Steady-state evoked potentials to study the processing of tactile and nociceptive somatosensory input in the human brain

Etude du traitement cortical des afférences somatosensorielles tactiles et nociceptives par enregistrement de potentiels évoqués stationnaires.

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ABSTRACT

The periodic presentation of a sensory stimulus induces, at certain frequencies of stimulation, a sustained electroencephalographic response of corresponding frequency, known as steady-state evoked potential (SS-EP). In visual, auditory and vibrotactile modalities, studies have shown that SS-EPs reflect mainly activity originating from early, modality-specific sensory cortices. Furthermore, it has been shown that SS-EPs have several advantages over the recording of transient ERPs, such as a high signal-to-noise ratio, a shorter time to obtain reliable signals, and the capacity to frequency tag the cortical activity elicited by concurrently presented sensory stimuli. Recently, we showed that SS-EPs can be elicited by the selective activation of skin nociceptors and that nociceptive SS-EPs reflect the activity of a population of neurons that is spatially distinct from the somatotopically-organized population of neurons underlying vibrotactile SS-EPs. Hence, the recording of SS-EPs offers a unique opportunity to study the cortical representation of nociception and touch in humans, and to explore their potential crossmodal interactions. Here, (1) we review available methods to achieve the rapid periodic stimulation of somatosensory afferents required to elicit SS-EPs, (2) review previous studies that have characterized vibrotactile and nociceptive SS-EPs, (3) discuss the nature of the recorded signals and its relationship with transient event-related potentials and (4) outline future perspectives and potential clinical applications of this technique.

RESUME

À certaines fréquences de stimulation, l'application d'un stimulus sensoriel évoque une réponse électroencéphalographique stationnaire soutenue, de fréquence identique à la fréquence de stimulation (potentiels évoqués stationnaires ou « *steady-state evoked potentials* », SS-EP). Selon plusieurs études, ces réponses pourraient être le reflet d'un phénomène de résonance entreprenant des populations de neurones impliquées dans les étapes précoces du traitement sensoriel cortical. Les potentiels évoqués stationnaires offrent plusieurs avantages, tel qu'un rapport signal sur bruit élevé, et la possibilité de marquer l'activité cortical générée par la présentation simultanée de plusieurs trains de stimulation (*frequency tagging).* Récemment, nous avons montré que des SS-EPs peuvent être obtenus par l'activation sélective des nocicepteurs cutanés et que le traitement cortical des

afférences somatosensorielles nociceptives et non-nociceptives fait intervenir des réseaux corticaux distincts. La technique des SS-EPs constitue donc une opportunité pour étudier les processus corticaux impliqués dans la perception de douleur ainsi que la perception vibrotactile chez l'homme, ainsi que pour caractériser les éventuelles interactions entre ces processus. Dans cet article de revue, (1) nous décrivons les différentes méthodes permettant de stimuler rapidement et périodiquement les afférences somatosensorielles, afin d'obtenir des SS-EPs ; (2) nous examinons les études antérieures par enregistrement de SS-EPs vibrotactiles et nociceptifs ; (3) nous discutons la nature des signaux enregistrés et (4) nous évoquons les perspectives futures et les applications cliniques potentielles de cette technique.

Keywords

Steady-state evoked potentials (SS-EP), Electroencephalography, Nociception, Somatosensory, frequency-tagging, vibrotactile.

Mots clés

Potentiels évoqués stationnaires, Electroencéphalographie, Nociception, Somatosensoriel, Frequency-tagging, vibrotactile.

Introduction

Since the first recording of electrical activity from the human brain by Hans Berger [7] a great number of investigators have used non-invasive electroencephalographic (EEG) techniques to study how the human brain processes sensory inputs. The majority of studies have relied on the recording of event-related brain potentials (ERPs), i.e., changes in the ongoing electrical brain activity time-locked to a transient external event like the sudden onset of a sensory stimulus [58,61].

In 1966, Regan introduced the technique of "steady-state evoked potentials" (SS-EPs) as an alternative approach to characterize stimulus-evoked activity in the ongoing EEG. Unlike conventional transient ERPs, which Regan described as *"the response to a kick in the system"*, SS-EPs reflect a sustained cortical response induced by the long-lasting periodic repetition of a sensory stimulus, described by Regan [61] as "*the response to a gentle shake of the system at a fixed repetition rate*". These steady-state responses remain constant in amplitude and phase over time, and are thought to result from an entrainment or resonance of a population of neurons responding to the stimulus at the frequency of stimulation [26,48,79] or from the linear superposition of independent transient responses elicited by the fast repetition of the sensory stimulus [9,11]. Whereas transient ERPs are identified in the time domain as a series of time-locked deflections following the onset of the stimulus, SS-EPs are identified in the frequency domain as peaks appearing at the frequency of the repeated stimulus and/or at harmonics of that frequency [61].

An increasing number of studies have used SS-EPs to explore the neural activity involved in the cortical processing of visual and auditory sensory modalities and, to a lesser extent, the somatosensory modality. These studies showed that SS-EPs

reflect, at least in part, activity originating from early, modality-specific sensory cortices [23,56,59,66,67,71].

Recently, we showed that it is possible to record SS-EPs in response to the rapid periodic thermal activation of cutaneous nociceptors in humans [46], as well as to the rapid periodic electrical stimulation of nociceptive intra-epidermal free nerve endings [13]. We found that the scalp topography of these nociceptive SS-EPs was maximal at the scalp vertex, and symmetrically distributed over both hemispheres, suggesting a radial source originating from midline brain structures (Figure 1). Most interestingly, at stimulation frequencies greater than 3 Hz, this midline scalp topography contrasted strongly with the lateralized scalp topography of the SS-EPs obtained by vibrotactile stimulation, which displayed a clear maximum over the parietal region contralateral to the stimulated side, suggesting a tangential source possibly originating from the contralateral primary somatosensory cortex (S1). Because the spatial distribution of nociceptive SS-EPs was significantly different from the spatial distribution of non-nociceptive vibrotactile SS-EPs, we hypothesized that nociceptive SS-EPs reflect the activity of a population of neurons spatially distinct from the somatotopically-organized population of neurons underlying vibrotactile SS-EPs.

As compared to methods based on the recording of transient ERPs, but also as compared to other non-invasive methods to sample brain activity in humans such as functional MRI, investigating brain function using SS-EPs offers several outstanding advantages. First, studies performed in other sensory modalities have shown that SS-EPs exhibit a high signal-to-noise ratio [42,54,79]. Hence, nociceptive SS-EPs could be used to sample neural activity that cannot be sampled reliably using other

techniques. Second, because SS-EPs have been shown to reflect, at least in part, neural activity originating from modality-specific sensory cortices, it is possible that nociceptive SS-EPs reflect cortical activity that is at least partly specific for nociception and the perception of pain [46]. Third, SS-EPs are not induced by the sudden onset of a stimulus, but by the sustained modulation of a long-lasting stream of sensory input. Hence, as compared to nociceptive ERPs, nociceptive SS-EPs are probably less imprinted by cortical activity related to stimulus-triggered attentional capture [27,36,46]. Fourth, different stimulation frequencies can be used to *tag* the different sensory inputs constituting a multimodal stimulus and, thereby, isolate the neural activity related specifically to each stream of input [43,61,74]. This frequency-tagging approach has been used successfully to characterize the neural activity involved in the multimodal integration of audiovisual stimuli, and its modulation by selective attention [16,22,23,31,49,50,64,73]. Hence, frequency-tagging of concomitant nociceptive and non-nociceptive somatosensory inputs could constitute a unique mean to characterize their respective neural representations, as well as to study how these sensory inputs integrate at cortical level.

For all these reasons, the recording of vibrotactile and nociceptive somatosensory SS-EPs could constitute a promising approach to study the cortical representation of touch and nociception in humans. Importantly, exploring fully this new line of research will require optimizing current stimulation techniques to achieve the rapid, periodic, selective and controlled activation of nociceptors required to elicit SS-EPs.

Here, we will review the use of SS-EPs as a technique to study the neural representation of touch and nociception in humans. Specifically, (1) we will describe

different methods to achieve the rapid periodic stimulation of somatosensory afferents required to elicit SS-EPs, (2) we will discuss the nature of the recorded signals and its relationship with transient ERPs, (3) we will review previous studies characterizing tactile and nociceptive SS-EPs and (4) we will discuss future perspectives and potential clinical applications of this technique.

1. Rapid periodic stimulation of somatosensory afferents

To elicit somatosensory SS-EPs, studies have relied on mechanical vibrotactile stimulation of mechano-sensitive cutaneous afferents [1,5,22,23,48,52,65,66,71,72], thermal stimulation of heat-sensitive nociceptive afferents [46] and direct electrical stimulation of sensory nerve fibres [2,13,34,41,46,54,60].

Mechanical vibrotactile stimulation. Several studies have devised stimulation methods to periodically activate low threshold mechanoreceptors by applying a light force onto the skin and, thereby, elicit somatosensory SS-EPs related to the perception of vibrotactile sensations. For example, Nangini et al. [52] developed an inflatable membrane connected to a pneumatic controller containing magnetic valves for switching the airflow to the membrane. Other investigators have relied on piezo-electric devices [65] or solenoid vibrators (e.g. [1]). One advantage of these methods of stimulation is that different frequencies of stimulation may be expected to preferentially activate different types of low-threshold mechanoreceptors, having different frequency response characteristics. For example, relatively low frequencies of stimulation should preferentially elicit neural activity related to the activation of Meissner corpuscles, whereas higher frequencies of stimulation should preferentially elicit activity related to the activation of Pacinian corpuscles [25,29]. Most studies

have used vibrotactile stimuli consisting of a greater than 100 Hz carrier frequency (i.e. frequencies at which Pacinian corpuscles are especially sensitive to vibration) periodically modulated at a frequency below 40 Hz. A disadvantage of these methods is that care must be taken to ensure that the mechanical vibration generated by the stimulator is not concomitantly transduced by sensory receptors of the ears (air and body conduction). It should be noted that, in principle, mechanical stimulation of the skin could also be used to periodically activate mechano-sensitive nociceptors and, thereby, elicit nociceptive SS-EPs, for example, using the pinprick device developed by Ziegler et al. [80]. However, because of the unavoidable concomitant activation of low-threshold mechanoreceptors, the technique would not be selective for nociceptive afferents, thus limiting the interpretability of the obtained EEG responses.

Thermal stimulation of heat-sensitive somatosensory afferents. Recently, we showed that it is possible to activate periodically heat-sensitive afferents of the skin using an infrared CO_2 laser stimulator [46]. Brief (20 ms) and focal (5 mm beam diameter) laser pulses were delivered to the hand and foot dorsum at a rate of 7 Hz. To avoid skin overheating and possible sensitization or habituation of the activated nociceptors, the target of the laser stimulus was displaced immediately after each pulse, using a flat mirror set on a two-axis computer-controlled device powered by two high-speed servomotors. The displacement followed a zigzag path, such that the same spot was stimulated only once in each train. The advantage of this approach is that it is entirely selective for heat-sensitive free nerve endings of the thermo-nociceptive system. Higher frequencies of stimulation could be obtained using, for example, a device driven by galvanometers, as these have switching times as short

as a few microseconds. In principle, it may also be possible to activate periodically heat-sensitive free nerve endings without displacing the stimulus, for example, using a Peltier-type contact stimulator having the capacity to both rapidly heat and cool the skin [33,68,78], or using an infrared laser stimulator able to adjust laser power output as a function of an online measurement of target skin temperature such as to account for the increasing baseline temperature [40]. One possible drawback for all approaches using thermal stimulation to elicit nociceptive SS-EPs is the fact that they rely on the transduction of the thermal stimulus into a neural impulse. Hence, the elicited responses can only reflect the activation of a subpopulation of short activation latency heat-sensitive afferents, able to preserve the periodicity of the afferent input. Furthermore, variations in the heat transfer to the skin, variations in transduction and variations in nerve conduction velocities could result in variations of the temporal dynamics of the elicited afferent input, possibly blurring its periodicity, in particular, at high frequencies of stimulation [46].

Electrical stimulation of somatosensory nerve fibres. An alternative approach to elicit somatosensory SS-EPs is to bypass transduction processes altogether, by depolarizing directly afferent sensory nerve fibres. A number of studies have relied on transcutaneous electrical stimulation of a nerve trunk to selectively and directly activate large diameter thickly myelinated Aβ-fibres involved in the perception of touch [2,13,34,41,46,54,60]. Similarly, we recently showed that intra-epidermal electrical stimulation to deliver very focal currents restricted to the epidermis can be used to activate nociceptive free nerve endings selectively [47] and, thereby, elicit nociceptive SS-EPs [13]. Several devices have been proposed, consisting of a small surface cathode surrounded by a cylindrical anode [28,30]. Importantly, the

selectivity of this technique relies on the difference in receptor depth of nociceptive and non-nociceptive somatosensory receptors [28,53] and, therefore, the technique is selective only at low intensities of stimulation [47]. An advantage of all approaches based on the direct electrical stimulation of afferent nerve fibres is that, as they bypass transduction, the periodicity of the afferent input may be better preserved and, hence, the elicited SS-EPs may be more robust, in particular, at high frequencies of stimulation. Furthermore, direct electrical stimulation of sensory afferents may ensure that the elicited responses are not related to the activation of only a small subpopulation of rapidly-adapting somatosensory receptors. A drawback of this approach is that the results can be difficult to interpret if the recorded signals are contaminated by an electrical stimulation artefact, appearing at the frequency of stimulation.

2. Nature of SS-EP signals and relationship with transient ERPs

How SS-EPs emerge within the human EEG, and its relationship with transient ERPs remains a matter of debate [11].

A first hypothesis is that SS-EPs are simply the result of the linear summation of successive transient responses elicited by the fast repetition of the sensory stimulus [9,11]. In this view, SS-EPs would result from the same neural activity underlying transient ERPs [11]. This hypothesis has been mainly tested in the auditory modality [3,9,10,14,63], and is suggested by the observation that the auditory SS-EP elicited by stimulation at 40 Hz can be largely explained by the linear sum of middle latency auditory ERPs (i.e. series of ERP waves appearing 8-80 ms after the onset of a brief auditory stimulus such as an auditory click) [20]. Building on this observation, a number of studies have attempted to demonstrate that SS-EPs emerge from the

linear superposition of transient responses by computing the sum of real or simulated transient responses and by examining how well they correlate with actual SS-EPs. While some studies have shown evidence in favour of the superposition hypothesis [24,69], others have failed to demonstrate a significant correlation between SS-EPs and transient ERPs [3,14,63]. To explain such discrepant results, it has been suggested that these approaches do not account for the influence of neural adaptation and/or refractoriness [9,11]. Using approaches accounting for this influence, investigators have succeeded in finding a linear relationship between SS-EPs and transient ERPs, both in the auditory domain [9] and in the visual domain [11].

A second hypothesis is that SS-EPs result from a stimulus-driven entrainment of a network of neurons responding to the periodically-modulated feature of the eliciting stimulus [26,79]. Therefore, at preferred frequencies of stimulation, the network – or part of the network - of neurons responding to that stimulus feature is hypothesized to resonate to the stimulus [26,79]. According to this hypothesis, SS-EPs would reflect the ability of the neurons to oscillate at particular frequencies, and to synchronize their activity to an external periodic event [19,26]. Compatible with this view, it has been shown that the magnitude of the SS-EPs elicited by a flickering visual stimulus in the human visual cortex is markedly greater for particular frequencies of stimulation than for adjacent frequencies of stimulation, indicating a preference of the underlying neuronal oscillators for given frequencies and their harmonics [26]. The preferred response frequencies of a given ensemble of neurons could be explained by the temporal characteristics of the axonal connexions constituting the resonating network. In other words, the resonance hypothesis

proposes that SS-EPs are the result of an emergent property of a network of interconnected neurons. In this view, the brain is considered as a non-linear system and, most importantly, the neural activity captured by SS-EPs may differ markedly from the neural activity reflected in transient ERPs [61].

In summary, whether or not SS-EPs can be entirely explained by a linear superposition of successive transient ERPs or whether they reflect a stimulus-driven entrainment of neurons resonating at the frequency of stimulation remains an open question, and the two hypotheses may coexist (i.e. SS-EPs elicited by a given stimulus presented at a given frequency could reflect mainly the superposition of transient ERPs while SS-EPs elicited by another type of stimulus or presented at another frequency could reflect mainly a stimulus-driven neuronal entrainment).

3. Vibrotactile somatosensory steady-state evoked-potential

Using either transcutaneous electrical stimulation [2,13,34,41,46,54,60] or mechanical stimulation of low threshold mechanoreceptors [1,5,22,23,48,52,65,66,71,72], several studies have aimed at characterising the SS-EPs related to the perception of vibrotactile sensations.

Using a carrier frequency to elicit a steady afferent somatosensory input (e.g. 128 Hz; [66]) modulated using a range of frequencies extending from 2 to 41 Hz [48,54,65,66,71,72], investigators have reported that vibrotactile stimulation of the hand palm elicits maximal SS-EPs at periodicities around 27 Hz [48], 26 Hz [66] or 21 Hz [71,72]. When stimulating the foot sole, maximal amplitudes were observed at

slightly lower modulation frequencies, around 19-25 Hz [72]. Hence, it appears that the preferred frequency to elicit somatosensory SS-EPs lies in the range of 20-30 Hz. This differs from the visual modality, where greatest SS-EP amplitudes are usually found between 10 and 18 Hz for flash stimuli and at even lower frequencies for patterned stimuli [61,71]. It also differs from the auditory modality, where greater SS-EP amplitudes originating from the cortex are usually obtained using modulation frequencies in the range of 40 Hz [19]. As discussed in the preceding section, these different frequency response properties have been interpreted as resulting from differences in the temporal characteristics of the connexions constituting the responding network [26,61]. It should be noted that single-cell recordings performed in animals have shown the existence, in S1, of neurons with exquisite responsiveness to high frequency vibrations (e.g. 127 Hz; [35]), probably encoding input transduced by Pacinian afferents. Given that several recent studies (e.g. [4]) have shown that EEG is able to sample high-frequency responses (500-600 Hz) to transient somatosensory stimuli originating from S1 (referred to as high-frequency bursts), future studies could examine the feasibility of recording high-frequency vibrotactile SS-EPs.

Whatever the method used to activate non-nociceptive somatosensory afferents, the scalp topography of the elicited SS-EPs displays a clear maximum over the parietal region contralateral to the stimulated side, and source analysis studies have yielded results compatible with activity originating from the primary somatosensory cortex contralateral to the stimulated side [22,23,46,60,66,71]. Single-cell recordings performed in animals have shown that rapidly-adapting afferent units, which encode vibrotactile somatosensory input, have strong projections to areas 3b and area 1 of

the contralateral S1 cortex [44], thus supporting the view that SS-EPs elicited by vibrotactile stimulation originate mainly from these regions. It should be noted that the scalp topographies of vibrotactile SS-EPs are highly similar to the scalp topographies of the early components of transient non-nociceptive somatosensory ERPs (e.g., the N20 wave following electrical stimulation of the median nerve) [15,61]. Nevertheless, through a direct comparison of both types of responses, Nangini et al. [52] suggested that early-latency somatosensory ERPs and vibrotactile SS-EPs may originate from slightly distinct subregions of area 3b.

In a recent study, we found that the scalp topography of vibrotactile SS-EPs differs when very low modulation frequencies are used (e.g. 3 Hz; [13]. Indeed, and contrasting with the lateralized parietal scalp topography obtained at higher stimulation frequencies, the scalp topography of the SS-EP elicited by 3-Hz stimulation was symmetrically distributed over both hemispheres, and maximal over the vertex and fronto-central regions. Furthermore, this scalp topography was similar to that of the late P2 wave of transient somatosensory ERPs [45]. Such as the late P2 wave [27], the magnitude of the 3-Hz SS-EP showed a marked habituation, suggesting that both responses reflect unspecific and non-obligatory stages of sensory processing, strongly dependent on the context within which the afferent sensory input occurred and possibly related to stimulus-evoked attentional capture [13,27,36,38].

4. Nociceptive somatosensory steady-state evoked-potential

Using EEG, investigators have relied mostly on the recording of transient laser-evoked brain potentials (LEPs) to study nociception and pain perception in humans

[12,21,76]. A large number of studies have suggested that LEPs reflect, at least partially, the neural processes by which the perception of pain emerges from nociceptive input [6,77]. As a consequence, it has been hypothesized that LEPs constitute a reliable approach to study how pain is “represented” in the brain [76]. However, there is also increasing evidence indicating that the largest part of LEPs could reflect cortical activity unspecific for nociception, such as multimodal cognitive processes involved in the detection and the orientation of attention toward the occurrence of a transient, salient sensory event [27,36; see also 37 in this issue].

As previous studies have shown that SS-EPs are effective to capture neural activity related to sensory processing, originating mainly from primary sensory cortices [32,57,66,71], the effective recording of nociceptive SS-EPs could constitute a novel mean to characterize the cortical processing of nociceptive input in humans.

We recently showed that it is possible to record nociceptive SS-EPs using rapidly-displaced laser pulses delivered to the skin at a 7-Hz periodicity [46]. Subsequently, we showed that nociceptive SS-EPs can also be obtained using intra-epidermal electrical stimulation to selectively activate epidermal free nerve endings [13], this time using a range of frequencies extending from 3 to 43 Hz.

Whatever the method used to activate nociceptive afferents selectively, and whatever the location of the stimulus (hand and foot dorsum), the scalp topographies of the recorded nociceptive SS-EPs were symmetrically distributed over both hemispheres, and displayed a clear maximum over midline, fronto-central regions [13,46]. Source analysis showed that the elicited responses could be satisfactorily

explained by a single radial source located in anterior midline brain structures such as the anterior cingulate cortex [46]. However, given the uncertainty inherent to EEG source analyses, a contribution from bilateral symmetrical sources located within operculo-insular cortices can clearly not be excluded. Whatsoever, our findings indicate that nociceptive SS-EPs reflect the activity of a cortical network that is distinct from the somatotopically organized cortical network involved in the generation of vibrotactile SS-EPs [13,46], (Figure 1). Consistent with the hypothesis that cortical activity originating from these regions contributes to the bulk of nociceptive SS-EPs, but not to vibrotactile SS-EPs, Dum et al. [18] showed that, unlike tactile somatosensory input, the primary target of nociceptive spino-thalamic input is not the contralateral S1, but the insular cortex, the secondary somatosensory cortex and, above all, the cingulate cortex.

Using low-energy laser stimuli to activate selectively low-threshold C-warm receptors of the skin, we also attempted to record SS-EPs related to the selective activation of unmyelinated C-fibres [46]. Although participants reported the clear perception of a diffuse and long-lasting warm sensation, laser stimuli applied at a frequency of 7-Hz did not elicit an identifiable C-fibre SS-EP. This lack of measurable EEG response could be explained by the fact that the magnitude of SS-EPs is not only determined by the magnitude of the underlying neural activity, but also by the constancy of it phase over the repeated stimulation cycles. Indeed, differences in the temporal properties of the C-fibre responses (response latency of C-fibre free nerve endings, variability in C-fibre nerve conduction velocity) elicited by each successive laser pulse could be expected to dampen or even abolish the periodicity of the C-fibre afferent input. Future studies should examine whether C-fibre SS-EPs can be recorded using lower frequencies of stimulation.

5. Future perspectives: frequency tagging of somatosensory SS-EPs

Several studies have shown that different stimulation frequencies can be used to *tag* the cortical responses elicited by each of several, concurrently applied, sensory stimuli [43,61,74). For example, simultaneously presenting an auditory stimulus modulated at frequency F1 and a visual stimulus modulated at frequency F2 elicits two distinct peaks in the EEG spectrum, at frequencies F1 and F2, respectively. This frequency-tagging approach has been used successfully to demonstrate top-down attentional modulation of visual [43,49], vibrotactile [22,23] and auditory [8,51] inputs, and to characterize the cortical activity involved in the multimodal integration of audiovisual stimuli [16,31,55,64,70,73]. Recently, in a preliminary and unpublished experiment, we have shown that distinct SS-EPs can be reliably recorded following concomitant nociceptive, vibrotactile and visual stimulation and that the elicited responses, appearing as three separate peaks in the EEG frequency spectrum, have distinct scalp topographies (Figure 2). Hence, frequency-tagging of the EEG responses to concomitant nociceptive and non-nociceptive somatosensory stimulation could constitute a unique mean to characterize their respective neural representations, as well as to study how these sensory inputs integrate at cortical level. Furthermore, the approach could be used to examine whether neural processes involved in the integration of nociceptive and non-nociceptive somatosensory stimuli can be revealed by the presence of cross-modulation frequencies in the EEG, appearing at frequencies $nF_1 \pm mF_2$, where n and m are integers and F1 and F2 are the frequencies of stimulation of two concurrent streams of sensory input. For example, concomitant nociceptive stimulation at frequency F1=7Hz and non-nociceptive stimulation at frequency F2=9Hz could elicit cross-

modulation SS-EPs appearing at F2+F1=16Hz and F2-F1=2Hz, and such responses would constitute an index of the activity generated by neuronal populations onto which the different sensory inputs converge [61,62]. A small number of studies have already shown cross-modulation SS-EPs induced by the integration of auditory and visual inputs [62]. Showing the presence of such cross-modulation frequencies constitutes unequivocal evidence for a non-linear process of convergence of the two sensory inputs. For example, such cross-modulation SS-EPs could reflect the activity of a population of neurons whose output corresponds to the product of the two input oscillations. Admittedly, whether or not the concomitant presentation of nociceptive and non-nociceptive somatosensory stimuli elicits cross-modulation SS-EPs remains to be determined, as such components have not yet been described. However, if such responses can be identified, they would open a new door to study directly the cortical mechanisms involved in multimodal perceptual integration [17,39].

6. Clinical applications

A small number of studies have highlighted the potential clinical usefulness of recording vibrotactile SS-EPs [54,60]. One advantage over the recording of transient ERPs is the high signal-to-noise ratio of the elicited responses and, hence, the short time required to obtain reliable signals. This could be potentially interesting, in particular, in circumstances where patient collaboration is poor (e.g. children, patients with cognitive impairment) or when it is crucial to obtain rapid estimates of the elicited responses (e.g. perioperative neuromonitoring of spinal cord function; [60]).

References

1 Adler, J., Muller, M.M., Giabbiconi, C.M., 2009. Shift of attention to the body location of distracters is mediated by perceptual load in sustained somatosensory attention. Biological Psychology 81, 77-85.

2 Astolfi, L., Fallani Fde, V., Cincotti, F., Mattia, D., Bianchi, L., Marciani, M.G., Salinari, S., Gaudiano, I., Scarano, G., Soranzo, R., Babiloni, F., 2009. Brain activity during the memorization of visual scenes from TV commercials: an application of high resolution EEG and steady state somatosensory evoked potentials technologies. J Physiol Paris 103, 333-341.

3 Azzena, G.B., Conti, G., Santarelli, R., Ottaviani, F., Paludetti, G., Maurizi, M., 1995. Generation of Human Auditory Steady-State Responses (Ssrs) .1. Stimulus Rate Effects. Hearing Research 83, 1-8.

4 Baker, S.N., Curio, G., Lemon, R.N., 2003. EEG oscillations at 600 Hz are macroscopic markers for cortical spike bursts. J Physiol 550, 529-534.

5 Bardouille, T., Ross, B., 2008. MEG imaging of sensorimotor areas using inter-trial coherence in vibrotactile steady-state responses. Neuroimage 42, 323-331.

6 Baumgartner, U., Tiede, W., Treede, R.D., Craig, A.D., 2006. Laser-evoked potentials are graded and somatotopically organized anteroposteriorly in the operculoinsular cortex of anesthetized monkeys. Journal of Neurophysiology 96, 2802-2808.

7 Berger, H., 1929. Über das electroenzephalogramm beim menschen. Arch Psychiatrie Nervenkrankheiten 87, 527-570.

8 Bidet-Caulet, A., Fischer, C., Besle, J., Aguera, P.E., Giard, M.H., Bertrand, O., 2007. Effects of selective attention on the electrophysiological representation of concurrent sounds in the human auditory cortex. J Neurosci 27, 9252-9261.

9 Bohorquez, J., Ozdamar, O., 2008. Generation of the 40-Hz auditory steady-state response (ASSR) explained using convolution. Clin Neurophysiol 119, 2598-2607.

10 Bohorquez, J., Ozdamar, O., Acikgoz, N., Yavuz, E., 2007. Methodology to estimate the transient evoked responses for the generation of steady state responses. Conf Proc IEEE Eng Med Biol Soc 2007, 2444-2447.

11 Capilla, A., Pazo-Alvarez, P., Darriba, A., Campo, P., Gross, J., 2011. Steady-State Visual Evoked Potentials Can Be Explained by Temporal Superposition of Transient Event-Related Responses. PLoS One 6.

12 Carmon, A., Mor, J., Goldberg, J., 1976. Evoked cerebral responses to noxious thermal stimuli in humans. Exp Brain Res 25, 103-107.

13 Colon, E., Nozaradan, S., Legrain, V., Mouraux, A., 2011. Steady-state evoked potentials to tag specific components of nociceptive cortical processing. Neuroimage.

14 Conti, G., Santarelli, R., Grassi, C., Ottaviani, F., Azzena, G.B., 1999. Auditory steady-state responses to click trains from the rat temporal cortex. Clin Neurophysiol 110, 62-70.

15 Cruccu, G., Aminoff, M.J., Curio, G., Guerit, J.M., Kakigi, R., Mauguiere, F., Rossini, P.M., Treede, R.D., Garcia-Larrea, L., 2008. Recommendations for the clinical use of somatosensory-evoked potentials. Clin Neurophysiol 119, 1705-1719.

16 de Jong, R., Toffanin, P., Harbers, M., 2010. Dynamic crossmodal links revealed by steady-state responses in auditory-visual divided attention. International Journal of Psychophysiology 75, 3-15.
17 Driver, J., Noesselt, T., 2008. Multisensory interplay reveals crossmodal influences on 'sensory-specific' brain regions, neural responses, and judgments. Neuron 57, 11-23.
18 Dum, R.P., Levinthal, D.J., Strick, P.L., 2009. The spinothalamic system targets motor and sensory areas in the cerebral cortex of monkeys. J Neurosci 29, 14223-14235.
19 Galambos, R., 1982. Tactile and Auditory-Stimuli Repeated at High-Rates (30-50 Per Sec) Produce Similar Event Related Potentials. Annals of the New York Academy of Sciences 388, 722-728.
20 Galambos, R., Makeig, S., Talmachoff, P.J., 1981. A 40-Hz auditory potential recorded from the human scalp. Proc Natl Acad Sci U S A 78, 2643-2647.
21 Garcia-Larrea, L., Frot, M., Valeriani, M., 2003. Brain generators of laser-evoked potentials: from dipoles to functional significance. Neurophysiol Clin 33, 279-292.
22 Giabbiconi, C.M., Dancer, C., Zopf, R., Gruber, T., Muller, M.M., 2004. Selective spatial attention to left or right hand flutter sensation modulates the steady-state somatosensory evoked potential. Brain Res Cogn Brain Res 20, 58-66.
23 Giabbiconi, C.M., Trujillo-Barreto, N.J., Gruber, T., Muller, M.M., 2007. Sustained spatial attention to vibration is mediated in primary somatosensory cortex. Neuroimage 35, 255-262.
24 Hari, R., Hamalainen, M., Joutsiniemi, S.L., 1989. Neuromagnetic steady-state responses to auditory stimuli. Journal of the Acoustical Society of America 86, 1033-1039.
25 Hashimoto, I., Mashiko, T., Kimura, T., Imada, T., 1998. Human somatosensory evoked magnetic fields to vibratory stimulation of the index finger: is there frequency organization in SI? Electroencephalogr Clin Neurophysiol 109, 454-461.
26 Herrmann, C.S., 2001. Human EEG responses to 1-100 Hz flicker: resonance phenomena in visual cortex and their potential correlation to cognitive phenomena. Exp Brain Res 137, 346-353.
27 Iannetti, G.D., Hughes, N.P., Lee, M.C., Mouraux, A., 2008. Determinants of laser-evoked EEG responses: pain perception or stimulus saliency? J Neurophysiol 100, 815-828.
28 Inui, K., Tran, T.D., Hoshiyama, M., Kakigi, R., 2002. Preferential stimulation of Adelta fibers by intra-epidermal needle electrode in humans. Pain 96, 247-252.
29 Johnson, K.O., 2001. The roles and functions of cutaneous mechanoreceptors. Curr Opin Neurobiol 11, 455-461.
30 Kaube, H., Katsarava, Z., Kaufer, T., Diener, H., Ellrich, J., 2000. A new method to increase nociception specificity of the human blink reflex. Clin Neurophysiol 111, 413-416.
31 Keitel, C., Schroger, E., Saupe, K., Muller, M.M., 2011. Sustained selective intermodal attention modulates processing of language-like stimuli. Experimental Brain Research 213, 321-327.
32 Kelly, E.F., Folger, S.E., 1999. EEG evidence of stimulus-directed response dynamics in human somatosensory cortex. Brain Res 815, 326-336.

33 Kenshalo, D.R., Bergen, D.C., 1975. A device to measure cutaneous temperature sensitivity in humans and subhuman species. Journal of Applied Physiology 39, 1038-1040.
34 Kourtis, D., Seiss, E., Praamstra, P., 2008. Movement-related changes in cortical excitability: a steady-state SEP approach. Brain Res 1244, 113-120.
35 Lebedev, M.A., Nelson, R.J., 1996. High-frequency vibratory sensitive neurons in monkey primary somatosensory cortex: entrained and nonentrained responses to vibration during the performance of vibratory-cued hand movements. Exp Brain Res 111, 313-325.
36 Legrain, V., Iannetti, G.D., Plaghki, L., Mouraux, A., 2011. The pain matrix reloaded A salience detection system for the body. Prog Neurobiol.
37 Legrain, V., Mancini, F., Sambo, C.F., Torta, D.M., Ronga, I., Valentini, E., 2012. Cognitive aspects of nociception and pain. Bridging neurophysiology with neuropsychology. Neurophysiologie clinique.
38 Lorenz, J., Garcia-Larrea, L., 2003. Contribution of attentional and cognitive factors to laser evoked brain potentials. Neurophysiol Clin 33, 293-301.
39 Macaluso, E., Driver, J., 2005. Multisensory spatial interactions: a window onto functional integration in the human brain. Trends in Neurosciences 28, 264-271.
40 Magerl, W., Ali, Z., Ellrich, J., Meyer, R.A., Treede, R.D., 1999. C- and A delta-fiber components of heat-evoked cerebral potentials in healthy human subjects. Pain 82, 127-137.
41 Manganotti, P., Formaggio, E., Storti, S.F., Avesani, M., Acler, M., Sala, F., Magon, S., Zoccatelli, G., Pizzini, F., Alessandrini, F., Fiaschi, A., Beltramello, A., 2009. Steady-state activation in somatosensory cortex after changes in stimulus rate during median nerve stimulation. Magn Reson Imaging 27, 1175-1186.
42 Meigen, T., Bach, M., 1999. On the statistical significance of electrophysiological steady-state responses. Documenta Ophthalmologica 98, 207-232.
43 Morgan, S.T., Hansen, J.C., Hillyard, S.A., 1996. Selective attention to stimulus location modulates the steady-state visual evoked potential. Proc Natl Acad Sci U S A 93, 4770-4774.
44 Mountcastle, V.B., Steinmetz, M.A., Romo, R., 1990. Frequency discrimination in the sense of flutter: psychophysical measurements correlated with postcentral events in behaving monkeys. J Neurosci 10, 3032-3044.
45 Mouraux, A., Iannetti, G.D., 2009. Nociceptive Laser-Evoked Brain Potentials Do Not Reflect Nociceptive-Specific Neural Activity. Journal of Neurophysiology 101, 3258 - 3269.
46 Mouraux, A., Iannetti, G.D., Colon, E., Nozaradan, S., Legrain, V., Plaghki, L., 2011. Nociceptive Steady-State Evoked Potentials Elicited by Rapid Periodic Thermal Stimulation of Cutaneous Nociceptors. Journal of Neuroscience 31, 6079-6087.
47 Mouraux, A., Iannetti, G.D., Plaghki, L., 2010. Low intensity intra-epidermal electrical stimulation can activate Adelta-nociceptors selectively. Pain 150, 199-207.
48 Muller, G.R., Neuper, C., Pfurtscheller, G., 2001. "Resonance-like" frequencies of sensorimotor areas evoked by repetitive tactile stimulation. Biomed Tech (Berl) 46, 186-190.

49 Muller, M.M., Andersen, S., Trujillo, N.J., Valdes-Sosa, P., Malinowski, P., Hillyard, S.A., 2006. Feature-selective attention enhances color signals in early visual areas of the human brain. Proc Natl Acad Sci U S A 103, 14250-14254.
50 Muller, M.M., Picton, T.W., Valdes-Sosa, P., Riera, J., Teder-Salejarvi, W.A., Hillyard, S.A., 1998. Effects of spatial selective attention on the steady-state visual evoked potential in the 20-28 Hz range. Brain Res Cogn Brain Res 6, 249-261.
51 Muller, N., Schlee, W., Hartmann, T., Lorenz, I., Weisz, N., 2009. Top-down modulation of the auditory steady-state response in a task-switch paradigm. Frontiers in Human Neuroscience 3, 1.
52 Nangini, C., Ross, B., Tam, F., Graham, S.J., 2006. Magnetoencephalographic study of vibrotactile evoked transient and steady-state responses in human somatosensory cortex. Neuroimage 33, 252-262.
53 Nolano, M., Simone, D.A., Wendelschafer-Crabb, G., Johnson, T., Hazen, E., Kennedy, W.R., 1999. Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation. Pain 81, 135-145.
54 Noss, R.S., Boles, C.D., Yingling, C.D., 1996. Steady-state analysis of somatosensory evoked potentials. Electroencephalogr Clin Neurophysiol 100, 453-461.
55 Nozaradan, S., Peretz, I., Mouraux, A., 2011. Steady-state evoked potentials as an index of multisensory temporal binding. Neuroimage 60, 21-28.
56 Pantev, C., Bertrand, O., Eulitz, C., Verkindt, C., Hampson, S., Schuierer, G., Elbert, T., 1995. Specific tonotopic organizations of different areas of the human auditory cortex revealed by simultaneous magnetic and electric recordings. Electroencephalogr Clin Neurophysiol 94, 26-40.
57 Pantev, C., Roberts, L.E., Elbert, T., Ross, B., Wienbruch, C., 1996. Tonotopic organization of the sources of human auditory steady-state responses. Hearing Research 101, 62-74.
58 Pfurtscheller, G., Lopes da Silva, F.H., 1999. Event-related EEG/MEG synchronization and desynchronization: basic principles. Clin Neurophysiol 110, 1842-1857.
59 Plourde, G., 2006. Auditory evoked potentials. Best Pract Res Clin Anaesthesiol 20, 129-139.
60 Pollok, B., Moll, M., Schmitz, F., Muller, K., Schnitzler, A., 2002. Rapid mapping of finger representations in human primary somatosensory cortex applying neuromagnetic steady-state responses. Neuroreport 13, 235-238.
61 Regan, D. (Ed.), 1989. Human brain electrophysiology. Evoked potentials and evoked magnetics fields in science and medicine. Elsevier, New York.
62 Regan, M.P., He, P., Regan, D., 1995. An audio-visual convergence area in the human brain. Exp Brain Res 106, 485-487.
63 Santarelli, R., Maurizi, M., Conti, G., Ottaviani, F., Paludetti, G., Pettorossi, V.E., 1995. Generation of Human Auditory Steady-State Responses (Ssrs) .2. Addition of Responses to Individual Stimuli. Hearing Research 83, 9-18.
64 Saupe, K., Schroger, E., Andersen, S.K., Muller, M.M., 2009. Neural mechanisms of intermodal sustained selective attention with concurrently presented auditory and visual stimuli. Frontiers in Human Neuroscience 3.
65 Severens, M., Farquhar, J., Desain, P., Duysens, J., Gielen, C., 2010. Transient and steady-state responses to mechanical stimulation of different

fingers reveal interactions based on lateral inhibition. Clin Neurophysiol 121, 2090-2096.
66 Snyder, A.Z., 1992. Steady-state vibration evoked potentials: descriptions of technique and characterization of responses. Electroencephalogr Clin Neurophysiol 84, 257-268.
67 Srinivasan, R., Russell, D.P., Edelman, G.M., Tononi, G., 1999. Increased synchronization of neuromagnetic responses during conscious perception. J Neurosci 19, 5435-5448.
68 Stuart, D.G., Ott, L.H., Cheshire, F.C., 1962. Thermal electrodes based on "Peltier effect". Electroencephalogr Clin Neurophysiol 14, 132-135.
69 Suzuki, T., Kobayashi, K., Umegaki, Y., 1994. Effect of natural sleep on auditory steady state responses in adult subjects with normal hearing. Audiology 33, 274-279.
70 Talsma, D., Doty, T.J., Strowd, R., Woldorff, M.G., 2006. Attentional capacity for processing concurrent stimuli is larger across sensory modalities than within a modality. Psychophysiology 43, 541-549.
71 Tobimatsu, S., Zhang, Y.M., Kato, M., 1999. Steady-state vibration somatosensory evoked potentials: physiological characteristics and tuning function. Clin Neurophysiol 110, 1953-1958.
72 Tobimatsu, S., Zhang, Y.M., Suga, R., Kato, M., 2000. Differential temporal coding of the vibratory sense in the hand and foot in man. Clin Neurophysiol 111, 398-404.
73 Toffanin, P., de Jong, R., Johnson, A., Martens, S., 2009. Using frequency tagging to quantify attentional deployment in a visual divided attention task. International Journal of Psychophysiology 72, 289-298.
74 Tononi, G., Srinivasan, R., Russell, D.P., Edelman, G.M., 1998. Investigating neural correlates of conscious perception by frequency-tagged neuromagnetic responses. Proc Natl Acad Sci U S A 95, 3198-3203.
75 Treede, R.D., Apkarian, A.V., Bromm, B., Greenspan, J.D., Lenz, F.A., 2000. Cortical representation of pain: functional characterization of nociceptive areas near the lateral sulcus. Pain 87, 113-119.
76 Treede, R.D., Kenshalo, D.R., Gracely, R.H., Jones, A.K.P., 1999. The cortical representation of pain. Pain 79, 105-111.
77 Treede, R.D., Kief, S., Holzer, T., Bromm, B., 1988. Late Somatosensory Evoked Cerebral Potentials in Response to Cutaneous Heat Stimuli. Electroencephalography and Clinical Neurophysiology 70, 429-441.
78 Valeriani, M., Le Pera, D., Niddam, D., Chen, A.C., Arendt-Nielsen, L., 2002. Dipolar modelling of the scalp evoked potentials to painful contact heat stimulation of the human skin. Neurosci Lett 318, 44-48.
79 Vialatte, F.B., Maurice, M., Dauwels, J., Cichocki, A., 2010. Steady-state visually evoked potentials: focus on essential paradigms and future perspectives. Prog Neurobiol 90, 418-438.
80 Ziegler, E.A., Magerl, W., Meyer, R.A., Treede, R.D., 1999. Secondary hyperalgesia to punctate mechanical stimuli. Central sensitization to A-fibre nociceptor input. Brain 122 (Pt 12), 2245-2257.

Figure legends

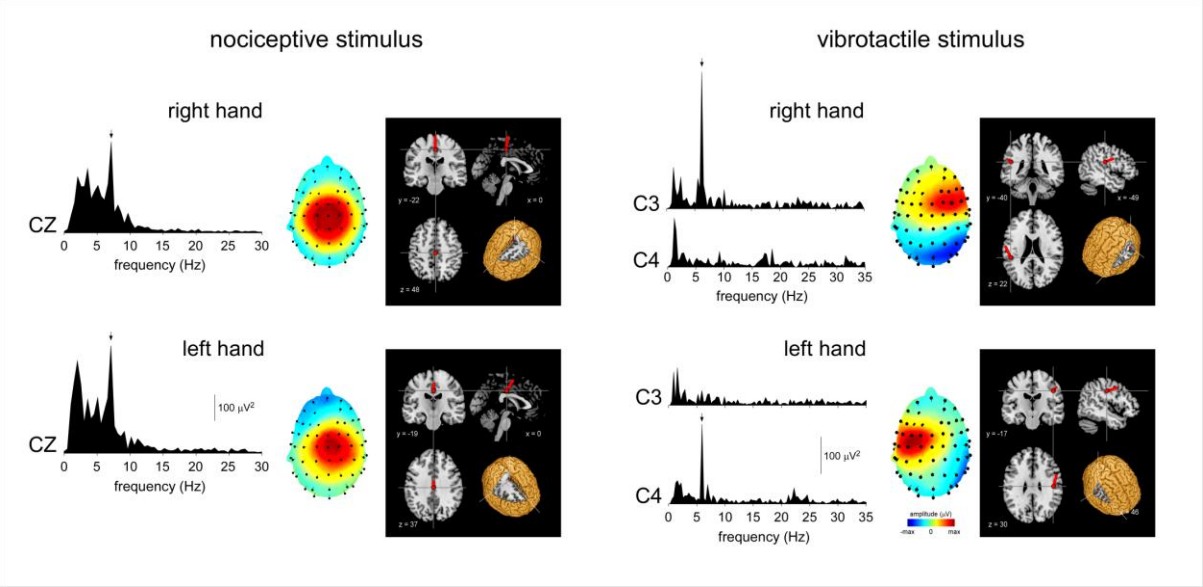

Figure 1. In this experiment, 2.3-s trains of nociceptive stimuli (7 Hz thermal CO_2 laser stimulation) were applied to the hand dorsum. The elicited responses were compared to those elicited by trains of vibro-tactile stimulation (6 Hz transcutaneous electrical stimulation of the superficial radial nerve). The left and right panels show the responses elicited by nociceptive and vibro-tactile stimulation, respectively. The left part of the panel represents the group-level average of the frequency spectrum of the EEG signals recorded at electrode Cz during the 7 Hz periodic stimulation of nociceptive fibres, and at electrodes C3 and C4 during the 6 Hz periodic stimulation of non-nociceptive fibres (noise-subtracted signal power, μv^2). Note that, for the two modalities and at all stimulus locations, the stimulus elicited a significant SS-EP at the corresponding frequency (marked by the vertical black arrows). The middle part of the panels represents the topographical distribution of the stimulus-induced increase in EEG signal power at the frequency of stimulation (group-level average). Note that, for all stimulus locations, the scalp topography of the nociceptive SS-EP was maximal at the vertex (electrode Cz), whereas the scalp topography of the vibro-tactile SS-EP was maximal over the parietal region contralateral to the stimulated side. The right part of the panels shows the location of a single equivalent dipole fitted to the group-level topographical maps of nociceptive and the vibro-tactile SS-EPs elicited by stimulation of the left and right hand. Note that, nociceptive SS-EPs were best modelled by a single radial dipole located near the midline, whereas non-nociceptive SS-EPs were best modelled by a single tangential dipole, located in the parietal lobe contralateral to the stimulated side. Figure adapted from Mouraux et al. (2011).

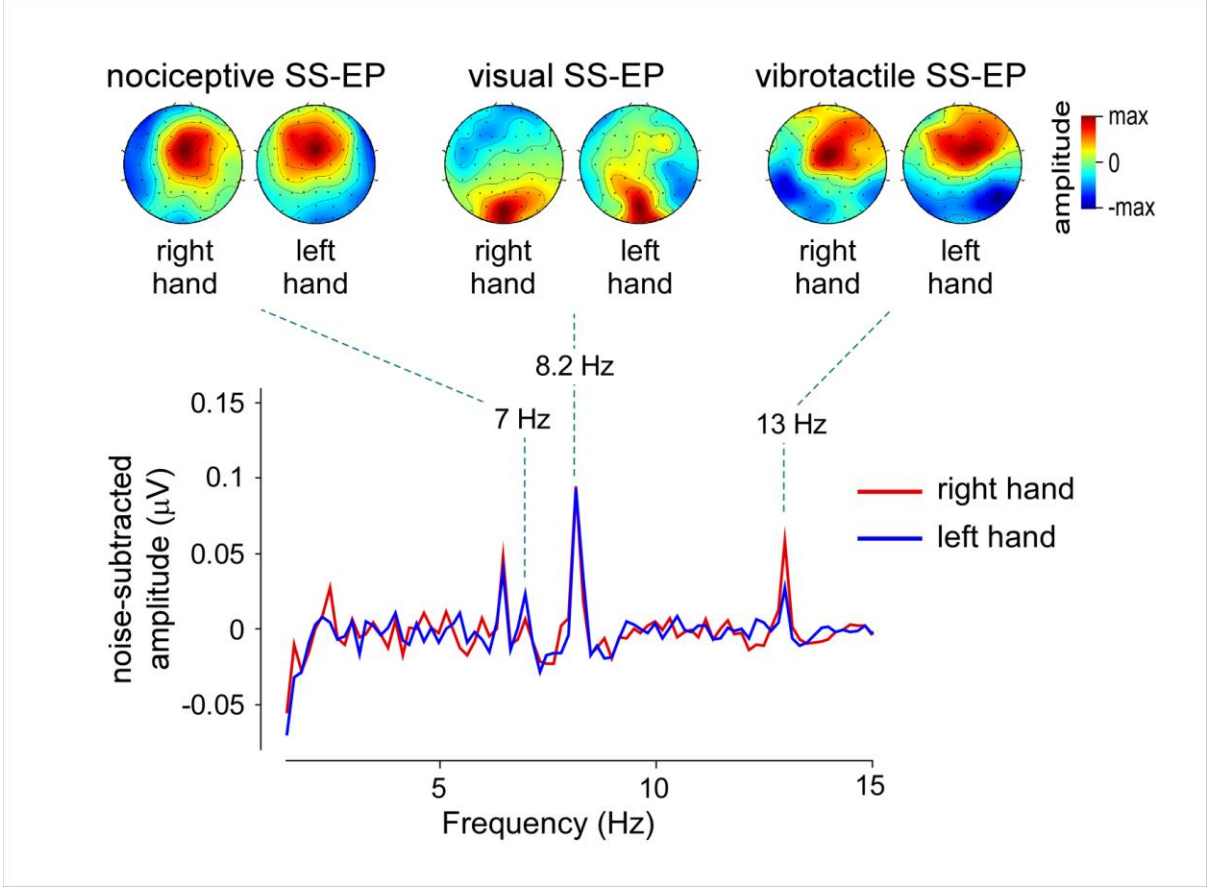

Figure 2. In this experiment, 5-s trains of nociceptive (7-Hz thermal CO_2 laser stimulation of the hand dorsum), tactile (13 Hz transcutaneous electrical stimulation of the superficial radial nerve) and visual (8.2 Hz visual stimulation using an electroluminescent diode placed above the hand dorsum) stimuli were concurrently delivered in blocks of 20 trains, to the left and right hand. The bottom panel represents the noise-subtracted EEG amplitude spectrum (μv), averaged across all subjects and all scalp electrodes, for the left (blue) and the right (red) hand. Note that all three stimuli elicited consistent and distinct SSEPs, appearing as three separate peaks in the EEG frequency spectrum at the corresponding stimulation frequencies (7, 8.2 and 13 Hz). Note also at 6.5 Hz, a peak corresponding to the subharmonic of the 13 Hz tactile SS-EP. The upper panel represents the group level average scalp topographies of nociceptive (7 Hz), tactile (13 Hz) and visual (8.2 Hz) SS-EPs elicited by stimulation of the left and right hand. Note that the scalp topographies of the elicited SS-EPs are distinct according to the modality. The nociceptive SS-EP (7 Hz) was maximal over the scalp vertex, whereas the tactile SS-EP (13 Hz) was maximal over the parietal region contralateral to the stimulated side and the visual SS-EP (8.2 Hz) was maximal over occipital regions. These results indicate that all three responses originate from distinct neuronal populations.