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Defensive peripersonal space: the blink reflex evoked by hand stimulation is increased when the hand is near the face

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Sambo CF, Liang M, Cruccu G, Iannetti GD. Defensive peripersonal space: the blink reflex evoked by hand stimulation is increased when the hand is near the face. *J Neurophysiol* 107: 880–889, 2012. First published November 16, 2011; doi:10.1152/jn.00731.2011.—Electrical stimulation of the median nerve at the wrist may elicit a blink reflex [hand blink reflex (HBR)] mediated by a neural circuit at brain stem level. As, in a Sherringtonian sense, the blink reflex is a defensive response, in a series of experiments we tested, in healthy volunteers, whether and how the HBR is modulated by the proximity of the stimulated hand to the face. Electromyographic activity was recorded from the *orbicularis oculi*, bilaterally. We observed that the HBR is enhanced when the stimulated hand is inside the peripersonal space of the face, compared with when it is outside, irrespective of whether the proximity of the hand to the face is manipulated by changing the position of the arm (*experiment 1*) or by rotating the head while keeping the arm position constant (*experiment 3*). *Experiment 2* showed that such HBR enhancement has similar magnitude when the participants have their eyes closed. *Experiments 4* and *5* showed, respectively, that the blink reflex elicited by the electrical stimulation of the supraorbital nerve, as well as the N20 wave of the somatosensory evoked potentials elicited by the median nerve stimulation, are entirely unaffected by hand position. Taken together, our results provide compelling evidence that the brain stem circuits mediating the HBR in humans undergo tonic and selective top-down modulation from higher order cortical areas responsible for encoding the location of somatosensory stimuli in external space coordinates. These findings support the existence of a "defensive" peripersonal space, representing a safety margin advantageous for survival.

body posture; brain stem circuits; cortex; space representation

REFLEXES ARE INVOLUNTARY AND stereotyped responses to external stimuli, usually mediated by fast, subcortical pathways. Despite their relatively simple neural circuits, in a Sherringtonian sense reflex responses may subserve an important protective function, by rapidly reacting to potentially aversive stimuli to avoid impending danger (Sherrington 1906). More sophisticated and nonstereotyped defensive behaviors are mediated by cortical mechanisms, occurring in associative areas such as the polysensory zone (PZ) in the precentral gyrus and the ventral intraparietal (VIP) area (Graziano and Cooke 2006). These cortical areas are also thought to encode the peripersonal space, i.e., the region of space surrounding the body (Macaluso and Maravita 2010). From an evolutionary point of view, an efficient coordination between these cortical and subcortical systems for reacting to aversive stimuli would maximize the chances of survival. Accordingly, reflexes should be regarded as integrated actions of the organism that may be modulated by higher centers to adapt to different purposes (Sherrington 1906).

The blink reflex (BR) is a prototypical defensive reflex that may be elicited by abrupt and intense stimuli in various sensory modalities (i.e., visual, auditory, and somatosensory). The BR elicited by the electrical stimulation of the trigeminal divisions has been extensively studied and consists of a short-latency (~10 ms), ipsilateral component (R1), and a subsequent (~30 ms), bilateral component (R2) detected in the electromyographic (EMG) activity recorded from the *orbicularis oculi* (Berardelli et al. 1999). The neural circuits for this reflex are entirely located in the brain stem: in the pons for R1 and in the reticular formation of the lower medulla for R2 (Cruccu et al. 2006). In humans, the closure of the eyelids is related to the R2 component only.

A few studies, both in neurological patients (Imamura et al. 1995; Miwa et al. 1995; Miwa et al. 1996; Valls-Solé et al. 1997) and healthy volunteers (Miwa et al. 1998; Alvarez-Blanco et al. 2009), have reported that the BR may also be elicited by electrical stimulation of the peripheral nerves of the limbs, primarily of the median nerve. Although it has been recently suggested that the hand-elicited BR involves also the mesencephalic reticular formation, the extratrigeminal BR is probably analogous to the R2 of the trigeminofacial BR (Leon et al. 2011; Miwa et al. 1995; Miwa et al. 1996; Valls-Solé et al. 1997). The BR elicited by peripheral nerve stimulation is larger when stimuli are applied to the upper than to the lower limb (Alvarez-Blanco et al. 2009; Miwa et al. 1995). This has been interpreted as being due to the shorter conduction distance and, consequently, the more synchronized afferent volleys generated by stimuli applied to the upper limb (Alvarez-Blanco et al. 2009). However, an additional explanation is possible: that the greater proximity of the upper limb to the face is responsible for the larger BR elicited by upper limb stimulation. Indeed, one may expect that the perceived threat of stimuli increases when the stimulated body part is closer to the face, thus resulting in a larger BR.

Here we tested this hypothesis with a series of experiments aiming to answer three main questions. First (*experiments 1* and *3*), we explored whether the magnitude of the BR elicited by electrical stimulation of the median nerve at the wrist [hand blink reflex (HBR)] is increased when the stimulated hand enters the peripersonal space surrounding the face. If the HBR is affected by hand position, this would suggest that the brain stem circuits mediating the HBR undergo top-down modulation from higher order cortical areas responsible for encoding the peripersonal space of the face, such as VIP and PZ.

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Notably, these areas are also thought to compute the location of somatosensory stimuli with respect to the position of the body parts in external space (Azanon et al. 2010; Lloyd et al. 2003; Medina and Coslett 2010). Second (*experiment 2*), because vision affects somatosensory processing and perception of limb position (Longo et al. 2008; Ro et al. 2004; Sambo et al. 2009; Taylor-Clarke et al. 2002; van Beers et al. 1999), we tested whether vision of the stimulated hand modulates the possible effect of hand position on the HBR. Third (*experiments 4* and *5*), we investigated at which level of the somatosensory pathway the top-down modulation of the HBR takes place. If this modulation consists in a facilitation at the level of the motoneurons innervating the *orbicularis oculi* muscle, then hand position would modulate not only the magnitude of the HBR, but also that of the BR elicited by the electrical stimulation of the supraorbital nerve (*experiment 4*). On the other hand, if the top-down modulation takes place in the cuneate nucleus, at the level of the first synapse of the lemniscal pathway (i.e., before the Aβ afferents from the hand enter the brain stem circuits subserving the HBR), then hand position would modulate not only the HBR but also the N20 wave of the somatosensory evoked potentials (SEPs), which represents the first arrival of the input transmitted by the Aβ pathway to the cortex (Mauguiere et al. 1999).

MATERIALS AND METHODS

Participants

Twenty healthy volunteers (nine women), aged between 20 and 40 yr (mean ± SD: 29 ± 5.4 yr), all right-handed, were recruited for this study. Participants gave written, informed consent before taking part in the study. The study was approved by the local ethics committee.

Stimulation and Recording

Electrical stimuli were delivered using a surface bipolar electrode placed on the median nerve at the wrist (*experiments 1*, *2*, *3*, and *5*) and on the supraorbital nerve (*experiment 4*). The stimulator was attached on the participants' wrist with a Velcro strap before the beginning of the recording, thus ensuring constant pressure across experimental conditions. In *experiments 1–4*, the stimulus intensity was adjusted, in each participant, to elicit a reproducible BR (20–80 mA, mean = 43.5 mA, in *experiments 1–3*, and 4–12 mA, mean = 7.5 mA in *experiment 4*). In these four experiments, the stimulus duration was 200 μs, and the interval between successive stimuli was 30 s. EMG activity was recorded from the *orbicularis oculi* muscle, bilaterally, using pairs of surface electrodes with the active electrode over the mid-lower eyelid and the reference electrode a few centimeters laterally to the outer canthus. Signals were amplified and digitized at a sampling rate of 8,192 Hz (ISA 1004, Micromed, Treviso, Italy), and stored for offline analysis.

In *experiment 5*, we measured the N20 wave of the SEPs according to the recommendations of the International Federation of Clinical Neurophysiology (Cruccu et al. 2008). The stimulus intensity was adjusted, in each participant, to cause a "reproducible muscle twitch" (7–13 mA, mean 9 mA)[1]. The stimulus duration was 200 μs, and the stimulus frequency was 4.5 Hz. Scalp electroencephalographic (EEG) activity was recorded from electrode C3 (i.e., placed over the contralateral somatosensory cortex) referenced to F_Z (positions defined according to the International 10–20 system). Signals were amplified and digitized at a sampling rate of 32,768 Hz (ISA 1004, Micromed, Treviso, Italy), and stored for offline analysis.

Procedures

Preliminary recordings. Participants were seated in a comfortable armchair. In each participant, we first determined the stimulus intensity able to elicit a well-defined and stable BR in response to electrical stimulation of the median nerve at the wrist (HBR). This was achieved by increasing the stimulus intensity until a clear HBR was observed in three consecutive trials, or the participant refused a further increase of stimulus intensity (Valls-Solé et al. 1997). Only participants showing a reproducible HBR (i.e., "responders", $N = 12$; five women, mean age ± SD: 29.2 ± 5.7 yr) underwent further testing. In these participants, we performed five separate experiments.

Experiment 1. To investigate the effect of "hand position" on the HBR, in the 12 responders, we recorded HBR responses in two experimental conditions. In the "far" condition, participants were sitting with their forearm resting on a pillow, at ~120° with respect to the arm, and with the hand close to the ipsilateral knee; such posture resulted in the wrist being at a distance of ~60 cm from the ipsilateral side of their face (Fig. 1, *top*); in the "near" condition, participants were sitting with their arm resting on a table, the forearm at ~75° with respect to the arm, and the wrist at a distance of ~4 cm from the ipsilateral side of their face (Fig. 1, *bottom*). These distances corresponded to hand positions clearly outside ("far" condition) and inside ("near" condition) the peripersonal space of the face (Duhamel et al. 1998; Farnè et al. 2005). In the near position, the palm of the hand and the fingers were above the upper limit of the visual field, so that the participants could not see the thumb twitching in response to the electrical stimulation. The fingers, the palm of the hand, the wrist, or any other part of the upper limb were never touching the face or the head. The hand not undergoing the postural manipulation was never stimulated, and the arm was held along the body throughout the duration of the experiment. A total of 40 electrical stimuli were delivered to the median nerve, 20 stimuli to the left wrist and 20 stimuli to the right wrist, in separate blocks. The order of blocks was balanced across participants. In each block, 10 stimuli were delivered in the far and 10 in the near condition, in alternating trials. Participants changed the position of their arm after each trial. One-half of the participants started the block with a stimulus in the far condition, and one-half with a stimulus in the near condition. Throughout each block, participants were instructed to keep their gaze on a small fixation cross (1.5 cm^2) placed at ~30 cm and 45° below eye level. White noise was played throughout the experiment to mask any sound possibly arising from the stimulation procedure.

Experiment 2. To investigate the contribution of the vision of the stimulated hand to the effect observed in *experiment 1*, in eight responders we recorded the HBR in the far and near experimental conditions, while they kept their eyes closed throughout each of the two recording blocks. The experimental procedures used were otherwise similar to *experiment 1*.

Experiment 3. To control whether the effect of hand position on the HBR was affected by the different proprioceptive input from the arm in the far and near conditions, in six responders we recorded the HBR while the position of both the hand and the arm was kept constant, and the proximity of the stimulated hand to the face was manipulated by rotating the head. Thus, the participants' forearm was kept flexed in the same near position all the time, and their head was either kept straight in anatomical position ("near-front" condition) or rotated sideways by 90° ("near-side" condition) (see Fig. 5). Similar to *experiment 1*, a total of 40 electrical stimuli were delivered to the median nerve, 20 stimuli to the left wrist and 20 stimuli to the right wrist, in separate blocks. The order of blocks was balanced across

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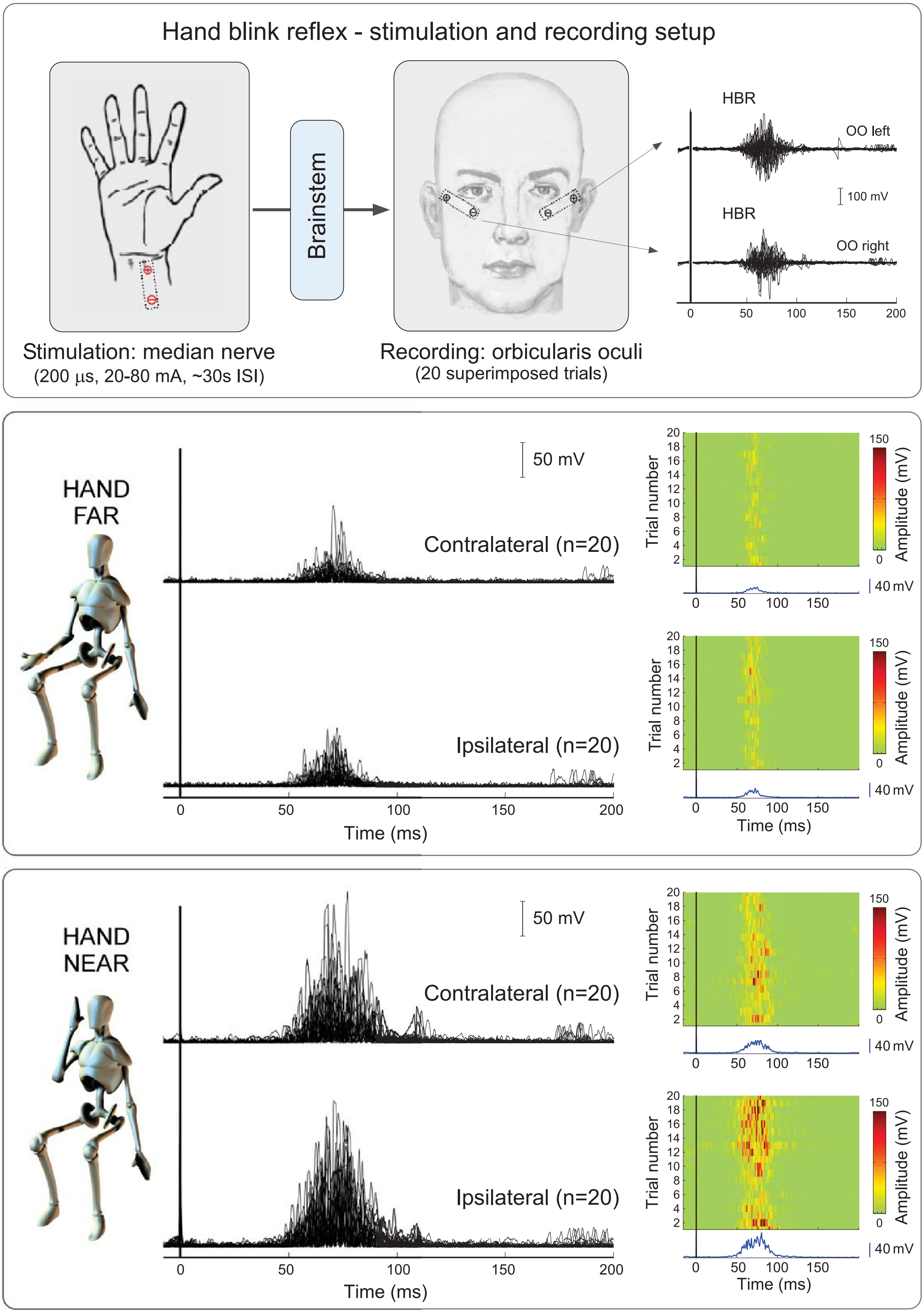
[1] Although different stimulus intensities were used to elicit the HBR and the N20 wave, both responses reflected exclusively the activation of Aβ afferents. Indeed, although the high intensity used to elicit the HBR may have also activated Aδ and C fibers besides Aβ fibers, the onset-offset latencies of the HBR (~45–100 ms; e.g., Fig. 2) rule out any contribution by Aδ- and C-fiber input to the response (Plaghki et al. 2010).


Hand blink reflex - stimulation and recording setup
Brainstem
HBR
OO left
100 mV
HBR
OO right
0
50
100
150
200
Stimulation: median nerve
(200 µs, 20-80 mA, ~30s ISI)
Recording: orbicularis oculi
(20 superimposed trials)
HAND FAR
50 mV
Contralateral (n=20)
Ipsilateral (n=20)
0
50
100
150
200
Time (ms)
Trial number
2
4
6
8
10
12
14
16
18
20
150
0
Amplitude (mV)
40 mV
Time (ms)
HAND NEAR
50 mV
Contralateral (n=20)
Ipsilateral (n=20)
Time (ms)

participants. In each block, 10 stimuli were delivered in the near-front and 10 in the near-side condition, in alternating trials.

Experiment 4. To further control whether the effect of hand position on the HBR reflected a modulation of the excitability of the *orbicularis oculi* muscle due to the change of upper-limb position, in six responders we recorded the BR elicited by the electrical stimulation of the supraorbital nerve, in the far and near experimental conditions, as in *experiments 1* and *2*. In this experiment, electrical stimuli were delivered, with equal probability, either to the right supraorbital nerve, or to the median nerve of the right hand (i.e., the hand undergoing the postural manipulation). Participants did not know in advance if the median or the supraorbital nerve would be stimulated: this was done to ensure that the position of the hand with respect to the face was as relevant as in *experiments 1–3* and *5*, where stimuli were always delivered to the median nerve of the hand undergoing the postural manipulation. A total of 40 stimuli were delivered, 20 to the supraorbital nerve and 20 to the median nerve and, for each site, 10 in the far and 10 in the near condition, in alternating trials. The stimuli were delivered in pseudorandom order, with no more than three consecutive stimuli delivered to the same body site.

Experiment 5. To rule out the possibility that the effect of hand position on the HBR resulted from a reduced presynaptic inhibition on the primary Aβ afferents, in six responders we recorded the N20 wave of the SEPs elicited by the electrical stimulation of the median nerve at the wrist, in the same two experimental conditions (far and near). In this experiment, 600 electrical stimuli were delivered to the right wrist, 300 in the far condition and 300 in the near condition, in alternating blocks of 100 stimuli each.

Data Analysis and Statistics

Both EMG and EEG signals were analyzed using Letswave (http://amouraux.webnode.com) (Mouraux and Iannetti 2008). EMG signals from each participant were high-pass filtered (55 Hz), full-wave rectified, and averaged separately for the far and near conditions (or near-front and near-side conditions in *experiment 3*) at the ipsilateral and contralateral recording sides. In each participant, we measured the onset latency, duration, and area of the HBR for each experimental condition and recording side. In *experiments 1* and 2, for each of these measures, we performed a two-way, repeated-measures ANOVA, with hand position (two levels: far and near) and "recording side" (two levels: "ipsilateral" and "contralateral") as experimental factors. In *experiment 3*, data were averaged across ipsilateral and contralateral recording sides.

Furthermore, to investigate the time course of the possible effects of hand position and recording side in *experiment 1*, we performed the same repeated-measures ANOVA, but using each time point of the averaged reflex response, as implemented in Letswave (Mouraux and Iannetti 2008). In those participants who took part in *experiment 2*, we also performed a point-by-point, two-way repeated-measures ANOVA, with hand position (two levels: far and near) and "vision" (two levels: "eyes open" and "eyes closed") as experimental factors. Each point-by-point ANOVA yields three waveforms expressing the significance of the effect of each of the two factors across time and their interaction. In *experiment 3*, we performed a similar point-by-point analysis, consisting in a paired *t*-test with "head position" (two levels: near-front and near-side) as experimental factor. In all analyses, a consecutivity threshold of 10 ms was chosen to account for multiple comparisons.

EEG signals from each participant were averaged separately for the far and near conditions. In each participant, we measured the peak amplitude and latency of the N20 wave of the SEPs for each condition.

In all statistical analyses, the significance level (α) was set at 0.05.

RESULTS

We observed a clear and reproducible HBR in 12 out of 20 participants (60%), using a stimulus intensity ranging between 4 and 53 times the individual perceptive threshold. These 12 participants were considered as responders and were included in further analyses.

Experiment 1

Figures 1 (*left*) and 2 show the HBR waveforms recorded in one representative participant (as superimposed single trials) and in all 12 participants (as single-subject averages), respectively, in the far and near conditions at the contralateral and ipsilateral recording sides. Importantly, as shown in the single-trial response plots (Fig. 1, *right*), although there was some degree of variability in the responses, which is expected for a reflex mediated by a polysynaptic circuit in the reticular formation (Cruccu et al. 2006), the magnitude of the HBR was not reduced throughout the recording. This indicates that, when using a constant intertrial interval of 30 s, the HBR does not habituate over time.

Effect of hand position and recording side on onset latency and duration of the HBR. The onset latency of the HBR was shorter when the stimulated hand was inside the area of peripersonal space surrounding the face (near: 45.5 ± 7.5 ms) than when it was outside (far: 50.3 ± 7 ms), and when the stimulated hand was ipsilateral (46.8 ± 7.2 ms) than contralateral (49 ± 7.3 ms) to the recording side. This was reflected in a significant main effect of hand position [$F_{(1,11)} = 19.07$, $P = 0.001$, $\eta^2 = 0.64$] and recording side [$F_{(1,11)} = 29.98$, $P = 0.001$, $\eta^2 = 0.73$] on the HBR onset latency (two-way, repeated-measures ANOVA). The interaction between the two factors was not significant ($P = 0.65$).

Likewise, the duration of the HBR was longer in the near (50.2 ± 9.3 ms) than in the far condition (40.1 ± 12.5 ms), and at the ipsilateral (47.6 ± 10.8 ms) than at the contralateral (42.7 ± 11 ms) recording side. There was a significant main effect of hand position [$F_{(1,11)} = 14.55$, $P = 0.003$, $\eta^2 = 0.57$] and recording side [$F_{(1,11)} = 9.61$, $P = 0.01$, $\eta^2 = 0.47$] on the HBR duration (two-way, repeated-measures ANOVA). The interaction between the two factors was not significant ($P = 0.19$).

Fig. 1. *Top*: schematic representation of the stimulation and recording setup (*experiments 1–3*). The hand blink reflex (HBR) was elicited by electrical stimulation of the median nerve at the wrist and recorded from the *orbicularis oculi* (OO) muscle. ISI, interstimulus interval. *Middle* and *bottom*: *experiment 1*. *Left*: rectified and superimposed single-trial HBR waveforms from one representative participant. *x*-axis, time (ms); *y*-axis, EMG activity (mV). *Middle*: "far" condition. The HBR was elicited while participants were sitting with their forearm at ~120° with respect to the arm, and with the hand close to the ipsilateral knee. *Bottom*: "near" condition. The HBR was elicited while participants were sitting with their forearm at ~75° with respect to the arm, and their hand close by ~4 cm to the ipsilateral side of their face. Note that the HBR has a significantly greater magnitude in the "near" than in the "far" condition, and at the "ipsilateral" than at the "contralateral" recording side. *Right*: to emphasize the lack of response habituation to repetitive stimulation, one bidimensional plot of single-trial responses is shown for each condition and recording side. Horizontal lines in the plot represent single-trial EMG responses, with signal amplitude color-coded at each time point. Responses are sorted vertically in order of occurrence, from *bottom* (first trial) to *top* (last trial). The waveform below each plot is the average of all responses.

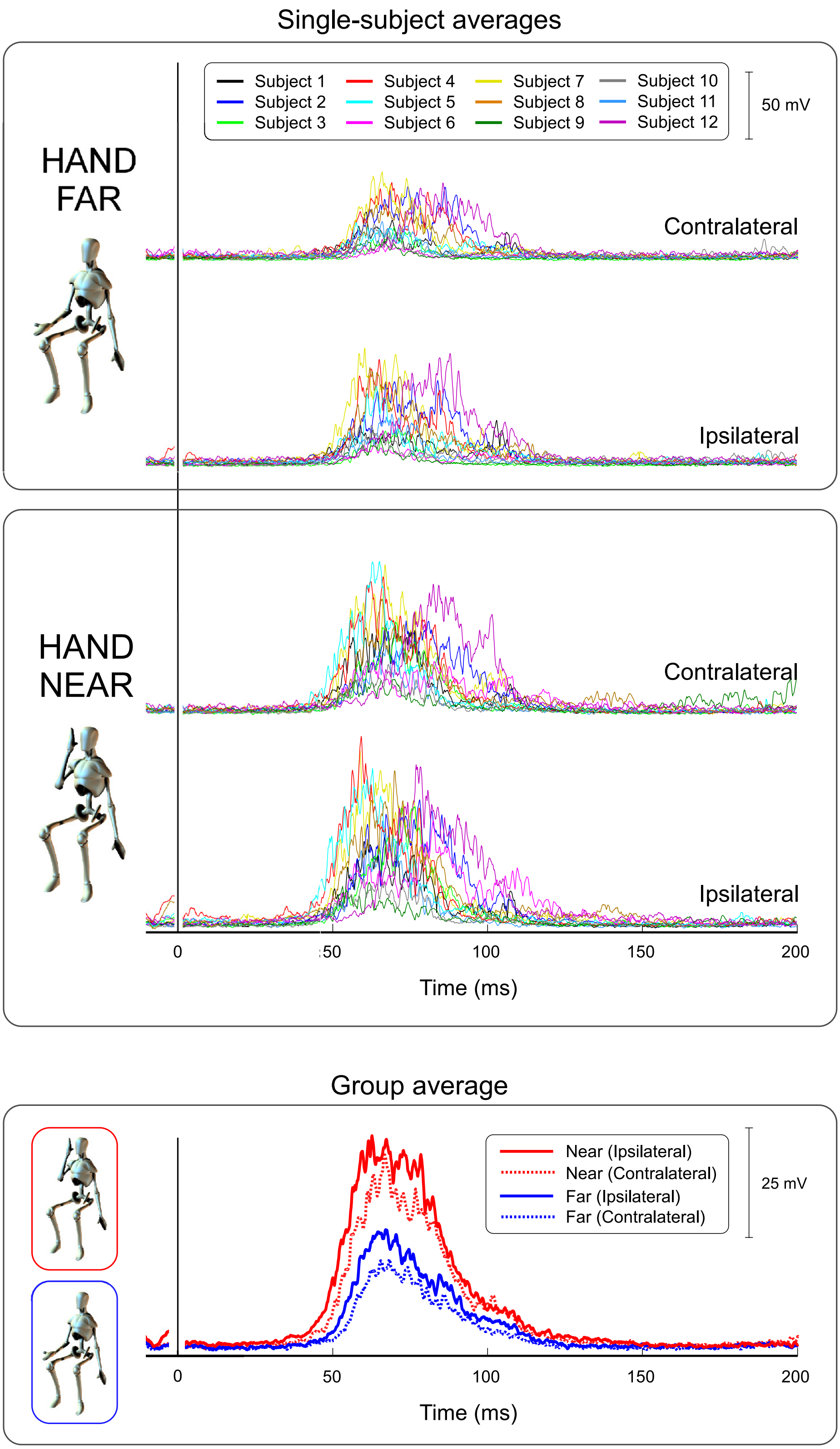


Fig. 2. *Experiment 1*. *Top*: rectified and superimposed average of each participant ($N = 12$) for the far and near conditions, at the contralateral and ipsilateral recording sides. Each participant is represented by a different color. *Bottom*: group-average HBR waveforms in the far and near conditions, at the contralateral and ipsilateral recording sides. x-axis, time (ms); y-axis, EMG activity (mV).

Effect of hand position and recording side on HBR magnitude. The magnitude of the HBR, as measured by the area under the curve (AUC), was significantly larger when the stimulated hand was inside the peripersonal space of the face [near: 128 ± 50, far: 71 ± 37 AUC arbitrary units; +99.3 ± 60.2%; main effect of hand position, $F_{(1,11)} = 75.70$, $P < 0.001$, $\eta^2 = 0.87$], and when the stimulated hand was ipsilateral to the recording side [ipsilateral: 111 ± 46, contralateral: 88 ± 40 AUC arbitrary units; +28.6 ± 19.2%; main effect of recording side, $F_{(1,11)} = 39.42$, $P < 0.001$, $\eta^2 = 0.78$]. The

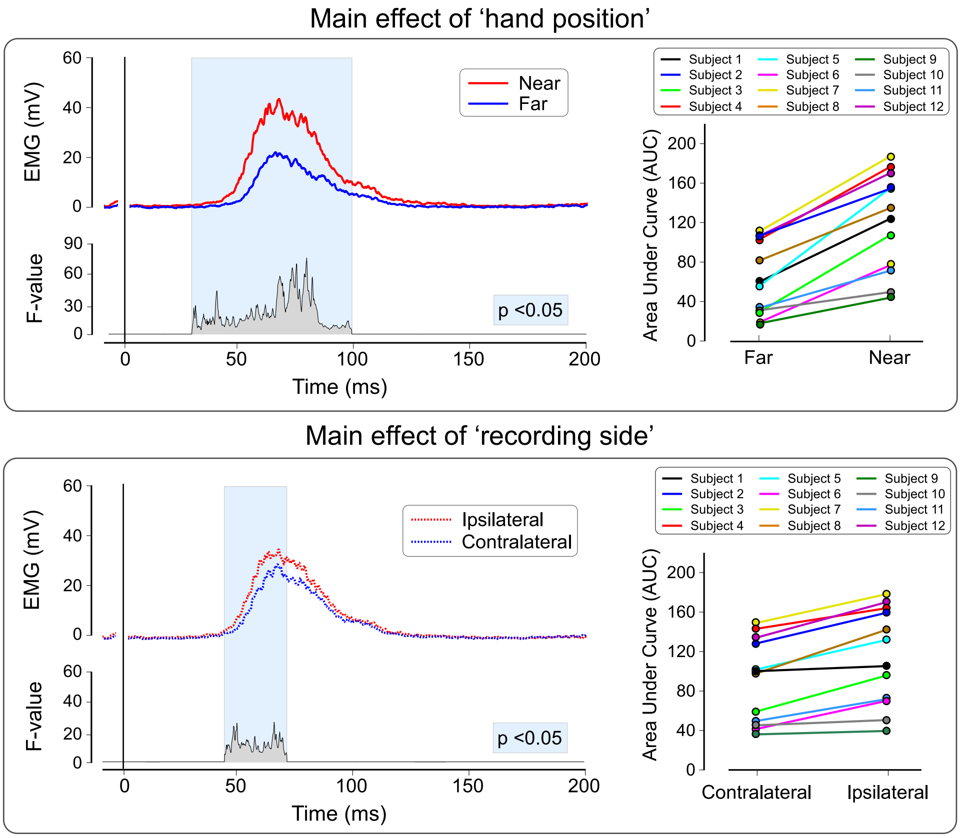


Fig. 3. *Experiment 1*. Main effects of the experimental factors "hand position" (two levels: far and near) and "recording side" (two levels: ipsilateral and contralateral). *Left*: the *top* waveforms are the rectified group-average HBR for the two levels of each factor. The *bottom* waveforms express the *F*-value of the two-way ANOVA for each time point, in the significant time windows ($P < 0.05$). *Right*: single-subject HBR magnitudes, expressed as area under the curve (AUC arbitrary units). Single subjects are color coded as in Fig. 2.

interaction between the two factors was not significant ($P = 0.18$).

Effect of hand position and recording side on HBR magnitude across time. To investigate the time course of the effects of the two experimental factors across the whole HBR response, we performed a two-way repeated-measures ANOVA for each time point of the average HBR. The factor hand position was a significant source of variance within the 30- to 98-ms time window (i.e., the HBR was significantly larger in the near than in the far condition; see time course of *F*-values in Fig. 3), and particularly in the second part of the HBR waveform (i.e., between 65 and 88 ms). In contrast, the factor recording side was a significant source of variance only in the early part of the HBR waveform (i.e., the HBR was significantly larger at the ipsilateral than at the contralateral recording side within the 44- to 66-ms time window; Fig. 3). The interaction between these two factors across time was not significant ($P > 0.05$; Fig. 3).

Experiment 2

Effect of hand position and recording side on onset latency and duration of the HBR without vision. Similarly to what was observed when participants had their eyes open (*experiment 1*), when they had their eyes closed the onset latency of the HBR was shorter in the near (43.3 ± 4.6 ms) than in the far (45.6 ± 4.4 ms) condition, and when the stimulated hand was ipsilateral (43.7 ± 4.2 ms) than contralateral (45.2 ± 4.8 ms) to the recording side. This resulted in a significant main effect of hand position [$F_{(1,7)} = 27$, $P = 0.001$, $\eta^2 = 0.79$] and recording side [$F_{(1,7)} = 11.45$, $P = 0.012$, $\eta^2 = 0.62$] on the HBR onset (two-way, repeated-measures ANOVA). The interaction between the two factors was not significant ($P = 0.71$).

In addition, the duration of the HBR was longer in the near (62.7 ± 13.4 ms) than in the far (51.8 ± 8.9 ms) condition, and at the ipsilateral (59.4 ± 10.5 ms) than at the contralateral (55.1 ± 9.9 ms) recording side. There was a significant main effect of hand position [$F_{(1,7)} = 7.7$, $P = 0.027$, $\eta^2 = 0.52$], and recording side [$F_{(1,7)} = 6.3$, $P = 0.040$, $\eta^2 = 0.47$] on the HBR duration (two-way, repeated-measures ANOVA). The interaction between the two factors was not significant ($P = 0.18$).

Effect of hand position and recording side on HBR magnitude without vision. Also similar to what observed in *experiment 1*, when participants had their eyes closed, the HBR magnitude was larger in the near condition [near: 173 ± 57, far: 101 ± 39 AUC arbitrary units; +94.8 ± 116.2%; main effect of hand position, $F_{(1,7)} = 12$, $P = 0.011$, $\eta^2 = 0.63$], and when the stimulated hand was ipsilateral to the recording side [ipsilateral: 153 ± 37, contralateral: 121 ± 45 AUC arbitrary units; +37.6 ± 41%; main effect of recording side, $F_{(1,7)} = 9.42$, $P = 0.018$, $\eta^2 = 0.57$]. The interaction between the two factors was not significant ($P = 0.14$).

Effects of hand position and vision on HBR magnitude across time. To investigate the possible effect of vision on the HBR across time, and whether and at what time point vision modulated the effect of hand position, in the eight participants of both *experiments 1* and *2*, we computed a two-way, repeated-measures ANOVA for each time point of the average HBR response. The factor hand position was a significant source of variance of the reflex waveform within a similar time window, as reported in *experiment 1* (i.e., the HBR was significantly larger in the near than in the far condition within the 43- to 88-ms time window) (Fig. 4). The factor vision was a significant source of variance of the EMG activity before and after the HBR, i.e., the background EMG activity was significantly larger in the eyes closed condition within the 14- to 27-ms and 152- to 220-ms time windows (Fig. 4), as expected because of the motoneuronal facilitation during the voluntary contraction caused by eye closure. The interaction between the two experimental factors across time was not significant ($P > 0.05$).

Experiment 3

Even when the proximity of the hand to the face was manipulated by rotating the head, i.e., while keeping both the hand and the arm in the same position all the time, the HBR was significantly enhanced (+96.5 ± 67.9%) when the stimulated hand was inside the peripersonal space of the face [$t_{(5)} = 2.92$, $P = 0.03$; paired *t*-test]. To investigate the time course of such effect, we performed a paired *t*-test for each time point of the average HBR response. The factor "head position" was a significant source of variance of the reflex waveform within the 58- to 90-ms time window ($P < 0.05$; Fig. 5).

Experiment 4

In striking contrast with what was observed in *experiments 1–3*, hand position did not modulate either the onset latency [far: 38.7 ± 5.7 ms, near: 38.3 ± 5.6 ms; $t_{(5)} = 0.8$, $P = 0.46$] or the duration [far: 48.4 ± 15.2 ms, near: 49.3 ± 16.1 ms; $t_{(5)} = -0.51$, $P = 0.63$] of the R2 response of the BR elicited by the electrical stimulation of the supraorbital nerve. Also, the magnitude of the R2 was similar in the two experimental conditions [far: 281 ± 32, near: 269 ± 24 AUC arbitrary units; $t_{(5)} = 0.38$, $P = 0.72$] (Fig. 6).

Experiment 5

Furthermore, hand position did not affect the amplitude [far: 1.85 ± 0.57 μV, near: 1.83 ± 0.63 μV; $t_{(5)} = 0.28$, $P = 0.79$] or the latency [far: 19.9 ± 2.1 ms, near: 19.9 ± 2 ms; $t_{(5)} = -0.41$, $P = 0.69$] of the N20 wave of the SEPs measured at scalp electrode C3, contralateral to the side of the stimulation (Fig. 7).

DISCUSSION

Our results show that the HBR is influenced by the proximity of the stimulated hand to the face. We observed four main findings. First, when the hand is placed inside the area of peripersonal space surrounding the face, the HBR has shorter onset latency, longer duration, and greater magnitude than when the hand is placed outside this area. This finding indicates that the brain stem circuits mediating the HBR undergo tonic top-down modulation, in that their excitability is increased when the stimulated hand enters the peripersonal space surrounding the face. Second, such HBR enhancement has similar magnitude when the participants have their eyes closed. This finding suggests that this effect relies on the proprioceptive information about stimulus location with respect to the face. Third, the effect of hand position on the HBR is observed when the proximity of the hand to the face is manipulated either by changing the position of the arm (while keeping the head position constant), or by rotating the head (while keeping the arm position constant). This finding rules out that the HBR enhancement relies on the different proprioceptive input from the stimulated arm. Fourth, neither the BR elicited by the stimulation of the supraorbital nerve or the N20 of the SEPs elicited by median nerve stimulation are affected by hand position. This finding indicates that the enhancement of the HBR is mediated by a selective preactivation of the brain stem circuits subserving the HBR, and not by facilitation of facial motoneurons or by presynaptic disinhibition of primary afferents of the hand.

Characteristics of the HBR

We observed that 60% of the subjects participating in our study had a reproducible HBR, i.e., a proportion considerably higher than that reported in previous investigations in healthy volunteers (e.g., 42.8% in Miwa et al. 1998; 27.3% in Alvarez-Blanco et al. 2009). This is likely to be due to the higher stimulation intensity we applied. The same reason might explain why we did not observe the previously reported habituation of the HBR after repeated stimulation (Fig. 1, *right*). Although Miwa et al. (1995), recording from a number of muscles (including the *orbicularis oris*, sternocleidomastoid, posterior neck, and *pectoralis maior*), have demonstrated that the HBR is different from a startle reaction, they did not completely rule out that it could still represent the initial part of a startle response. Our observation of a clear lack of habituation of the HBR provides further evidence that the HBR is fundamentally different from a startle response.

Other features of the HBR recorded in the present study (Figs. 1 and 2) are similar to what has been previously reported, e.g., an onset latency of ~45 ms and an earlier onset, longer duration, and greater magnitude in the orbicularis muscle ipsilateral to the stimulated hand. Furthermore, using a novel statistical approach to analyze each point of the HBR, we showed for the first time that the latter effect is limited to the first part of the response (Figs. 2 and 3).

Effect of Hand Proximity to the Face

The BR elicited by electrical nerve stimulation has been reported to be more frequent (Miwa et al. 1995; Miwa et al. 1998) and larger (Alvarez-Blanco et al. 2009) when stimuli are applied to the upper limb than to the lower limb. It has been suggested that stimuli applied to the lower limb are less effective in eliciting a clear BR because they trigger less synchronized afferent volleys due to the longer peripheral distance (Alvarez-Blanco et al. 2009). However, from these studies, it cannot be determined whether the distance of the stimulated body part from the face in external spatial coordinates may also modulate the occurrence and magnitude of this response. Here we show that the HBR is dramatically affected by the proximity of the stimulated hand to the face, being enhanced when the hand is located in the peripersonal space surrounding the face (Figs. 1–5). This finding is important, as it indicates that the excitability of subcortical neural circuits, entirely located in the brain stem and mediating a basic defensive reflex, undergo significant top-down modulation from the neocortex.

Converging evidence from behavioral, neuropsychological, and neuroimaging studies supports the view that the location of somatosensory stimuli is initially encoded in a somatotopic frame of reference (i.e., relative to the skin surface) and then automatically remapped into an external, egocentric frame of reference after the relative position of the body parts is taken

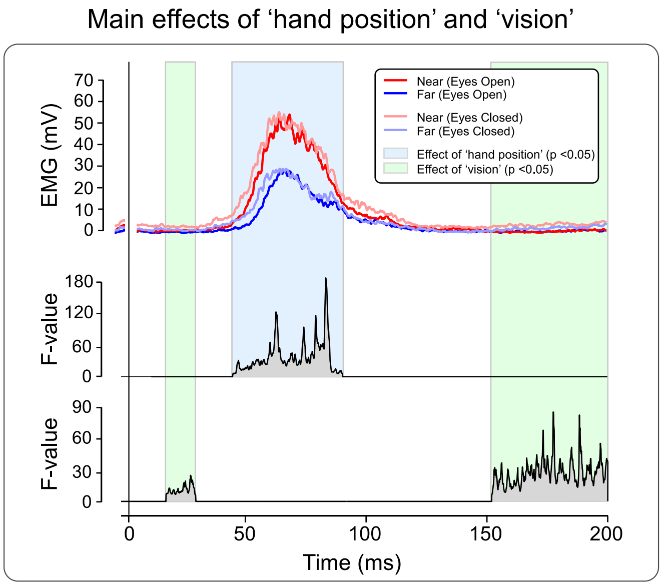


Fig. 4. *Experiment 2*. Group-average HBR waveforms in the far and near conditions (averaged across ipsilateral and contralateral recording sides), when participants had their eyes open and closed. *x*-axis, time (ms); *y*-axis, EMG activity (mV). The two bottom waveforms express the *F*-value of the two main effects (hand position two levels: far and near; vision two levels: "eyes open" and "eyes closed"), for each time point, in the significant time windows ($P < 0.05$, two-way ANOVA). Note that the factor hand position was a significant source of variance of the HBR within a similar time window, as reported in *experiment 1* (Fig. 3), and that the factor vision was a significant source of variance only of the EMG activity before and after the HBR. The interaction between these two experimental factors across time was not significant ($P > 0.05$).

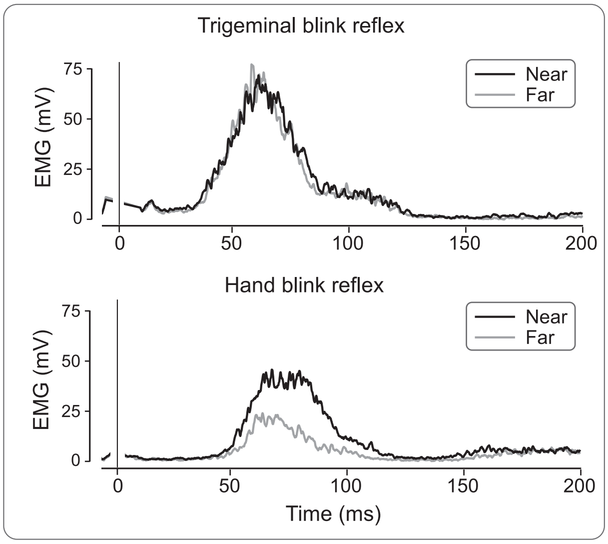


Fig. 6. *Experiment 4*. Group-average blink reflex waveforms elicited by the electrical stimulation of the supraorbital nerve (*top* waveforms) and the median nerve at the wrist (*bottom* waveforms) in the far and near conditions (averaged across ipsilateral and contralateral recording sides). *x*-axis, time (ms); *y*-axis, EMG activity (mV). Note that onset latency, duration, and magnitude of the R2 are remarkably similar in the two experimental conditions, while the effect of hand position is present for the HBR (as in *experiments 1* and *2*).

into account (Azanon and Soto-Faraco 2008; Kitazawa 2002; Röder et al. 2004). Studies in both human and nonhuman primates propose that frontoparietal cortical areas, such as the PZ in the precentral gyrus and the VIP area, and their human homologous, are responsible for such remapping (Azanon et al. 2010; Graziano and Gross 1995; Lloyd et al. 2003). Furthermore, this frontoparietal network is thought to subserve the multisensory representation of peripersonal space (Fogassi et al. 1996; Làdavas et al. 1998; Macaluso and Maravita 2010), as well as to respond to aversive visual stimuli presented within the peripersonal space, including stimuli representing potential threats to others (Avenanti et al. 2005; Costantini et al. 2008; Lloyd et al. 2006). Here, given that the HBR, like the R2 component of the trigeminofacial BR, entirely relies on subcortical circuitry (Cruccu et al. 2005; Leon et al. 2011; Miwa et al. 1996), our finding suggests that these higher order association areas, involved in the transformation of spatial coordinates and in the detection of aversive stimuli delivered in the peripersonal space, preset the brain stem circuits underlying the HBR to be more responsive when the stimulated body part is located inside the peripersonal space surrounding the face. Such increased responsiveness of the brain stem circuits would facilitate the transmission of the signal to the facial motoneurons, resulting in an HBR of shorter latency and larger magnitude when the hand is near the face, and thus a more efficient defensive response. These results provide the first

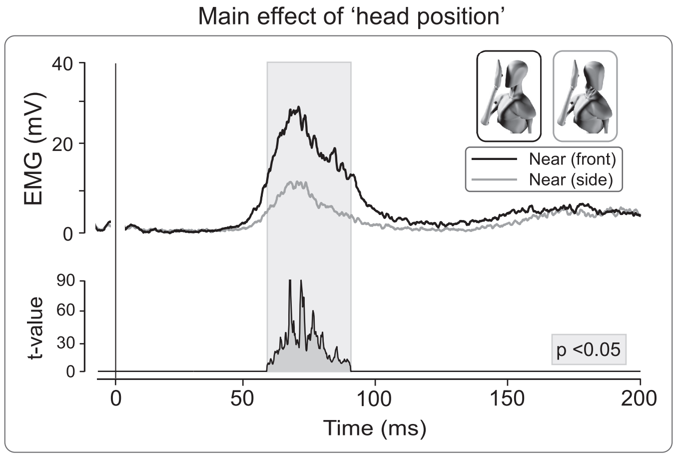


Fig. 5. *Experiment 3*. Group-average HBR waveforms in the "near-front" and "near-side" conditions (averaged across ipsilateral and contralateral recording sides). *x*-axis, time (ms); *y*-axis, EMG activity (mV). The participants' forearm was kept flexed in the same near position for the entire duration of the recording blocks, while their head was either kept straight in anatomical position (near-front condition) or rotated sideways by 90° (near-side condition). The *bottom* waveform expresses the *t*-value of the effect of "head position" (two levels: near-front and near-side), for each time point, in the significant time window ($P < 0.05$, paired *t*-test).

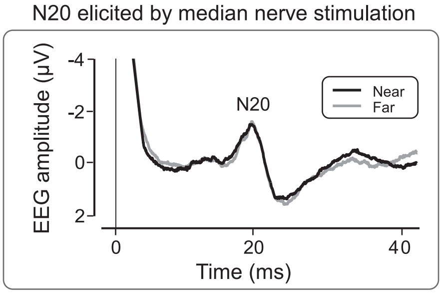


Fig. 7. *Experiment 5*. Group-average somatosensory evoked potentials (SEP) waveforms elicited by the electrical stimulation of the median nerve at the wrist, in the far and near conditions. *x*-axis, time (ms); *y*-axis, EEG amplitude (μV). Note that the latency and amplitude of the N20 wave, which represents the first arrival of the Aβ pathway to the cortex, are remarkably similar in the two experimental conditions.

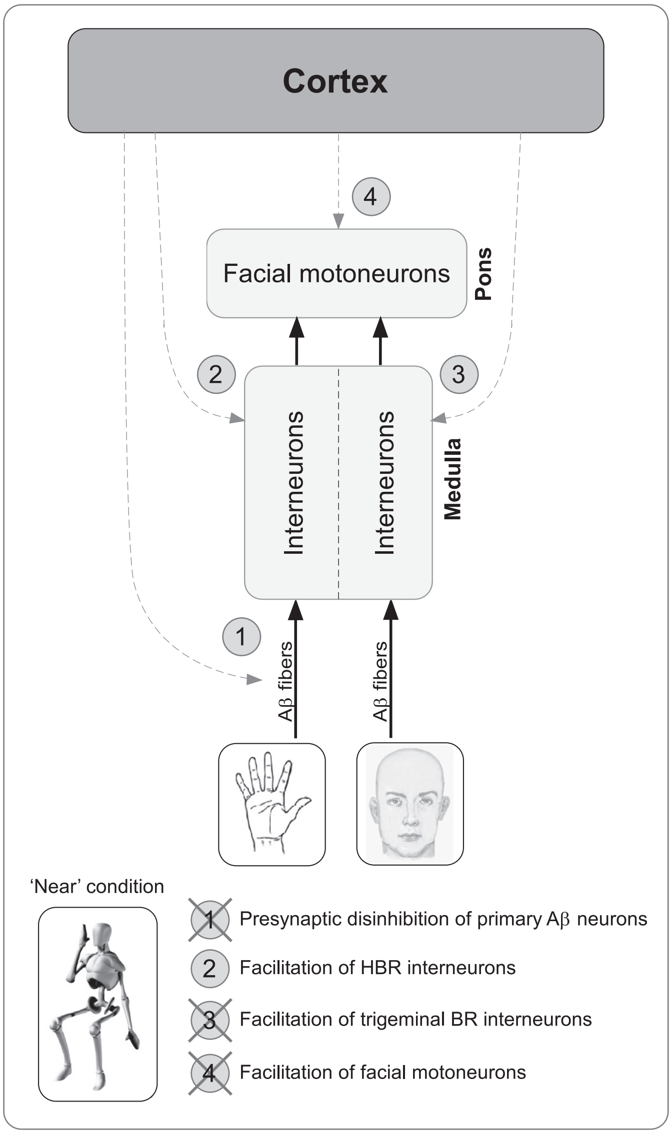


Fig. 8. Putative model of sites of cortical modulation in the near condition. Our results rule out top-down modulation of the primary sensory Aβ afferents through presynaptic disinhibition (*action site 1*; *experiment 5*), and of either the interneurons of medullary circuit underlying the trigemino BR (*action site 3*; *experiment 4*) or the motoneurons innervating the OO (*action site 4*; *experiment 4*). Instead, our results are compatible with a selective top-down facilitation of the interneurons of the circuit underlying the HBR (*action site 2*; *experiments 1*, *2*, and *3*). Note that the medullary circuits processing somatosensory input from the median and the trigeminal nerves might not consist of entirely distinct interneuronal populations.

evidence in support of the protective function of the peripersonal space in humans. This "defensive" peripersonal space, not necessarily corresponding to the peripersonal space related to multisensory integration for guiding action (Macaluso and Maravita 2010), would represent a "safety margin" advantageous for survival (Cooke and Graziano 2003; Graziano and Cooke 2006). That is, whenever a threatening stimulus approaches or enters such peripersonal space, the individual would engage in actions aimed at preserving this safety margin.

What could be the neural mechanisms underlying the observed HBR modulation (Fig. 8)? We provide evidence that the proximity of the stimulated hand to the face enhances specifically the BR elicited by hand stimulation. Indeed, the R2 component of the trigeminofacial BR was not dependent on hand position (*experiment 4*). This finding, besides providing further evidence that the circuits for the two reflexes are functionally independent, rules out the possibility that the effect of hand position on the HBR is due to a change of excitability of either the motoneurons innervating the *orbicularis oculi* muscle or the circuit mediating trigeminofacial BR. Thus the effect could be mediated by a selective disinhibition of the first synapse of the Aβ afferents from the hand, which activate the brain stem circuits subserving the HBR. An alternative possibility is that the neocortex selectively increases the excitability of the subset of brain stem interneurons responsible for integrating the afferent information arising from the hand placed inside the peripersonal space of the face and projecting to the facial nucleus in the pons. The finding that the N20 wave of the SEPs, which represents the first arrival of the Aβ pathway to the cortex (Mauguiere et al. 1999), was not affected by hand position (*experiment 5*) indicates that the latter possibility is most likely, as it shows that the top-down modulation of the HBR must take place after the first synapse of the lemniscal pathway in the cuneate nucleus (Fig. 8).

Functional Heterogeneity of the HBR

The effect of hand position was stronger in the second part of the HBR (Fig. 3). Together with the observation that the effect of recording side was exclusively present in the first part of the response (Fig. 3), this finding suggests that the HBR is not a unitary physiological phenomenon, but it is mediated by a complex circuit that has two functionally distinct components, undergoing differential modulation. Indeed, the early recording side effect is likely due to an intrinsic anatomophysiological property of the reticular bulbopontine circuit projecting to the facial nucleus, where a higher number of synapses is necessary to reach the nucleus contralateral to the stimulated side. In contrast, the top-down hand position effect observed across the whole HBR (and stronger in its later part, Fig. 3) is likely to be explained by a cortical projection on the HBR reticular interneurons. Thus we suggest that two physiologically distinct populations of neurons subserve these two experimental effects.

Effect of Hand Position on the HBR Persists in Absence of Vision

The HBR was significantly increased when the stimulated hand was close to the face, even when participants kept their eyes closed (Fig. 4). This finding might seem in contrast with previous studies that have shown that vision of the body is crucial for proprioceptive localization (van Beers et al. 1999) and attentional selection (Sambo et al. 2009), and, furthermore, that external frames of reference used for localizing somatosensory stimuli are dominated by vision (Eimer 2004; Röder et al. 2004). However, it is possible that the absence of current visual information does not suppress the effect of hand position on the HBR because our study did not involve a fine discrimination of the stimulus location and the stimulated hand in space, and the participants had previously seen the two postures.

Conclusion

We show that the HBR is dramatically enhanced when the stimulated hand enters the peripersonal space of the face. Importantly, such effect is mediated by tonic and selective top-down modulation from higher order cortical areas involved in the representation of peripersonal space on the interneurons of the brain stem circuits subserving the HBR. These results are important as they provide compelling evidence that the nervous system is able to adjust its output in a very specific and fine-grained manner, even at the level of seemingly stereotyped defensive reflex responses. These findings are entirely in line with Sherrington's theory of reflexes as the result of integrated activities of the nervous system and support the existence of a defensive peripersonal space representing a safety margin advantageous for survival (Plaghki et al. 2010).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.F.S., M.L., G.C., and G.D.I. conception and design of research; C.F.S. performed experiments; C.F.S. analyzed data; C.F.S., M.L., G.C., and G.D.I. interpreted results of experiments; C.F.S. and G.D.I. prepared figures; C.F.S. and G.D.I. drafted manuscript; C.F.S. and G.D.I. edited and revised manuscript; C.F.S., M.L., G.C., and G.D.I. approved final version of manuscript.

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