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To blink or not to blink: fine cognitive tuning of

the defensive hand blink reflex

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Abstract

The blink reflex elicited by the electrical stimulation of the median nerve at the wrist (hand blink reflex, HBR) is a subcortical, defensive response that is enhanced when the stimulated hand is inside the peripersonal space of the face. Such enhancement results from a tonic, top-down modulation of the excitability of the brainstem interneurons mediating the HBR. Here we aim to (1) characterize the somatotopical specificity of this top-down modulation and (2) investigate the effect of cognitive expectations on such modulation. Experiment 1 showed that the somatotopical specificity of the HBR enhancement is not only heterosegmental, but also partially homosegmental, i.e. the enhancement is greater for the HBR elicited by stimulation of the hand located inside the peripersonal space of the face, as compared to the HBR elicited by the stimulation of the other hand, always kept far from the face. Experiment 2 showed that the top-down modulation of the HBR is triggered only when the participants expect to receive stimuli on the hand placed inside the peripersonal space of the face, and is thus strongly dependent on cognitive expectations. Taken together, these findings indicate a fine somatotopical and cognitive tuning of the excitability of brainstem circuits subserving the HBR, whose strength is adjusted depending on the context in a purposeful manner.

Introduction

Defensive reflexes are primitive and involuntary motor responses to potentially dangerous stimuli, mediated by fast, subcortical pathways. For example, the blink reflex (BR) is consistently elicited by the electrical stimulation of the trigeminal territory, but also, although less frequently, by the intense electrical stimulation of the median nerve (hand-blink reflex, HBR) (Alvarez-Blanco et al., 2009; Sambo et al., 2012). The HBR has an onset latency of approximately 45 ms and, similarly to the R2 component of the trigemino-facial BR, is entirely mediated by subcortical circuits at brainstem level (Miwa et al., 1996; Valls-Sole et al., 1997; Leon et al., 2011).

We demonstrated that the HBR is modulated by the proximity of the stimulated hand to the face, being dramatically increased when the hand is inside the peripersonal space surrounding the face (Sambo et al., 2012). This modulation, reflecting the protective function of the BR, results from a tonic, top-down modulation of the brainstem circuits mediating the HBR. We proposed that such modulation is exerted by associative cortical areas (such as the premotor cortex and the ventral intraparietal area, VIP) involved in representing the peripersonal space and remapping the location of somatosensory stimuli into an external frame of reference. Such areas would pre-activate the brainstem HBR circuits when the stimulated hand is located within the facial peripersonal space. Importantly, this increased excitability is specific for the brainstem interneurons mediating the HBR, but not for those mediating the BR elicited by trigeminal stimulation, or for the facial motoneurons innervating the *orbicularis oculi* (Sambo et al., 2012).

However, two important questions remain to be addressed. First, is such cortical modulation specific for the HBR elicited by the stimulation of the hand located inside the peripersonal

space of the face, or could it be observed also in the response elicited by the stimulation of the hand contralateral to the one near the face? In other words –having already demonstrated a somatotopical specificity of such top-down modulation of the circuits mediating the BR elicited by the stimulation of two heterosegmental territories (i.e. hand vs. face) – is this specificity also homosegmental? Second, do participants' expectations about whether or not the hand placed inside the facial peripersonal space could be stimulated affect the strength of the cortical modulation of the HBR circuit excitability?

Here we addressed these issues with two separate experiments. We reasoned that if there is no homosegmental somatotopical specificity, then the cortex would always enhance the excitability of the HBR circuits when one of the hands is placed near the face, irrespective of which one is actually stimulated. In contrast, if the effect is also homosegmentally specific, then the cortex would enhance the excitability only of the circuits mediating the HBR elicited by the stimulation of the hand placed inside the peripersonal space of the face. Finally, we hypothesized that the top-down modulation of the HBR circuits would be dependent on cognitive expectations, being more effective when participants have higher expectations that the hand inside the peripersonal space of the face will be stimulated.

Materials and Methods

Participants

Ten healthy volunteers (five women) aged between 25 and 37 years (mean ±SD: 30.3 ±3.9), all right-handed, participated in this study. They were chosen from a sample of 15 'responders', i.e. participants showing a reproducible hand blink reflex (HBR) (see (Sambo et

al., 2012)). 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 to the median nerve at the wrist using a surface bipolar electrode attached on the participants' wrist with a velcro strap. The stimulus intensity was adjusted, in each participant, to elicit a clear HBR in three consecutive trials (20-80 mA, mean = 42.5 mA) (Sambo et al., 2012). The stimulus duration was 200 μsec, and the interval between successive stimuli was 30 sec.

Electromyographic (EMG) activity was recorded from the *orbicularis oculi* muscle, bilaterally, using two pairs of surface electrodes with the active electrode over the mid lower eyelid and the reference electrode a few cm 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 off-line analysis.

Procedures

All participants performed the two experiments described below. In both experiments we recorded HBR responses while only one of the two hands underwent the 'far' and 'near' postural manipulation used in our previous study (Sambo et al., 2012). In the 'far' position participants were sitting with their forearm resting on a pillow, at approximately 120 degrees in respect to the arm, and with the hand close to the ipsilateral knee (Figs. 1 and 2); in the 'near' position, participants were sitting with their arm resting on a table, the forearm at approximately 75 degrees in respect to the arm, and the hand at a distance of approximately 4 cm from the ipsilateral side of their face (Figs. 1 and 2). The fingers, 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 always kept on the pillow in the 'far' position. Throughout each block, participants were instructed to keep their gaze on a fixation cross (1.5x1.5 cm) placed at approximately 30 cm and 45 degrees below eye level. White noise was played throughout the experiment to mask any auditory cue possibly arising from the stimulation procedure.

In Experiment 1 electrical stimuli were delivered, with equal probability, either to the hand undergoing the postural (i.e. 'far' and 'near') manipulation ('moving hand') or to the other hand ('non-moving hand') (Fig. 1). Participants did not know in advance which hand would be stimulated. In two separate blocks, either the left or the right hand underwent the postural manipulation. A total of 64 electrical stimuli were delivered as follows: 32 stimuli to the 'moving hand' (16 in the 'far' condition and 16 in the 'near' condition) and 32 stimuli to the 'non-moving hand', always kept in the 'far' position throughout the block (16 while the other hand was in the 'far' condition and 16 while the other hand was in the 'near' condition). The stimuli were delivered in pseudo-random order, with no more than two consecutive stimuli delivered to the same hand.

In Experiment 2 electrical stimuli were only delivered to the hand contralateral to the one undergoing the postural manipulation ('non-moving hand'), while the 'moving hand' was never stimulated (Fig. 2). In separate blocks, 16 stimuli were delivered to the left wrist and 16 to the right wrist, for a total of 32 stimuli. The order of blocks was balanced across participants. In each block, 8 stimuli were delivered while the 'moving hand' was kept in the 'far' position and 8 while this hand was in the 'near' position, in alternating trials.

Data Analysis and Statistics

EMG signals in both experiments were analyzed using Letswave (http://amouraux.webnode.com) (Mouraux and Iannetti, 2008). EMG signals from each participant were high-pass filtered (55 Hz) and full-wave rectified. In both experiments, data were averaged across ipsilateral and contralateral recording sides.

In Experiment 1, HBR responses elicited by the stimulation of the 'moving hand' and the 'non-moving hand' were averaged separately, according to the position ('far' vs. 'near') of the hand undergoing the postural manipulation ('moving hand'), resulting in four HBR average waveforms for each subject. In Experiment 2, HBR responses elicited by the stimulation of the 'non-moving hand' (in this Experiment, the only hand to be stimulated) were averaged according to the position of the 'moving hand', resulting in two HBR average waveforms for each subject.

In each participant, we measured the area under the curve (AUC) of each HBR average waveform. In Experiment 1, we performed a two-way, repeated-measures ANOVA, with 'position of the moving hand' (two levels: 'far' and 'near') and 'stimulated hand' (two levels: 'moving hand' and 'non-moving hand') as experimental factors. In Experiment 2, we performed a paired t-test to compare the HBR waveforms in the 'far' and 'near' positions.

Results

Somatotopical specificity of the HBR enhancement

When the electrical stimuli were delivered to either the 'moving hand' or the 'non-moving hand' with equal probability (Experiment 1), the magnitude of the HBR was significantly larger when the stimulated hand was inside the peripersonal space of the face (main effect of

'position of the moving hand', $F_{(1, 9)} = 36.59$, $p < 0.001$), and when the HBR was elicited by the stimulation of the hand undergoing the postural manipulation ('moving hand') compared to the hand always kept in the 'far' position ('non-moving' hand) (main effect of 'stimulated hand', $F_{(1, 9)} = 11.42$, $p = 0.008$). Crucially, the interaction between the two factors was significant ($F_{(1, 9)} = 11.27$, $p = 0.008$), indicating that the increase of HBR magnitude in the 'near' condition was significantly larger for the HBR elicited by the stimulation of the 'moving hand' (+68.2 ±56.3 % increase) compared to the HBR elicited by the stimulation of the 'non-moving hand' (+38.1 ±33.1 % increase) ($t_{(9)} = 3.36$, $p = 0.008$; paired t-test).

Effect of cognitive expectations on the HBR enhancement

When participants were aware that the electrical stimuli would be only delivered to the 'non-moving hand' (i.e. the probability of receiving stimuli on the 'moving hand' was 0%; Experiment 2), the HBR magnitude was identical regardless of whether the contralateral, 'moving hand' was placed inside or outside the peripersonal space of the face ($t_{(9)} = .22$, $p = 0.83$; paired t-test).

Discussion

In this study we characterized the somatotopical specificity of the HBR enhancement due to the manipulation of the spatial proximity between the stimulated hand and the face, and we investigated the effect of cognitive expectations on such enhancement.

Somatotopical specificity of the HBR enhancement by hand-face proximity

We have previously reported that (1) the enhancement of the HBR observed when the stimulated hand is placed near the face (Sambo et al., 2012) is mediated by a tonic, top-down modulation exerted by cortical areas responsible for encoding the space surrounding the face on the brainstem interneurons mediating the HBR, and (2) such modulation is specific for the interneurons mediating the HBR, but not for those mediating the trigeminal BR (i.e. the modulation is heterosegmentally-specific). The results of the present study characterize further the somatotopical specificity of this modulation, as they show that such top-down modulation is also partially specific when comparing inputs arising from homologous segments. Indeed, the cortex enhances preferentially the excitability of the circuits mediating the HBR elicited by the stimulation of the hand placed inside peripersonal space of the face (resulting in an HBR enhancement of +68.2%) compared to those mediating the HBR elicited by the contralateral hand (resulting in an HBR enhancement of +38.1%) (Fig. 1). This finding highlights a remarkable ability of the neocortex to finely and selectively tune the excitability of the different subcortical circuits mediating primitive defensive responses. Indeed, considering the defensive nature of the BR, it makes functional sense that when one hand is located inside the peripersonal space of the face, only the circuits mediating the BR in response to the afferent input from that hand become more excitable. In fact, a general increase of excitability of the BR elicited by stimuli applied to body districts other than those located close to the face would be evolutionary disadvantageous as it would trigger abnormal eye closure, which would not only be unnecessary and without a clear defensive benefit, but would also disrupt the effectiveness of the behavior in response to a potentially dangerous situation.

What could be the neural mechanism responsible for the effects observed? Two alternative hypotheses may be put forward (Fig. 3). First, the top-down cortical modulation might be directed both to the brainstem circuits mediating the HBR elicited by the stimulation of the

hand entering the peripersonal space of the face and to the circuits mediating the HBR elicited by the stimulation of the contralateral hand, although the modulation of the latter would be less strong (Fig. 3, *top*). This hypothesis assumes that the interneurons of the brainstem circuits mediating the HBR elicited by the stimulation of the two hands are entirely independent, and it implies two distinct modulations, exerted with different strengths: one, stronger, on the circuit mediating the HBR elicited by right hand stimulation and one, weaker, on the circuit mediating the HBR elicited by left hand stimulation. Alternatively, the cortical modulation might be directed uniquely to the brainstem interneurons mediating the HBR elicited by the stimulation of the hand placed inside the peripersonal space of the face, while the smaller enhancement of the HBR elicited by the stimulation of the contralateral hand would be a byproduct due to the neural architecture of the brainstem circuits subserving the two responses (Fig. 3, *bottom*). This second hypothesis assumes that the brainstem circuits mediating the HBR elicited by the stimulation of the two hands share a subset of their interneurons.

While it has not been investigated whether the circuits mediating the HBR elicited by the stimulation of the two hands are overlapping or separate, it has been shown that the R2 component of the BR elicited by the stimulation of the trigeminal nerve on one side is inhibited by a preceding stimulus delivered on the opposite side, whereas the R1 response is preserved (Kimura and Harada, 1976). This finding suggests an at least partial overlap of the interneurons mediating the R2 response evoked by stimuli delivered to the right and the left trigeminal nerve. Importantly, the observation that the R1 is facilitated both when the test stimulus is preceded by the stimulation of the contralateral trigeminal nerve (Kimura and Harada, 1976) and of the ipsilateral median nerve (Miwa et al., 1998) indicates that the R2 suppression is not consequent to an inhibition of the facial motoneurons, but of the medullary interneurons. Thus, considering also the similarity between the R2 component of the

trigeminal BR and the HBR (Miwa et al., 1995; Valls-Sole et al., 1997; Leon et al., 2011) and the evidence suggesting that the circuits mediating the HBR and the trigeminal BR may interact at pre-motor level (Miwa et al., 1998), the results by Kimura and Harada (1976) provide support to the second of the hypotheses above, i.e. that the cortical modulation triggered by the proximity between the hand and the face is *homosegmentally* specific, and that the smaller enhancement of the HBR elicited by the stimulation of the hand contralateral to that placed near the face (Fig. 3) is the unavoidable consequence of the partial overlap between the interneurons mediating the HBR elicited by the stimulation of the left and right hand. According to this account, the small enhancement of the HBR elicited by the hand always kept in the far position would have no functional significance related to protection.

Effect of cognitive expectations on the modulation of the HBR by hand position

When participants knew that the electrical stimuli would never be applied to the hand undergoing the postural manipulation (Experiment 2), the magnitude of the HBR elicited by the stimulation of the hand always kept in the 'far' position was not modulated by the position of the hand changing its proximity to the face (Fig. 2). This finding indicates that the top-down modulation reported in (Sambo et al., 2012) and replicated here (Experiment 1) is crucially dependent on *expectations*, i.e. it is only triggered when participants are aware that stimuli will be (as in (Sambo et al., 2012)), or could be (as in Experiment 1), delivered to the hand entering the peripersonal space of the face. Furthermore, when participants do not have an *a priori* knowledge about which hand would receive the electrical stimuli (i.e. when the hand always kept far from the face and the hand changing its proximity to the face were stimulated with equal probability; Experiment 1), the enhancement of the HBR elicited by the stimulation of the hand entering the peripersonal space of the face was smaller than that obtained in (Sambo et al., 2012) (+68.2% vs. +99.3%, on average). Taken together, these

findings indicate that the cortex is able to finely adjust the level of excitability of the brainstem circuits as a function of the *probability* of stimulus occurrence.

We previously suggested that the perceived threat of stimuli applied to the hand increases when the hand is inside the peripersonal space surrounding the face, which results in the enhancement of the HBR (Sambo et al., 2012). Thus, we identified a 'defensive' peripersonal space in humans, functionally distinct from the peripersonal space defined by multisensory integration and action control (Macaluso and Maravita, 2010). This defensive peripersonal space would represent a 'safety margin' to protect the individual from external danger (Cooke and Graziano, 2003; Graziano and Cooke, 2006): whenever a potentially threatening stimulus approaches or enters the defensive peripersonal space, the individual would detect and react to such stimuli more effectively, to restore this safety margin. Here we suggest that perceived threat is modulated not only by the position of the stimulus in respect to the peripersonal space, but also by the expectation of stimulus occurrence, with perceived threat being increased at high probabilities of stimulus occurrence. Accordingly, the HBR enhancement, reflecting the level of perceived threat, was maximal when the probability that the hand close to the face was going to be stimulated was set at 100% (Sambo et al., 2012), and reduced when the probability was set at 50% (Experiment 1). Moreover, the HBR was not modulated by the proximity between hand and face when the probability of the hand close to the face being stimulated was set to 0% (Experiment 2).

Conclusions

In two experiments we have (1) characterized the spatial specificity of the tonic, top-down modulation exerted by cortical areas representing the peripersonal space on the brainstem circuits mediating the HBR when the stimulated hand changes its proximity to the face, and (2) investigated its dependence on expectations. The present findings highlight the staggering

ability of the neocortex to finely tune the excitability of brainstem circuits mediating ancient reflex responses. This fine tuning is reflected in two remarkable effects: the *somatotopical selectivity* for those circuits receiving the afferent input from the body territory located close to the muscle effectors (e.g. in the peripersonal space of the face when considering the blink reflex) and the *threat-dependent modulation* of the level of excitability of those circuits triggered by the participants' expectations about whether or not the hand located inside the peripersonal space of the face could be stimulated.

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Figures Capture

Figure 1. Group-average, rectified HBR waveforms elicited by the electrical stimulation of the hand undergoing the 'far' and 'near' manipulation ('moving hand') and of the other hand ('non-moving hand'), always kept in the 'far' position. x-axis, time (ms); y-axis, EMG activity (mV). Note that all waveforms are averaged according to the position ('far' vs. 'near') of the 'moving hand'. Thus, in the 'near' condition (red), one hand was placed near the face ('moving hand', full line) and the other was kept far from the face ('non-moving hand', dashed line), and stimuli were delivered to either hand with equal probability. In the 'far' condition (blue) both hands were kept far from the face, and stimuli were delivered, with equal probability, to the 'moving hand' (full line) and to the 'non-moving hand' (dashed line). Crucially, participants did not know in advance which of the two hands would be stimulated. Note that the HBR is significantly greater in the 'near' than in the 'far' condition, particularly in response to stimuli applied to the 'moving hand'.

Figure 2. Group-average, rectified HBR waveforms elicited by the electrical stimulation of the hand always kept in the 'far' position ('non-moving hand'). x-axis, time (ms); y-axis, EMG activity (mV). Note that the waveforms are averaged according to the position ('far' vs. 'near') of the 'moving hand', although this hand was *never* stimulated. Crucially, participants knew in advance that only the 'non-moving hand' would be stimulated. Note that the HBR magnitude is remarkably similar in the 'near' and the 'far' condition.

Figure 3 (Experiment 1). Two possible neural mechanisms responsible for the HBR enhancement observed when the 'moving hand' hand (red) is placed inside the peripersonal space of the face ('near' position) and the 'non-moving' hand (blue) is kept in the 'far' position. *Top panel*: The interneurons of the brainstem circuits mediating the HBR elicited by the stimulation of the two hands are entirely independent. This model implies two distinct cortical modulations, exerted with different strengths: one, stronger, on the circuit mediating the HBR elicited by the stimulation of the 'moving hand' and the other, weaker, on the circuit mediating the HBR elicited by the stimulation of the 'non-moving hand'. *Bottom panel*: The brainstem circuits mediating the HBR elicited by the stimulation of the two hands share a subset of interneurons. This model allows a single top-down modulation directed uniquely to the brainstem interneurons mediating the HBR elicited by the stimulation of the 'moving hand'. According to this model, the smaller enhancement of the HBR elicited by the stimulation of the 'non-moving hand' would be a byproduct of the neural architecture of the brainstem circuits subserving the two responses.
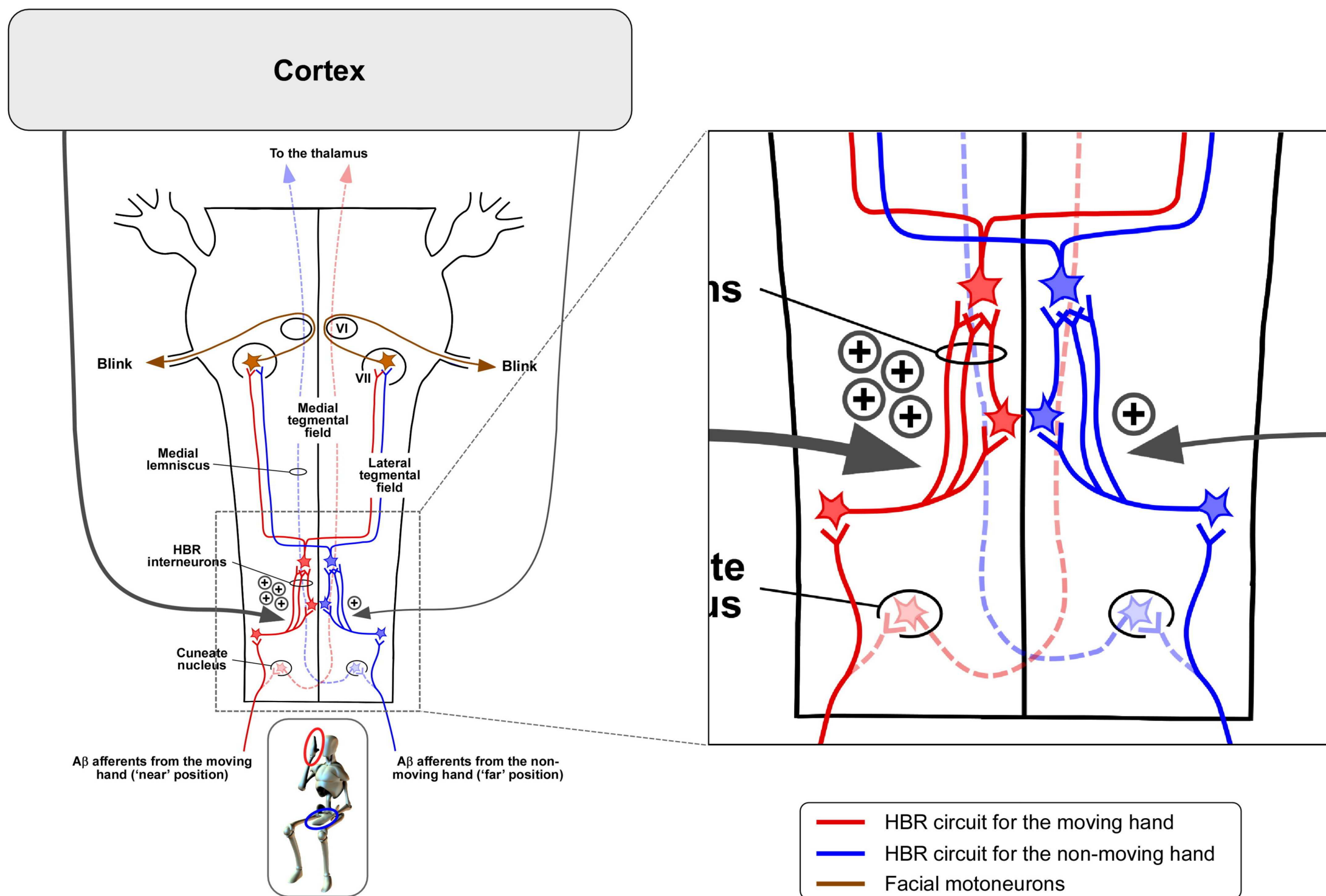
Somatotopical specificity of HBR enhancement (Experiment 1)
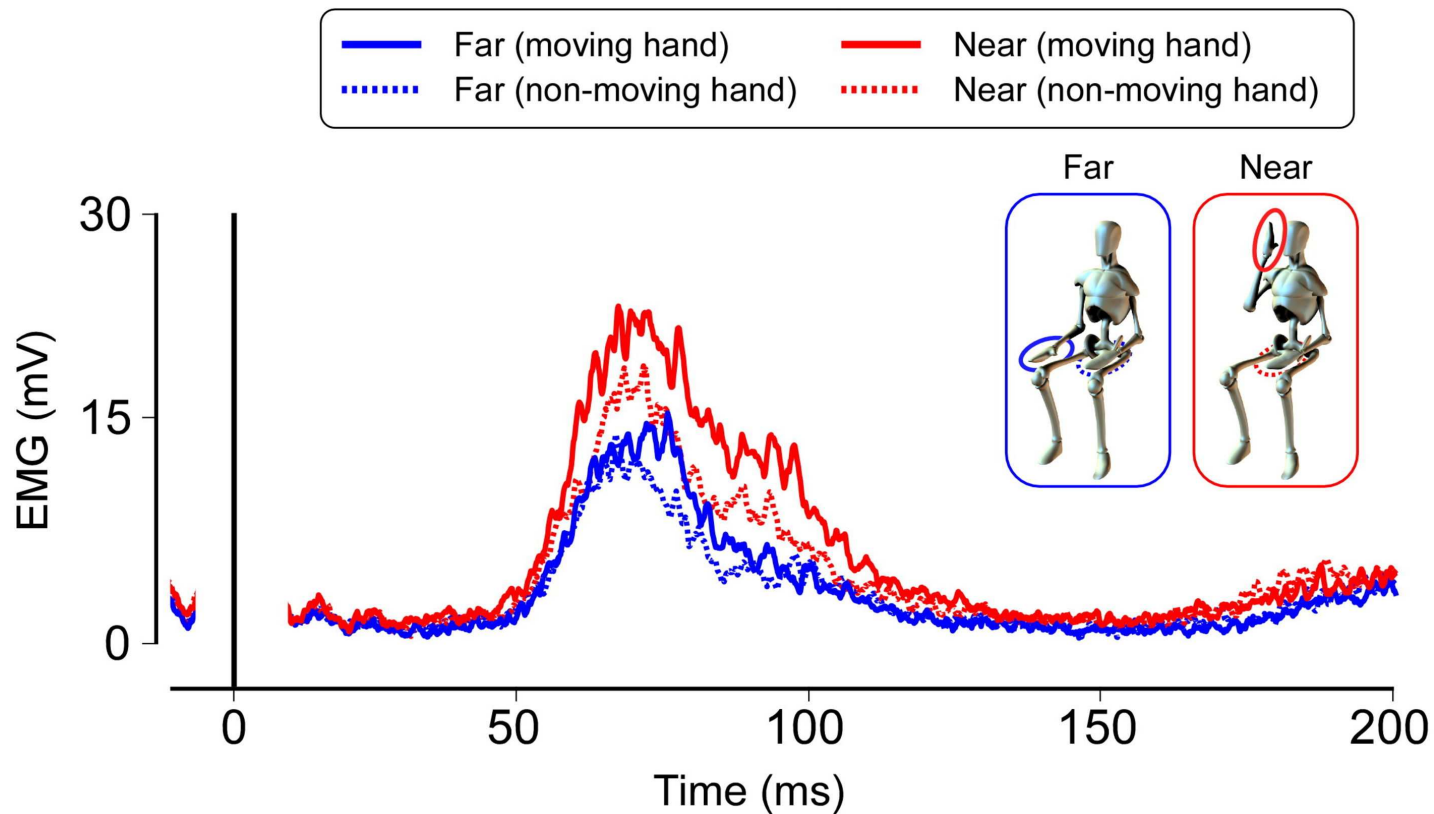
Effect of cognitive expectations on HBR enhancement (Experiment 2)
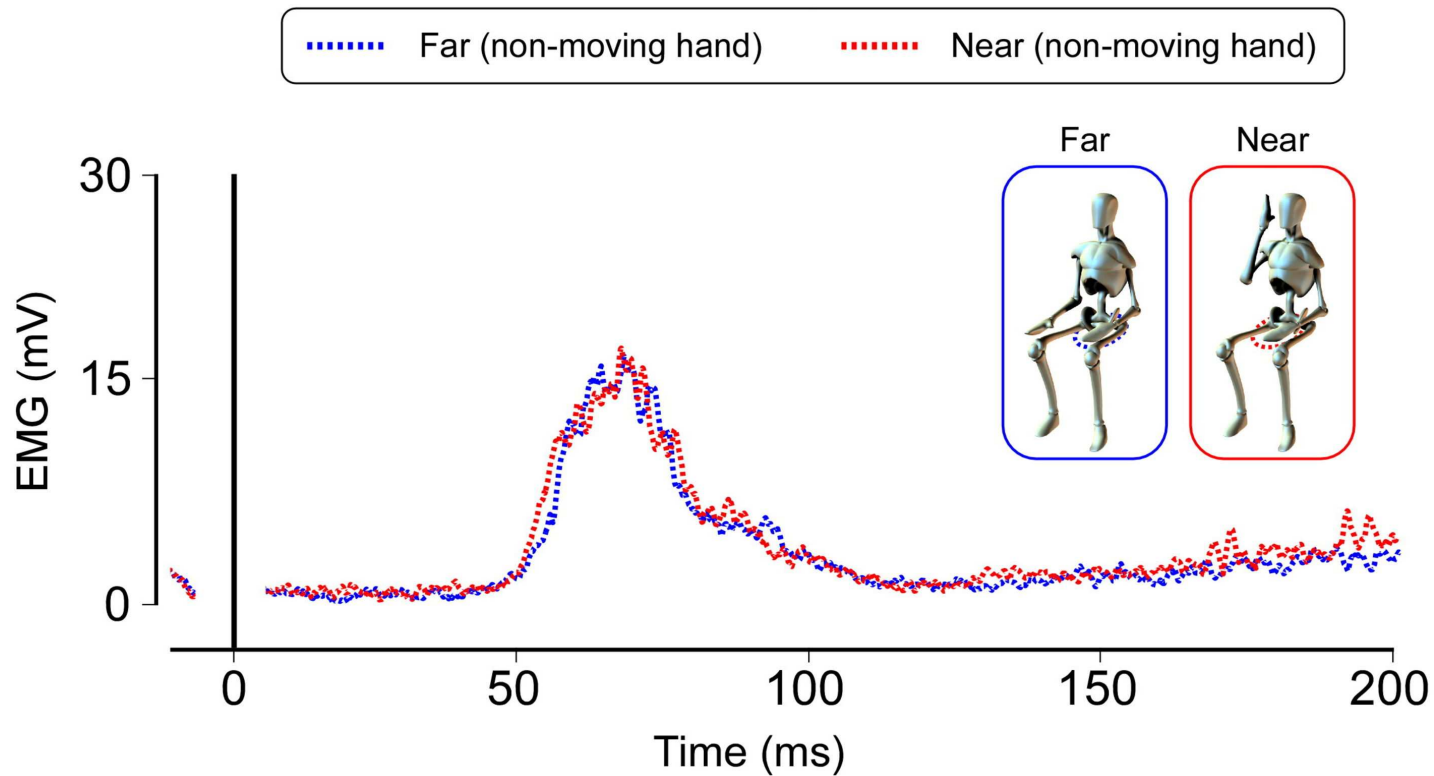
Two independent top-down modulations (of different strength) of the interneurons mediating the HBRs elicited by stimulation of the 'near' hand and of the non-moving hand

Cortex

To the thalamus

Blink

Blink

Medial tegmental field

Medial lemniscus

Lateral tegmental field

HBR interneurons

Cuneate nucleus

Aβ afferents from the moving hand ('near' position)

Aβ afferents from the non-moving hand ('far' position)

—— HBR circuit for the moving hand
—— HBR circuit for the non-moving hand
—— Facial motoneurons

One single top-down modulation of the interneurons mediating the HBR elicited by stimulation of the 'near' hand

Cortex

To the thalamus

Blink

Blink

Medial tegmental field

Medial lemniscus

Lateral tegmental field

HBR interneurons

Cuneate nucleus

Aβ afferents from the moving hand ('near' position)

Aβ afferents from the non-moving hand ('far' position)

—— HBR circuit for the moving hand
—— HBR circuit for the non-moving hand
—— Shared HBR interneuron(s)
—— Facial motoneurons