

391

392 **Figure 2: Example of raw data from respiratory recordings for a representative participant.** The
393 expiration phase is detected when a threshold (represented by the red line) is crossed. Inspiration
394 phase is detected when a threshold (represented by the blue line) is crossed. The odorant is
395 triggered immediately after the expiration phase threshold and diffuses for 5 s, as shown by the
396 green line. The TMS pulse is triggered with a delay (600 ms) after the inspiration phase threshold.

397

398 **Figure 3: Example of typical raw recordings from the right FDI muscle of a participant.** (A) spTMS
399 condition with positive odorant (green), negative odorant (orange), and the no-odorant
400 conditions (gray). (B) dsTMS condition, with positive odorant (green), negative odorant (orange)
401 and the no-odorant conditions (gray). (C) MEP ratios obtained after the normalization procedure
402 for a representative participant. The three MEP ratios are below 1, indicating an inhibitory
403 influence of the DLPFC on M1. Raw MEP traces represent a single trial recording. Bar graphs show
404 the mean, the standard deviation, and the individual MEP value of the 20 trials obtained in each
405 condition.

406

407 **DISCUSSION:**

408 The protocol above describes a novel method combining the use of a breath-synchronized
409 olfactometer with single- and dual-coil TMS to investigate changes in corticospinal excitability
410 and effective connectivity depending on the hedonic value of the odorants. This setup will allow
411 for objectively discriminating the pleasantness value of an odorant in a given participant,
412 indicating the biological impact of the odorant on brain effective connectivity and reactivity. The
413 critical steps in this protocol involve both TMS parameters (placement, intensities) and
414 olfactometer parameters (odorant selection, timing relative to respiratory phases).

415

416 This combination of spTMS and dsTMS with an olfactometer can be adapted in many ways,
417 depending on the user's needs, and has clear methodological advantages. As mentioned in the
418 introduction, two methodological aspects seemed crucial for a more in-depth investigation of the
419 mechanistic basis of the interactions between the olfactory and motor systems. The first was the
420 possibility of presenting different odor conditions (pleasant/unpleasant/no odor) within the
421 same experimental phase. This is now feasible as it is possible to specify on a trial-by-trial basis
422 which odorant will be delivered to the subject at a constant intensity. This is a crucial point, as it
423 allows us to eliminate the systematic intra-individual changes in MEPs amplitude within and
424 between stimulus blocks observed in previous studies, even at relatively long interstimulus
425 intervals^{48,49}.

426

427 Indeed, the application of a TMS pulse to M1 allows the quantification of the observed changes
428 in corticospinal excitability with undeniable temporal accuracy. However, a very large number of
429 factors can modulate corticospinal excitability, and these need to be controlled as much as
430 possible. For example, the simple fact of voluntary inspiration or exhalation (a motor act)
431 modifies the corticospinal excitability of non-respiratory finger muscles⁵⁰.

432

433 The second was the possibility to control and synchronize several factors with the respiratory
434 phases. These include the precise duration and timing of odor diffusion to the participants and
435 the timing of the TMS pulse. More importantly, these different parameters can be modified
436 according to the user's needs, opening the way for future studies.

437

438 The method presented here opens the way for a wide range of future research and broader
439 questions in the field of olfaction. First, no study has yet examined the temporal precision of the
440 modulation of corticospinal excitability in response to an olfactory stimulus. Is this modulation
441 very early (i.e., before the emergence of perceptual odor representations, estimated to be

442 between 300 ms and 500 ms after odor onset⁴⁵) or later (i.e., when odor representations are
443 extended to larger areas associated with emotional, semantic, and memory processing⁴⁵)? Is the
444 timing of changes in corticospinal excitability the same depending on the hedonic value of the
445 odor? Unpleasant odors, such as pain, often signal potential danger, elicit a faster response to
446 quickly avoid or escape negative situations^{51,52}, and thus modulate corticospinal excitability
447 earlier than positive odors. However, this remains speculative. By delivering the TMS pulse at
448 different times after the onset of both positive and negative odors and comparing the changes
449 in corticospinal excitability, the current protocol can address this question. Moreover, while the
450 focus of the present protocol was on the modulation of corticospinal excitability by targeting M1,
451 the TMS technique, due to its high temporal resolution, can also be used to investigate the causal
452 brain-behavior relationships and the time course of other areas during olfactory processes⁵³.
453 Similarly, in the current protocol, we evaluated the effective connectivity between the DLPFC and
454 M1 because there is evidence in the literature that modulations of this connectivity may occur
455 during odor perception. However, other cortico-cortical or cortico-subcortical-cortical networks
456 may be modulated during olfaction or motor control processes, and the connectivity within these
457 networks can be easily assessed with this new method. The only change would then be the
458 location of the coils toward the targeted cortical areas. For example, the orbitofrontal cortex has
459 been shown to be involved in coding for odor hedonic value and odor perception⁵⁴, and a recent
460 dual-site TMS study showed that this area has an inhibitory influence on M1 at rest¹². Probing
461 changes in the effective connectivity between the orbitofrontal cortex and M1 during perception
462 of positive and negative odors is an interesting avenue of study for a better understanding of the
463 mechanisms behind the interactions between olfactory and motor systems.

464
465 In addition, this method proposes a new way to reliably assess odor hedonic perception in a non-
466 verbal or conscious manner. This could pave the way for clinical investigations aimed at
467 understanding abnormal interactions between processing in the olfactory and motor systems.
468 For example, the current method could be used in patients with neuropsychiatric disorders such
469 as major depressive disorder (MDD), which has been associated with alterations in olfactory
470 function, including hedonic perception of odors and maladaptive approach and avoidance
471 behaviors⁵⁵. Furthermore, since the left DLPFC has been shown to be hypoactive in MDD
472 patients⁵⁶ and the DLPFC-M1 connectivity is modulated during approach-avoidance behaviors¹⁹,
473 the combination of TMS and an olfactometer may be a promising potential tool to elucidate
474 neurophysiological indicators of dysfunctional connectivity between DLPFC and M1 in MDD
475 patients. Neurophysiological findings can then be correlated with clinical symptomatology, such
476 as the severity of depression or the olfactory anhedonia score, defined as the reduced ability to
477 experience pleasure, found in patients with MDD⁵⁷. Finally, if abnormalities in effective
478 connectivity are revealed in these patients using the method presented here and correlate with
479 clinical symptoms, dual-site TMS could be used repeatedly to neuromodulate DLPFC-M1
480 connectivity and improve clinical symptoms, a protocol called paired-associative cortico-cortical
481 stimulation^{58,59}.

482
483 Although the present method and results provide a proof of concept for future investigations
484 into the neural mechanisms underlying the interactions between the olfactory and motor
485 systems, some limitations and considerations must be mentioned. First, to increase the reliability

486 and reproducibility of the measurements, the targeted brain areas should be precisely based on
487 anatomical and functional areas (this is especially true for the DLPFC target). Second, as
488 mentioned above and as demonstrated by E-field computational modeling, the scalp-based
489 targeting method used to position the coils is suboptimal compared to MRI guidance⁶⁰. To
490 maximize the accuracy and precision of TMS positioning, a neuronavigation system that co-
491 registers the patient's head and structural magnetic resonance imaging (MRI) scan and provides
492 real-time feedback on the coil position should be used³⁸. In addition, computational E-field
493 dosimetry has been shown to provide more efficient and focused stimulation by determining the
494 individual coil placement that maximizes E-field delivery to a specific brain target⁶¹. A third point
495 concerns the interpretation of the results: the amplitude of the MEPs. Indeed, it is known that
496 MEP amplitude reflects the intrinsic different neural inputs to the corticospinal cells, including
497 transcortical elements, and the activity of the spinal motoneuron pool^{62–64}. Therefore, the
498 modulation of corticospinal excitability and effective connectivity observed during the exposure
499 to a pleasant odor provides a partial picture of the more complex supraspinal and spinal networks
500 that are likely to be involved in the modulation of the MEP amplitude. Results should be
501 interpreted with caution.

502

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508

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514

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