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**Dynamic shaping of the defensive peripersonal space through predictive motor mechanisms:
When the “near” becomes “far”**

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(Article begins on next page)

1 **Dynamic shaping of the defensive peripersonal space through predictive motor mechanisms:**
2 **when the “near” becomes “far”**

3

4 **Running title:** Hand blink reflex and motor prediction

5

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34

35 **Abstract**

36 The Hand Blink Reflex (HBR) is a subcortical defensive response, known to dramatically increase
37 when the stimulated hand is statically positioned inside the defensive peripersonal space (DPPS) of
38 the face. Here, we tested in a group of healthy human subjects the HBR in dynamic conditions,
39 investigating whether the direction of the hand movements (up-to/down-from the face) could
40 modulate it. We found that, on equal hand position, the response enhancement was present only when
41 the hand approached to (and not receded from) the DPPS of the face. This means that, when the hand
42 is close to the face but the subject is planning to move the hand down, the predictive motor system
43 can anticipate the consequence of the movement: the “near” becomes “far”. We found similar results
44 both in passive movement condition, when only afferent (visual and proprioceptive) information can
45 be used to estimate the final state of the system, and in motor imagery task, when only efferent
46 (intentional) information are available to predict the consequences of the movement. All these
47 findings provide evidence that the DPPS is dynamically shaped by predictive mechanisms run by the
48 motor system and based on the integration of predictive feedforward and sensory feedback signals.

49

50 **Significance Statement**

51 The defensive peripersonal space (DPPS) has a crucial role for survival and its modulation is
52 fundamental when we interact with the environment, as when we move our arms. Here, we focused
53 on a defensive response, the Hand Blink Reflex (HBR), known to dramatically increase when a static
54 hand is stimulated inside the DPPS of the face. We tested the HBR in dynamic conditions (voluntary,
55 passive and imagined movements) and we found that, on equal hand position, the response
56 enhancement was present only when the hand approached to (and not receded from) the DPPS of the
57 face. This suggests that, through the integration of efferent and afferent signals, the safety boundary
58 around the body is continuously shaped by the predictive motor system.

59

60 **Introduction**

61 The peripersonal space (PPS) is the space directly surrounding the body (Rizzolatti et al., 1997) within
62 we can act and interact. According to a recent review (de Vignemont and Iannetti, 2015), there is not
63 a single representation of PPS but a dual model of peripersonal space. This is based on a functional
64 distinction between goal-directed action and bodily protection. In the present study we focused on
65 the latter concept, that is the defensive peripersonal space (D)PPS (Cooke and Graziano, 2003;
66 Graziano and Cooke, 2006). The DPPS has been recently investigated in humans by recording the
67 Hand Blink Reflex (HBR), which is a subcortical response at the brainstem level elicited by the
68 electrical stimulation of the median nerve at the wrist and recorded from the orbicularis oculi muscles
69 (Sambo et al., 2012a, 2012b; Sambo and Iannetti, 2013; Fossataro et al., 2016). In static condition,
70 the HBR is modulated by the hand position in space: the response dramatically increases when the
71 stimulated hand is located close to the face, inside the DPPS.

72 When we interact with the surrounding environment the modulation of the DPPS can become
73 fundamental to prevent potentially dangerous situations. During voluntary movements, the central
74 nervous system can estimate the final hand position using either motor outflow or sensory inflow
75 (i.e., visual and proprioceptive inputs). These two sources of information can be combined in a
76 predictive model, according to which, once the motor program is selected and sent to the periphery,
77 an efference copy is formed to predict the future body state and the consequences of the movement,
78 that, in turn, are compared with the actual state detected from the sensory feedback (Wolpert et al.,
79 1995; Blakemore et al., 2002; Haggard, 2005).

80 Understanding the role of the predictive motor system in modulating the DPPS during movement
81 might be a first important step toward a full comprehension of the defensive mechanisms in ecological
82 contexts when humans move in a possible dangerous environment. To this aim, we investigated the
83 role of predictive motor mechanisms in dynamically shaping the DPPS during upper limb voluntary
84 movements. To this goal, we recorded HBR when participants were asked to move their right forearm

85 up towards the face (up-moving condition) or down far from the face (down-moving condition).
86 Indeed, movements in different directions could allow us to investigate the response to a dangerous
87 stimulus entering or leaving our DPPS. In each condition, the HBR was elicited during the forearm's
88 movement when the amplitude of the elbow angle reached three pre-defined values. In turn, these
89 three positions determined three hand distances with respect to the face (far, intermediate and near).
90 We hypothesized that the predicted final consequence of the movement (either close to or far from
91 the face) could affect the reflex response amplitude. Thus, on equal hand positions, comparing the
92 up-moving with the down-moving condition, we expected to find a different modulation of the HBR
93 depending on the direction of the hand movement.

94 During voluntary movements, intentional outflow and sensory inflow are both available to estimate
95 the final position of the hand. Thus, to investigate the relative roles of these complementary sources
96 of information in dynamically modulating HBR amplitude during movement, we designed two
97 experiments, employing either passive movements (where only sensory inflow is present) or motor
98 imagery (where, on the opposite, only intentional outflow is present). In the former experiment, the
99 subjects were asked to stay relaxed while the examiner passively moved their right arm up towards
100 or down far from their face; in the latter, the subjects stayed still, keeping their right hand in the
101 intermediate position while imaging to move it up-to or down-from the face.

102

103 **Materials and Methods**

104 *Participants*

105 Thirteen participants, naive to the purpose of the experiment, were recruited for this study. They
106 reported no previous history of neurological disorders or orthopedic problems for the right-dominant
107 hand, as determined by the Edinburgh Handedness Inventory (Oldfield, 1971).

108 Firstly, participants were tested to assess whether they showed a reproducible HBR (Sambo et al.,
109 2012a) (see Preliminary experiment). Ten of them (about the 77% of the total number, 6 females and
110 4 males, mean age \pm std = 22.4 \pm 2.3) satisfied this requirement and were thus chosen to advance to the
111 next stages of the experimental procedure. Participants gave written informed consent before taking
112 part in the study. The study has been approved by the local ethics committee and was conducted in
113 accordance with the Declaration of Helsinki.

114

115 *Experimental set up*

116 The HBR response was elicited by administering transcutaneous electrical stimuli to the median nerve
117 at the right wrist, using a surface bipolar electrode attached with a velcro strap and connected to a
118 Digitimer constant current stimulator (DS7AH HV, Digitimer Ltd, UK). As the stimulator provided
119 constant current pulses, the trial-to-trial variability of the intensity of stimulation was negligible.
120 Stimulus intensity was adjusted to elicit in each participant clear HBR responses (mean stimulus
121 intensities were 26.2 \pm 4.6 mA, range 15-30 mA). None of the participants reported painful sensations
122 elicited by the stimulation. The stimulus duration was 200 μ s and the inter-stimulus interval was ~30
123 s. A twin-axis electronic goniometer (TSD130B, BIOPAC System, Inc.) connected to a BIOPAC
124 MP100 system was used to measure and record the elbow angle during movement execution. In
125 Experiment 1 and in Experiment 2 this device allowed the automatic delivery of the electrical
126 stimulation when the elbow angle corresponded to one of the three pre-determined stimulation
127 positions.

128 EMG activity was recorded by means of two MP100 BIOPAC EMG channels from the orbicularis
129 oculi muscle bilaterally, using two pairs of bipolar surface electrodes with the active electrode over
130 the mid lower eyelid and the reference electrode laterally to the outer canthus. Signals were amplified
131 and digitized at 1 kHz (BIOPAC MP100).

132

133 *Experimental Procedure*

134 The experiments took place in four different sessions. In the first session participants performed the
135 Preliminary experiment during which the HBR responses were acquired in Static condition.
136 Participants who showed a reproducible HBR in Static condition advanced to the next stages of the
137 study. In the second session the selected participants executed Experiment 1 (Voluntary movement).
138 Experiment 2 (Passive movement) and the Experiment 3 (Motor imagery) were randomly executed
139 in two other different sessions. At least one week passed between one experimental session and the
140 following.

141 Participants were seated on a comfortable chair and kept the right elbow at the limit of a table, in a
142 position allowing the right wrist to be in front of the ipsilateral eye while moving the forearm towards
143 the face, but never touching it. The electrical stimulation was delivered, in static condition or during
144 voluntary and passive movements, while participant's stimulated hand was located at three different
145 positions relative to the face. In particular, when the elbow angle was 10° less than the maximal arm
146 extension (far position, α_1), the half of the difference between the angles of maximal arm extension
147 and flexion (intermediate position, α_2), and when the angle was 10° more than the maximal elbow
148 flexion (near position, α_3). Throughout the experiment participants were instructed to keep their gaze
149 on a fixation point placed at 60 cm from the eyes.

150

151 Preliminary experiment: Static condition. This experiment aimed to make an initial selection of those
152 participants who showed a reproducible HBR response. These subjects were admitted to the next
153 sessions of the study. Further, with this study we would like to test the reliability of our set up by
154 replicating the results known in literature. Participants were instructed, trial by trial, to put the arm in
155 one of the three positions previously identified. After a randomly variable delay, the subject received
156 the electrical stimulation, which was manually delivered by the experimenter. Twenty-four

157 acquisitions were performed, 8 for each hand position. The order of the hand positions at which the
158 participant received the electrical stimulus was pseudo-random.

159

160 Experiment 1: Voluntary movement (Figure 1A). The aim of the present experiment was to assess
161 whether the HBR response was modulated during voluntary movement execution and was influenced
162 by movement direction. Participants were asked to perform two sequences of movements with the
163 right arm: elbow flexion-extension (block A) and elbow extension-flexion (block B). These two
164 blocks were introduced to avoid that participants could predict the instant of the stimulation, and the
165 order of blocks execution was balanced across participants. In both blocks, the electrical stimulation
166 was delivered in each trial (flexion-extension or extension-flexion movements) during either elbow
167 flexion (afterwards called Up-moving condition) or elbow extension (afterwards called Down-
168 moving condition), when the angle measured by the goniometer reached one of the pre-set angle
169 values (α_1 , α_2 , α_3). At this time, an electrical signal was automatically generated by the goniometer
170 and triggered the onset of the electrical stimulation. Ninety-six trials (2 blocks, 3 angles, 2 movement
171 directions and 8 repetitions) were acquired. A minimum time of 30 sec was kept as inter-trial interval.
172 During this interval the subjects were asked to keep the arm relaxed. The Voluntary movement
173 condition was preceded and followed by a HBR recording session in Static condition (4 repetitions x
174 3 stimulation positions before and after voluntary movement, for a total of 24 trials). This latter
175 condition was introduced here and in each of the following experiments to test whether subjects' HBR
176 responses in the three stimulation positions were comparable in the different days. Furthermore, this
177 evaluation allowed testing possible effects on HBR amplitude due to habituation.

178

179 Experiment 2: Passive movement (Figure 1B). This experiment was performed to test the role that
180 afferent (i.e., visual and proprioceptive signals) inputs could play in modulating the HBR response.
181 Participants were asked to keep the right arm completely relaxed in a plastic splint while the

182 experimenter moved it by means of a transparent wire connected to the splint and a pulley system.
183 The passive movement was an elbow flexion-extension of the right arm. A second transparent wire
184 was attached to the opposite site of the hand support (hand back) and adjusted in length to avoid that
185 the distance between the hand and the face of the participant was less than 4 cm. At the beginning,
186 the experimenter asked the participant to perform an elbow flexion and extension movement at natural
187 velocity. When the experimenter thought to have understood participant's natural movement velocity,
188 she moved participants' forearm and verbally questioned the subjects if he/she felt the movement
189 velocity similar to his/her velocity. After participant's agreement the experiment started. No
190 differences were found between angular movement velocities evaluated in the Experiment 1 and the
191 Experiment 2 (mean±SD: Experiment 1, 100.13±37 deg/s and Experiment 2, 99.58±23 deg/s;
192 $p=0.95$).

193 The electrical stimulation was delivered during the passive movement when the angle measured by
194 the goniometer reached the pre-set angle values (α_1 , α_2 , α_3) during either elbow flexion (Up-moving
195 condition) or extension (Down-moving condition) movements. As in the Experiment 1, an electrical
196 signal, automatically generated by the goniometer, triggered the onset of the electrical stimulation.
197 Differently from the Experiment 1, in order to reduce participants' expectancy, we introduced catch
198 trials. We didn't introduce the blocks paradigm used in the Experiment 1 because in that condition
199 no significant difference was found between the two blocks (see Results section). This allowed us to
200 dramatically reduce the number of trials. Excluding the catch trials, a total of 48 trials (3 angles, 2
201 directions, 8 repetitions) were acquired. The Passive movement condition was preceded and followed
202 by a HBR recording session in Static condition (4 repetitions x 3 stimulation positions before and
203 after passive movement, for a total of 24 trials).

204

205 Experiment 3: Motor imagery (Figure 1C). Before starting the experimental procedure, all the
206 participants completed the Italian version of the Movement Imagery Questionnaire (MIQ-R; (Hall

207 and Martin, 1997)) to assess their ability to form kinesthetic and visual images. The MIQ-R is an 8-
208 item self-report questionnaire, in which participants rated the vividness of their mental representations
209 using two 7-point scales (associated to visual and kinesthetic imagery): 1 means “really easy to
210 feel/see” whereas 7 corresponds to “really difficult to feel/see”. All participants considered it fairly
211 easy to form motor images and the scores indicated that they possessed good motor imagery abilities
212 (mean \pm SD = 18.8 \pm 5.55). After that, they were instructed to put the arm in $\alpha 1$ and $\alpha 3$ positions in
213 order to memorize them. Then, they were asked to keep the right arm in the position corresponding
214 to $\alpha 2$ (intermediate position) and to kinesthetically imagine the right arm making a flexion (Up-
215 moving condition, from $\alpha 2$ to $\alpha 3$) or an extension (Down-moving condition, from $\alpha 2$ to $\alpha 1$) movement
216 at spontaneous velocity. At the beginning, for few trials, participants had to verbally report when the
217 upper arm reached $\alpha 1$ or $\alpha 3$. When the experimenter learnt the time used by the participant to imagine
218 to move towards one of the two positions, the experiment started. After each trial the subject was
219 questioned whether the electrical stimulation was administered in correspondence to the position set
220 for the current trial ($\alpha 1$ or $\alpha 3$), and imagined by the subject. In case of mismatch, the trial was
221 repeated. As in the Experiment 2, catch trials were introduced to reduce participants’ expectancy of
222 the stimulus. Excluding the catch trials, a total of 16 trials were recorded for each subject (2 imagined
223 directions, 8 repetitions). The Motor imagery condition was preceded and followed by a HBR
224 recording session in Static condition (4 repetitions x 3 stimulation positions before and after motor
225 imagery, for a total of 24 trials).

226 --- Figure 1 here ---

227

228 *Data processing and statistical analysis*

229 A custom made MatLab software was used to process the EMG signals. EMG signals from each
230 participant were filtered and rectified. HBR responses were averaged separately in each condition
231 and for each participant. Trials with an abnormal EMG activity preceding the HBR response were

232 discarded by the analysis. The area under the curve (AUC, mV*ms) of each HBR average waveform
233 was considered as outcome parameter. To compute AUC in each averaged EMG trace the software
234 automatically analyzed a 130 ms-time interval from the stimulus onset that always contained the
235 subject's blink. The resulting curve was then integrated to compute AUC. In all experiments, data
236 were averaged across ipsilateral and contralateral recording sides (right and left eyes) according to
237 the previous analyses proposed in literature (Sambo et al., 2012a).

238 In the Preliminary experiment, AUC values acquired in Static condition were compared by mean of
239 a repeated-measure ANOVA with POSITION (3 levels: α_1 , α_2 and α_3) as within-subject factor. This
240 analysis was used to confirm the literature and thus assess the reliability of our experimental set up.
241 Further, it allowed us to identify the participants who showed a reproducible HBR response.

242 In Experiment 1, Experiment 2 and Experiment 3 the HBR values acquired in Static condition, before
243 and after the different "dynamic" conditions, were subjected to three repeated-measure ANOVA
244 (RM-ANOVA) with POSITION (3 levels: α_1 , α_2 and α_3) and TIME (2 levels: pre, post), as within-
245 subject factors.

246 Furthermore, in order to compare the AUC values associated to the Static condition in Experiment 1,
247 2 and 3 with those of the Preliminary experiment, the AUC values in the pre and post conditions of
248 Experiment 1, 2 and 3 were averaged. Then, these data were statistically compared by means of a
249 RM-ANOVA with POSITION (3 levels: α_1 , α_2 and α_3) and SESSION (4 levels: Preliminary
250 experiment, Experiment 1, Experiment 2 and Experiment 3) as within-subject factors.

251 In the Experiment 1, AUC data were analyzed by mean of RM-ANOVA, with POSITION (3 levels:
252 α_1 , α_2 and α_3), BLOCK (2 levels: A and B), and MOVEMENT DIRECTION (2 levels: Up-moving,
253 Down-moving), as within-subject factors. Furthermore, the HBR responses during voluntary
254 movements (AUC values averaged over the blocks) were compared to those acquired in the
255 corresponding Static condition (data were obtained by averaging AUC values evaluated in the pre
256 and post conditions) by means of a RM-ANOVA with CONDITION (3 levels: Static, Up-moving,

257 Down-moving) and POSITION (3 levels: α_1 , α_2 and α_3), as within-subject factors. In the Experiment
258 2, AUC values were statistically analyzed by mean of a RM-ANOVA with POSITION (3 levels: α_1 ,
259 α_2 and α_3) and MOVEMENT DIRECTION (2 levels: Up-moving, Down-moving). In order to
260 compare AUC values evaluated during passive movement with those in Static condition (data were
261 obtained by averaging AUC values evaluated in the pre and post conditions), a RM-ANOVA with
262 CONDITION (3 levels: Static, Up-moving, Down-moving) and POSITION (3 levels: α_1 , α_2 and α_3),
263 as within-subject factors, was performed. In the Experiment 3, a paired t-test (2 levels: Up-moving,
264 Down-moving), was adopted to evaluate HBR responses during the imagination of flexion and
265 extension movements. Further, we performed an additional analysis where a baseline condition,
266 during which the HBR response in Static condition corresponding to α_2 , was directly compared to
267 HBR amplitudes during MI in both up-moving and down-moving conditions (RM-ANOVA, within
268 factor CONDITION, 3 levels: Static α_2 , MI Up-moving, MI Down-moving). This allowed us to go
269 deeper insight the mechanisms regulating the reflex response when the arm is actually in a Static
270 condition but the motor system is involved in movement planning. Newmann-Keuls post hoc analysis
271 was used to interpret significant interactions. Data in the text are reported as mean \pm SE.

272

273 **Results**

274 Preliminary experiment: Static condition (Figure 2). The statistical analysis showed a significant
275 effect of the factor POSITION ($F(2,18)=7.49$, $p=0.004$). Post hoc tests revealed a significant increase
276 of AUC values in α_3 (22.33 ± 2.55 mV*ms) with respect to α_1 (17.86 ± 2.32 mV*ms, $p=0.02$) and α_2
277 (15.06 ± 1.26 mV*ms, $p=0.003$). These results confirmed the literature showing that when the
278 stimulated arm is close to the face, inside the DPPS, the HBR magnitude is significantly higher than
279 those evoked when the arm is in farther positions.

280

--- Figure 2 here ---

281 Comparison among the Static conditions acquired in the four testing sessions. The results of the RM-
282 ANOVA on AUC values related to the Static conditions acquired in each testing session showed a
283 significant effect of POSITION ($F(2,18)=17.87$, $p=0.00005$), due to the significant increase of α_3
284 with respect to the other stimulation positions (p always < 0.004). No differences appeared among
285 the SESSIONS ($p=0.33$).

286 Experiment 1: Voluntary movement. Single-subjects averaged data in each conditions are shown in
287 Figure 3. RM-ANOVA analysis showed that MOVEMENT DIRECTION ($F(1,9)=5.66$, $p=0.04$) as
288 well as POSITION ($F(2,18)=6.94$, $p=0.006$) significantly affected the amplitude of the HBR
289 responses and a significant interaction between these two factors was found ($F(2,18)=8.34$, $p=0.003$).
290 Post hoc analysis showed that during an elbow flexion movement (Up-moving condition) there was
291 a significant increase of the HBR value in α_3 (12.3 ± 1.7 mV*ms) with respect to α_2 (8 ± 1.1 mV*ms,
292 $p=0.0003$) and α_1 (8.7 ± 1 mV*ms, $p=0.001$) (Figure 4A). Differently, during elbow extension
293 movements (Down-moving condition) no difference in the AUC values was found in the three
294 stimulation positions ($\alpha_1=9.3\pm 1.3$ mV*ms, $\alpha_2=8.9\pm 1.1$ mV*ms, $\alpha_3=9.3\pm 1.1$ mV*ms, p always > 0.7)
295 (Figure 4B). Further, the HBR response in α_3 when moving up to the face was significantly higher
296 than that observed at the same position when the hand moved far from the face ($p=0.001$). Finally,
297 no difference between the two experimental blocks (i.e., elbow flexion-extension and elbow
298 extension-flexion) was found ($p=0.23$).

299 RM-ANOVA on AUC values evaluated in the Static condition revealed an effect of the factor
300 POSITION ($F(2,18)=11.21$, $p=0.0006$) due to the significant increase of the AUC values in α_3
301 (25.24 ± 3.34 mV*ms) with respect to α_2 (18.15 ± 3.3 mV*ms, $p=0.0009$) and α_1 (19.56 ± 3 mV*ms,
302 $p=0.002$). No differences appeared in the HBR responses acquired in static condition before and after
303 voluntary movement ($p=0.59$).

304 Further, when we compared HBR responses in Static condition and during voluntary movement a
305 significant interaction between CONDITION and POSITION ($F(4,36)=4.52$, $p=0.005$) was found.

306 Post doc analysis revealed that AUC values in static condition and in each arm's position were
307 significantly higher than those obtained during voluntary movement, regardless of movement
308 direction (p always < 0.05).

309 --- Figure 3 here ---

310 --- Figure 4 here ---

311

312 Experiment 2: Passive movement. The statistical analysis showed a significant interaction between
313 POSITION and MOVEMENT DIRECTION ($F(2,18)=6.91$, $p=0.006$). As in the case of voluntary
314 movement, during passive elbow flexion movements (Up-moving condition) the magnitude of the
315 HBR responses significantly increase when the hand was near the face ($\alpha_3=11.8\pm 1.8$ mV*ms) with
316 respect to far ($\alpha_1=6.5\pm 0.9$ mV*ms, $p=0.007$) and intermediate ($\alpha_2=7.3\pm 0.6$ mV*ms, $p=0.02$)
317 positions (Figure 5A), whereas during extension movements (Down-moving condition) no difference
318 was found among the three hand positions ($\alpha_1=9.6\pm 0.9$ mV*ms, $\alpha_2=8.1\pm 0.8$ mV*ms, $\alpha_3=8.2\pm 0.9$
319 mV*ms, p always > 0.3) (Figure 5B). Finally, the HBR response in α_3 when the arm was passively
320 moved up-to the face was significantly higher than that observed at the same position when the arm
321 was moved far from the face ($p=0.03$).

322 RM-ANOVA on the Static condition showed an effect of the factor POSITION ($F(2,18)=8.19$,
323 $p=0.002$), with AUC values in α_3 (21.66 ± 2.95 mV*ms) significantly higher than those in α_2
324 (18.83 ± 2.9 mV*ms, $p=0.006$) and α_1 (19.14 ± 2.7 mV*ms, $p=0.006$). No differences appeared in the
325 HBR responses acquired in static condition before and after passive movement condition ($p=0.18$).

326 When we compared HBR responses in Static condition and during Passive movement, a significant
327 interaction between CONDITION and POSITION ($F(4,36)=4.14$, $p=0.007$) was found. Post doc
328 analysis showed that, regardless of movement direction and arm's position, AUC values were
329 significantly higher in Static condition (p always < 0.001).

330

--- Figure 5 here ---

331

332 Experiment 3: Motor imagery (Figure 6). The results of the paired t-test showed a significant effect
333 of the direction of the imagined movement: when participants imagined a flexion movement (Up-
334 moving condition), from the intermediate to the near position, the HBR responses were significantly
335 higher (10.9 ± 0.9 mV*ms) than when they imagined to extend their arm towards the far position
336 (9.9 ± 0.7 mV*ms) ($t=3.04$, $p=0.01$). Further, when these conditions were directly compared to a
337 situation in which the subject kept the arm fixed in α_2 , the ANOVA showed a significant effect of
338 the factor CONDITION ($F(2,18)=15.09$, $p=0.0001$). The post hoc analysis revealed that MI
339 conditions, irrespective to the imagined movement directions, induced a reduction of the HBR
340 response (p always < 0.0007).

341 Finally, the statistical analysis on the data acquired in Static condition showed a significant effect of
342 the factor POSITION ($F(2,18)=5.31$, $p=0.01$); AUC values in α_3 (17.34 ± 1.34 mV*ms) were
343 significantly higher than those in α_2 (14.68 ± 1.3 mV*ms, $p=0.02$) and α_1 (15.10 ± 1.1 mV*ms,
344 $p=0.02$). No differences appeared in the HBR responses acquired in Static condition before and the
345 motor imagery task ($p=0.29$).

346

--- Figure 6 here ---

347

348 **Discussion**

349 In this study, we sought for evidence that the predictive motor system can modulate a defensive
350 response, the Hand Blink Reflex (HBR). The data discussed here confirm previous evidences on HBR
351 modulation in static condition and provide some important new findings on its modulation in dynamic
352 conditions.

353 The results of the Preliminary experiment performed in Static condition are in agreement with the
354 previously described “hand position” effect, demonstrating that the HBR is significantly enhanced
355 when one’s own stimulated hand is located inside the DPPS of the face (Sambo et al., 2012a, 2012b;
356 Sambo and Iannetti, 2013).

357 The Experiment 1 extended beyond the previous research on DPPS passing from static to dynamic
358 conditions. Firstly, we found a significant HBR enhancement in the near position also in dynamic
359 conditions, i.e., when the moving stimulated hand entered inside the DPPS of the face. It is worth
360 noting that, as previously suggested for Static condition (Sambo and Iannetti, 2013), the HBR
361 response was not linearly enhanced through the three positions (far, intermediate and near), but a
362 safety boundary exists: inside this boundary, the stimulus is potentially dangerous; outside, it is safe.
363 Indeed, in both static and dynamic conditions, comparable HBR responses were found when stimuli
364 were received in far (α_1) and intermediate (α_2) positions. But mostly relevant is the HBR
365 enhancement in the near (α_3) position with respect to α_1 and α_2 only when the hand was moving
366 towards the face and not when the hand was moving down-from the face. In other words, HBR was
367 modulated not only by the position of the hand when receiving the electrical stimulus but also by the
368 direction of the hand movement. This strongly points out that, when the hand is close to the face but
369 the subject is planning to move it far away, the predictive system may anticipate the consequences of
370 the movement. This means that the space representation is dynamically shaped by the movement: the
371 “near” becomes “far”. Thus, what is crucial in HBR modulation it is not the actual position of the
372 stimulated hand, but the future position where the hand is expected to be during the movement. On
373 the other hand, the lack of an increasing of the HBR value when the hand from the far position moves
374 towards the face might suggest that the dynamic shaping of the DPPS interacts with other aspects
375 defining the safety boundary around the body. In particular, the HBR enhancement has a not linear
376 trend: i.e., a significant difference was present only between near position and the other positions.
377 Thus, coherently, the directional modulation could occur only in the near position.

378 During voluntary arm movements, two sources of information are available to the motor system in
379 order to estimate the final state of the movement: the sensory inflow, like the information coming
380 from vision and proprioception, and the motor outflow (i.e., the copy of the motor commands). Thus,
381 the Experiment 1 results could not disambiguate between the role of these kinds of information in
382 modulating the defensive response. One possibility is that this directional effect only pertains to the
383 voluntary movements, when both the afferent and the efferent information are present. Alternatively,
384 the directional effect could be present also when the afferent and the efferent sources of information
385 are dissociated, as in the passive movements (Experiment 2) and in the motor imagery task
386 (Experiment 3). Our data verified this second hypothesis, showing a directional effect on the HBR
387 modulation both during passive movements and motor imagery task.

388 Similarly to the voluntary movement, in the Experiment 2, dealing with passive movements, the HBR
389 response increased when the hand received the stimulus near to the face only in the Up-moving
390 condition, while no difference among the three hand positions was found in the Down-moving
391 condition. This means that the modulation of the HBR response occurred also when only the afferent
392 information (coming from vision and proprioception) was available to the system for predicting the
393 consequence of the movement. Van Beers and colleagues (van Beers et al., 1999) showed that a
394 moving hand is localized by a predictive model that describes a multisensory integration as a
395 direction-dependent weighting of the proprioceptive and visual information. In particular, there are
396 at least two different hypotheses about how the weights given to each modality are determined.
397 According to one hypothesis, the weights are determined by the precision of the information in each
398 modality (Pick et al., 1969; Welch et al., 1979); according to second hypothesis, they are related to
399 the attention that is directed to each modality (Canon, 1970, 1971; Uhlarik and Canon, 1971; Kelso
400 et al., 1975; Warren and Schmitt, 1978). In the present study, it would have been also interesting to
401 manipulate the vision versus proprioception in order to investigate the role of these two afferent inputs
402 in modulating HBR separately. However, in a previous study it has been demonstrated that in static

403 condition the effect of hand position on the HBR persists in absence of vision, thus suggesting a
404 crucial role of proprioception in shaping DPPS (Sambo et al., 2012b).

405 The results of the motor imagery task (Experiment 3) showed that the HBR response was significantly
406 greater when the subject imagined to move up-to than down-from the face, although the arm was kept
407 in the intermediate position and the position remained unchanged during the experiment. Therefore,
408 whether during voluntary and passive movements no modulation was observed in α_2 , here the mere
409 movement planning was sufficient to evoke a different HBR response, suggesting the crucial role
410 played by movement intention. Converging evidences suggest that imagined and actual movements
411 trigger similar motor representations (Jeannerod and Decety, 1995; Gentili et al., 2004; Gandrey et
412 al., 2013) and share overlapping neural substrates (Grèzes and Decety, 2001; Jeannerod, 2001; Lotze
413 and Halsband, 2006; Filimon et al., 2007; Hanakawa et al., 2008). In particular, motor imagery would
414 engage the same internal forward models, i.e., the neural mechanisms that mimic the causal flow of
415 the physical process by predicting the future sensorimotor state, that are involved in action execution
416 (Wolpert and Flanagan, 2001). Looking at the present findings, we might propose that the modulation
417 of HBR response obtained during voluntary movements can be fully explained only when considering
418 both the sensory inflow and the motor outflow. Results from the motor imagery task strongly support
419 that the modulation of the defensive response occurred also when no actual movements were
420 executed, but when only the efferent information (i.e., the efference copy of the motor program) was
421 available to predict the final consequences of the movement. These results point out that the cortical
422 brain activations evoked during the motor imagery task affect the brainstem HBR circuits. As
423 suggested by Sambo and colleagues (Sambo et al., 2012b), the HBR enhancement may result from
424 the modulation of the excitability of brainstem circuits mediating the HBR by associative cortical
425 areas in the prefrontal gyrus and in the ventral intraparietal area, involved in the representation of
426 peripersonal space and in the detection of potentially dangerous stimuli near the face. Motor imagery
427 is known to activate a frontoparietal network (Grèzes and Decety, 2001; Bonzano et al., 2016) which
428 includes some of these associative cortical regions identified as top-down modulators of the activity

429 of the HBR circuitry (Sambo et al., 2012b). Therefore, we might hypothesize that the activation of
430 these areas when the subjects imagine to move the hand in different positions of the DPPS might
431 activate the descending pathways from the associative cortical areas to the medulla in the brainstem
432 and influence the amplitude of the reflex response.

433 Finally, the higher values of HBR response observed in the Preliminary experiment with respect to
434 the other experiments might suggest the use of two distinct neural mechanisms in modulating HBR
435 when the limb is statically positioned or when the motor system is involved, as in voluntary
436 movement, passive movement and motor imagery. Indeed, for each stimulation position, a
437 significantly reduced HBR was found in dynamic conditions with respect to static conditions. The
438 decrease of HBR response during movement can be explained as consequence of the sensory
439 attenuation, according to which the sensory effects generated by one's own actions are attenuated
440 compared to the same effects generated externally (Blakemore et al., 1999; Tsakiris and Haggard,
441 2003; Bays et al., 2006; Weiss et al., 2011). It is important to note that, in our experimental context,
442 during voluntary and passive conditions, the participant's movement triggered the electrical
443 stimulator. Thus, the resulting stimulus can be interpreted as a self-generated sensory effect. In the
444 context of DPPS, this means that a stimulus on which I can exert a direct control is interpreted as less
445 dangerous and produces a lower defensive physiological response with respect to an externally
446 generated stimuli, that is, by definition, outside from the subject's control (REF FRA). A similar
447 explanation could account for the results of the motor imagery condition. Indeed, recent studies
448 showed that the sensory attenuation occurs not only during action execution, but also during action
449 preparation and planning (e.g., Lange, 2009). Alternatively, the movement-related HBR decrease can
450 be explained by the different processing of the proprioceptive inputs in static and dynamic conditions.
451 We can suggest that in the static condition the proprioceptive inputs can give an exact information
452 about the position of the hand with respect to the face. Conversely, when a subject is moving, the
453 rapidly changing inputs generated during hand movements can make more difficult to estimate the
454 hand position and therefore might reduce the amplitude of the reflex response. It is worth noting that

455 the movement-related HBR decrease is particularly relevant for the motor imagery condition, where,
456 although the subjects did not actually move, the mere movement planning was sufficient in order to
457 modify the reflex response. Indeed, since motor imagery activates neural circuits partially overlapped
458 to those active during voluntary movement, the mechanisms engaged during the sensory information
459 processing might be similar to those used during the voluntary movement rather than those involved
460 in a static condition. As consequence, the modulation of the HBR response observed during motor
461 imagery might be similar to that activated by an overt movement execution but reduced with respect
462 to the static condition.

463 Taken together, these findings provide physiological evidence for the role of the predictive motor
464 system in dynamically shaping the defensive peripersonal space during movement.

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539

540 **Figure captions**

541 **Figure 1. Experimental set up.** Panel A refers to Experiment 1, during which participants
542 voluntarily performed either a flexion-extension or an extension-flexion movement of the elbow:
543 while moving up towards (Up) or down far from (Down) the face they received an electrical
544 stimulation in three pre-set positions (far position, α_1 ; intermediate position, α_2 ; near position, α_3).
545 Panel B refers to Experiment 2 and shows the pulley system used by the experimenter to induce the
546 passive flexion-extension (Up) and extension-flexion (Down) movements to participants' right
547 forearm. The electrical stimulation was delivered in the same conditions as in Experiment 1. Panel
548 C refers to Experiment 3 and shows the subject while keeping a static position corresponding to α_2
549 and imagining either an elbow flexion movement towards the face (Up, from α_2 to α_3) or an elbow
550 extension movement (Down, from α_2 to α_1).

551

552 **Figure 2. Preliminary experiment: Static condition.** Group-average, rectified HBR waveforms
553 (left panel) and group-average HBR amplitudes (right panel, AUC, mV*ms) recorded when the arm
554 was placed in the three stimulation positions: far (α_1), intermediate (α_2) and near (α_3). Error bars
555 refer to the standard error of the mean. ** refer to $p < 0.01$.

556

557 **Figure 3. Experiment 1: Voluntary movements.** Rectified and superimposed average EMG traces
558 (mean over the blocks and recording sites) of each participant for the near (α_1), intermediate (α_2)
559 and far (α_3) positions when participants performed up movement towards the face (Up-moving) or
560 down movement far from the face (Down-moving).

561

562 **Figure 4. Experiment 1: Voluntary movements.** On the upper (A) and lower (B) panels are
563 represented the group-average, rectified HBR waveforms (left panel) and the group-average HBR

564 amplitudes (right panel, AUC, mV*ms) in the Up-moving and Down-moving conditions in the
565 three stimulation positions: far (α_1), intermediate (α_2) and near (α_3). Error bars refer to the standard
566 error of the mean. ** refer to $p < 0.01$.

567

568 **Figure 5. Experiment 2: Passive movements.** On the upper (A) and lower (B) panels are
569 represented the group-average, rectified HBR waveforms (left panel) and the group-average HBR
570 amplitudes (right panel, AUC, mV*ms) in the Up-moving and Down-moving conditions in the
571 three stimulation positions: far (α_1), intermediate (α_2) and near (α_3). Error bars refer to the
572 standard error of the mean. * and ** refer to $p < 0.05$ and $p < 0.01$, respectively.

573

574 **Figure 6. Experiment 3: Motor imagery.** Group-average, rectified HBR waveforms (left panel)
575 and group-average HBR amplitudes (right panel, AUC, mV*ms) when participants were asked to
576 imagine to move the forearm from the intermediate to the near position (α_2 to α_3) and from the
577 intermediate to the far position (α_2 to α_1). Dashed lines indicate the HBR waveform (left panel) and
578 response amplitude (right panel) obtained in static condition corresponding to α_2 . Error bars refer
579 to the standard error of the mean. ** refer to $p < 0.01$.