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Bilateral activations in operculo-insular area show temporal dissociation after peripheral electrical stimulation in healthy adults

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ABSTRACT

Interhemispheric transfer is necessary for sensory integration and coordination of body sides. We studied how somatosensory input from one body side may reach both body sides. First, we investigated with 17 healthy adults which uni- and bilateral brain areas were involved in consecutive stages of automatic sensory processing of non-nociceptive peripheral stimulation. Somatosensory evoked fields (SEFs) to electrical stimulation were recorded with 306-channel magnetoencephalography in two conditions. First, SEFs were registered following sensory radial nerve (RN) stimulation to dorsal surface of the right hand and second, following median nerve (MN) stimulation at the right wrist. Cortical activations were located in contralateral postcentral gyrus after MN and RN stimulations and in bilateral operculo-insular area after RN stimulation. First component occurred earlier after MN than RN stimulation. Middle latency components had similar latencies with stronger activation in contralateral postcentral gyrus after MN than RN stimulation. Interestingly, long latency components located in bilateral operculo-insular area after RN stimulation showed latency difference between hemispheres, i.e. activation peaked earlier in contralateral than in ipsilateral side. Additional experiments comparing novel intracutaneous nociceptive, RN and

MN electrical stimuli confirmed bilateral long latency activation elicited by each stimulus type and highlighted latency differences between hemispheres. Variations in activation of bilateral operculo-insular areas may corroborate their role in pain network and in multisensory integration. Our findings imply that these areas present a relay station in multisensory stimulus detection.

INTRODUCTION

Long held key conception in cortical sensory physiology is that primary somatosensory cortex (SI) responds exclusively or mainly to tactile and other somatosensory stimulation from contralateral side of the body. This is evidenced in textbooks and noninvasive studies (Kakigi et al., 2000a; Noback et al., 2005; Penfield & Rasmussen, 1950) and yet interhemispheric transfer is necessary for sensory integration and coordination of both sides of the body. The most prominent structure responsible for interhemispheric transfer is corpus callosum (CC), which may have, depending on task requirements, inhibitory or excitatory function (Fling et al., 2013; van der Knaap & van der Ham, 2011). To date, the exact nature of this transfer is not known however, for example, the importance in limiting spreading of excitatory function via CC in severe epileptic seizure prevention with corpus callosotomy has been well established for decades (Englot et al., 2017). Neuropsychological literature has presented evidence in brain-damaged patients of e.g. simultaneous bilateral tactile sensations produced by unilateral peripheral stimuli suggesting that the normally occurring inhibitory mechanism limiting the sensation to contralateral side is damaged in such a patient (Medina & Rapp, 2008). In order to understand the effects of specific brain damages it may be necessary to acknowledge, in addition to the dominant neurophysiological viewpoint of contralateral representation of somatosensory input in SI, more context-dependent integration of somatosensory inputs from both body sides. The secondary somatosensory cortex (SII) has

been shown to contain bilateral functions and, furthermore, solid functional interaction between SI and SII has been well demonstrated (Forss et al., 2001; Hagiwara et al., 2014; Kakigi et al., 2000a; Karhu & Tesche, 1999; Raij et al., 2008; Wasaka et al., 2007). For instance, Hagiwara et al. (2014) elegantly demonstrated synchrony between two areas in specific time points in bilateral SIIs following unilateral somatosensory stimulation utilizing functional connectivity analysis, applying a method called weighted phase-lag index for cortico-cortical synchrony, and showed increased amount of local functional coupling in elderly participants compared to young participants. As the cortical processing of somatosensory stimuli appears to be modulated by task, lesion or age, then also the perception of intensity of stimulation needs to be considered. The transition from sensory electrical stimulus to nociceptive electrical stimulus may be small current-wise (Omori et al., 2013) but considering cortical processing it may be substantial as distinctly painful stimuli activates cortical pain network (Apkarian et al., 2005). Pain processing network includes primary somatotopic representation of painful stimuli in primary somatosensory cortex (Omori et al., 2013) and a matrix of cortical areas in humans including secondary somatosensory cortices, insula, anterior cingulate cortex, thalamus, prefrontal cortex and posterior parietal cortex (Apkarian et al., 2005; Kakigi et al., 2000b; Tarkka & Treede, 1993). Especially intriguing region in this pain network is the insula, which has been implicated in a large number of mainly sensory functions, including pain perception, but even social emotions and other cognitive processes have been placed in insula. As insula receives afferents from some of the sensory thalamic nuclei, amygdala and limbic and association cortical areas, it is plausible that it has a role in tactile recognition (Augustine, 1996) and it may also be essential in formation of multimodal sensory integration (Nieuwenhuys, 2012). For multimodal sensory integration and further associative processes it would be necessary to integrate functions in both hemispheres.

Our aim was to investigate which uni- and bilateral brain areas are involved in consecutive stages of automatic sensory processing of non-nociceptive peripheral electrical stimulation in healthy adults. The data was recorded with whole head magnetoencephalography (MEG) and the somatosensory evoked fields (SEF) from sensory radial nerve (RN) stimulation were compared with those of median nerve (MN) stimulation. Additional experiments were performed to elucidate the differences in bilateral brain activation first observed in comparing RN and MN stimulations. Cortical fields elicited by novel intracutaneous stimuli were compared with those of RN and MN stimuli in a strict fashion. Noninvasively detected sources of SEFs after sensory and mixed nerve stimulations were evaluated in this setting where non-nociceptive and nociceptive stimuli were delivered in the same body region activating same peripheral pathways but resulting in differences in activated brain network.

MATERIALS AND METHODS

Participants

Seventeen healthy adults participated in the study (for participant characteristics see table 1). Participants had no history of neurological or psychiatric diseases or alcoholic or narcotic addictions. RBDI mood questionnaire (Raitasalo, 2007), a depression scale developed for use in Finland based on the short version of the Beck Depression Inventory (Beck et al., 1961), was used to determine that none of the participants had any depressive and/or anxiety symptoms. All participants were right-handed. Before recording, participants were seated in the MEG device and a short recording was done to ensure that e.g. no metal objects in the head or upper body were present to generate artifacts or contaminate MEG recording. Additional experiments were performed with 5 subjects (see table 2). The research plan for original study and additional experiments was approved by the Ethics Committee of the

University of Jyväskylä and the tenets of Helsinki Declaration were followed. All participants gave written informed consent prior to participation.

(Tables 1 and 2 about here)

Experiment

The study was conducted under two conditions. In condition I, somatosensory evoked fields (SEFs) were registered from sensory radial nerve (RN) stimulation to the dorsal surface of the right hand prior to a motor task of the same hand. A weak electric shock (Digitimer Ltd., model DS7A, Welvyn Garden City, UK) was applied 80 times to the dorsal surface of the right hand randomly with 4-6 s interstimulus intervals (ISI). A motor task was instructed as reaction-time movement where the electric stimuli served as a go-stimulus and the participant was asked to react with index finger abduction to each stimulus while keeping the forearm and hand as relaxed as possible. The requested task occupied subject's attention during RN stimulation. Previous research has reported that spatial attention towards stimulated hand does not modulate the source strengths of early SEF components (Mauguière et al., 1997a). The stimulating electrodes (diameter 1 cm) were placed on the proximal end of the first metacarpal (anode) and on the distal head of the ulna (cathode). The stimulus intensity was set to twice individual sensory threshold (mean 7.7 ±2.2 mA) and stimulus was a monophasic square-wave current pulse of 0.2 ms duration. The stimulus did not induce any reported pain. The randomization of the stimulus intervals was generated by pre-programmed computer script. In condition II, SEFs were registered following median nerve (MN) stimulation at right wrist. Weak electric shocks were applied to the wrist above median nerve. Stimulus intensity was set to individual motor threshold (mean 5.8 ±1.4 mA) producing weak thumb

movement and the stimulus was a monophasic square-wave current pulse of 0.2 ms duration, applied with ISI of 5 Hz for a total of 300 stimuli. The whole hand and forearm were relaxed throughout MN stimulation period. Even though RN condition included a voluntary movement, only the early stages of cortical stimulation processing, well before the onset of voluntary movement, were compared between RN and MN.

For additional experiments RN, MN and a novel intracutaneous electrical stimuli (modified from Kochs et al., 1996) were applied, all with similar randomized ISI (4-6 s) and same number of repetitions (80) without attentional task. Nociceptive intracutaneous stimuli (NOCI) were delivered to the tip of right middle finger. The superficial epidermal layers of the glabrous skin were removed with a small stainless steel drill with a diameter of 1.5 mm. A non-magnetic copper tip electrode, diameter 1 mm, height 2 mm, was placed to the small skin hole and attached with surgical tape. A non-magnetic metal ring return electrode was placed in the metacarpophalangeal joint of the middle finger. The delivered current pulse of 0.2 ms duration activated palmar digital branch of the median nerve and likely activated superficial nociceptive nerve terminals. The perception of this stimulus was described as a stinging pain. The individual pain threshold was tested and for data collection stimulus intensity (mean 4.6 ±2.2 mA) was set to produce 6 in visual-analog pain scale (VAS 0-10).

Recordings were carried out while the participants were seated in the magnetically shielded room (Vacuumschmelze, GmbH, Hanau, Germany). Eye movements and blinks were recorded with electro-oculogram (EOG) with a bandpass filter of 0.1-330 Hz and a gain set to 2000. The participants were instructed to avoid blinking, voluntary eye movements and other unnecessary movements during recording. Five head position indicators (HPI) were placed on the scalp. The HPI coil locations in relation to three anatomical landmarks (nasion and

bilateral preauricular points) were measured with a 3-D digitizer (Fastrak®, Polhemus, Vermont, USA) with additional points from scalp, forehead and nose crest for more accurate representation of the individual head shape. MEG was recorded with the helmet-shaped 306-channel device (Elekta Neuromag®, Triux™, Stockholm, Sweden). MEG signals were recorded using a bandpass filter of 0.1-330 Hz. MEG and EOG signals were stored for later offline processing and analysis.

Data analysis

First, MEG data was filtered with MaxFilter software (Elekta Neuromag®, Stockholm, Sweden) using signal space separation (SSS) in order to reduce artifact components that are not caused by brain activity while preserving signals originating from the brain (Taulu & Simola, 2006; Taulu et al., 2004). Further data preprocessing and analysis was conducted with Brainstorm software (version released 15 Feb 2017) (Tadel et al., 2011) and Statistical Parametric Mapping v. 12 (SPM12) software (Litvak et al., 2011) available in http://www.fil.ion.ucl.ac.uk/spm running under Matlab 2015a (The Mathworks Inc. Natick, MA, USA). Since no individual magnetic resonance images (MRI) were available, an anatomy template (ICBM152) provided by Brainstorm was used. According to MEG guidelines recommendation (Gross et al., 2013), an accurate digitization of the individual head shape is an appropriate method for further source location analysis of electromagnetic activity. This shape can be used, instead of the individual MRI, to approximately align the subject's head to a template head (Holliday et al., 2003) to allow for averaging across subjects. Anatomy templates were aligned and warped for each subject with HPI data collected before MEG recording (Darvas et al., 2006). Event markers for the electrical stimulation were recorded simultaneously with MEG registration in all recordings. Artifacts from eye movements and blinks were identified and cleaned using signal-space projection

(SSP) method (Uusitalo & Ilmoniemi, 1997) available in Brainstorm. After artifact removal, the data was segmented to epochs according to the stimulus onset. The complete time window of a single epoch was from -10 ms to +180 ms, with zero marking the stimulus onset. Segmentation process was the same for original RN and MN data as well as additional experiments. First 10 ms (-10 to -1 ms) of the time window was used as a baseline. Separate averages of all conditions were computed for each participant, including additional experiments, from all artifact-free epochs and in the original experiment grand averages were formed.

Source modeling was done utilizing Brainstorm with distributed models. The forward model was computed with overlapping spheres where one local sphere was assigned to each sensor. Source models were generated from each participant's averaged epochs using minimum norm estimate in dynamic statistical parametric mapping (dSPM). Orientations of source dipoles were constrained normally to cortical surface and all gradiometer sensors of MEG recording were included.

The identified regions of interest (ROI), indicated by dSPM, were analyzed using Brainstorm's scout function for temporal analysis and SPM12 software for regional and source strength analysis. In Brainstorm, the scouts were applied for each participant's averaged source maps. The specific locations of the scouts were determined in the source maps by singular maximum amplitudes within four separate time periods indicated by averaged gradiometer waveform components: 15 - 25 ms, 25 - 35 ms, 60 - 80 ms and 100 - 140 ms. Each scout was set to cover 20 vertices, corresponding to 2.97 cm^2 on average on the cortical surface. One scout represented mean activity in each source location and the scout waveforms were used to compare brain activities between conditions in temporal domain

using time points of peak source field strengths and mean amplitudes over 10 ms time windows after source activation onsets.

Source strengths, regional, and hemispheric differences in ROIs were compared between conditions in SPM12 utilizing extension toolbox WFU PickAtlas (version 3.05) (Maldjian et al., 2003). Volumetric statistical parametric maps of the t-statistics were computed from each individual's average source map for 5 ms (short latency components) or 10 ms (middle and long latency components) time windows. These time windows were picked according to mean peak source strengths identified from the scout waveforms. Atlas-based ROI masks (Lancaster et al., 1997; Lancaster et al., 2000) including bilateral postcentral gyrus and bilateral insula were used for voxel-based statistical comparison.

Statistical analysis

Statistical analysis was performed with IBM SPSS 24 (IBM, Armonk, NY, USA) for temporal analysis and with SPM12 software for regional field strength analysis. Temporal variables were compared with paired samples t-test, with the significance threshold set at $p < 0.05$. All group analysis of MEG data was done in source space. Group level differences between identified brain regions were detected by voxel-level statistical analysis in SPM12 with two-sample t-test. Primary threshold was set to $p < 0.001$ or $p < 0.005$ (uncorrected for multiple comparisons) and corrected for multiple comparisons by the false discovery rate (FDR) method. Clusters were regarded as significant when falling below FDR-corrected cluster-level threshold of 0.05. No minimum cluster size was determined.

RESULTS

Main brain activations were identified from gradiometer waveforms and dSPM activation maps. First component after stimulus onset in RN condition had mean peak amplitude at 32 (±4.9) ms and in MN condition at 20 (±2.0) ms, both locating in postcentral gyrus. In both conditions, middle latency component was identified in postcentral gyrus with the mean peak amplitude at 67 (±4.8) ms in RN condition and 65 (±7.0) ms in MN condition. In RN condition, long latency bilateral posterior operculo-insular area activation was identified with the mean peak amplitude at 112 (±11.6) ms in contralateral side to stimulation and at 130 (±21.7) ms in ipsilateral side. It is noteworthy that MN stimulation did not show corresponding long latency activations when ISI was 5 Hz. RN (purely sensory) condition (7.7 ±2.2 mA) had slightly but statistically significantly stronger stimulus intensity than MN (mixed sensory and motor) condition (5.8 ±1.4 mA) ($p = 0.001$, $t = 5.44$, $df = 16$). For data visualization, the grand average MEG waveforms of RN SEF and MN SEF from gradiometers covering left parietal area are shown in figure 1 with whole head topographies at mean peak component time points, middle latency component illustrated at 66 ms for both conditions (maximal values: MN 65 ms and RN 67 ms). Mean reaction time in motor task after RN stimulation was 221 (±51) ms indicating that no on-going motor activity was present during the analyzed time window of -10 to 180 ms.

(Figure 1 about here)

Mean MNI coordinates and spatial differences for peak activations identified with scouts for each component are shown in table 3. Spatial distances for short- and middle latency coordinates in contralateral postcentral gyrus were calculated and revealed 4.5 mm distance between MN SEF 20 ms and RN SEF 32 ms components and 1.8 mm distance within MN

condition between SEF 20 ms and SEF 65 ms components. Moreover, 6.8 mm distance was detected within RN condition between SEF 32 ms and SEF 67 ms components and finally 9.3 mm distance was detected between MN SEF 65 ms and RN SEF 67 ms components.

(Table 3 about here)

Temporal analysis showed differences between conditions in first components after stimulation. Mean peak activation of the first component in MN SEF occurred earlier, at 20 (±2.0) ms, than in RN SEF, at 32 (±4.9) ms ($p = 0.001$, $t = 9.63$, $df = 16$). Mean time points of peak activities of the middle latency components showed no statistically significant differences between components of MN SEF at 65 (±7.0) ms and RN SEF at 67 (±4.8) ms ($p = 0.40$, $t = 0.87$, $df = 16$). Long latency activation in the posterior operculo-insular area following RN SEF showed hemispheric temporal difference where contralateral peak activation, in relation to the stimulated hand, occurred earlier (112 ±11.6 ms) than in the ipsilateral side (130 ±21.7 ms) ($p = 0.001$, $t = -4.45$, $df = 16$). This difference was also present in the initiation of bilateral posterior operculo-insular area activations as the contralateral activation started significantly earlier ($p = 0.003$, $t = 3.51$, $df = 16$). Figure 2 shows activation maps at mean peak time points and corresponding time courses of these sources.

(Figure 2 about here)

ROI analysis in SPM12 with extension tool WFU PickAtlas indicated highest active clusters, i.e. highest source strengths, when comparing activations between conditions. MN condition showed stronger activation in short latency component when comparing MN SEF 20 ms and

RN SEF 32 ms within 5 ms analysis window around the mean peak time points and showed highest active clusters in contralateral postcentral gyrus and secondary somatosensory area (SII). MN condition showed also stronger activation in middle latency component when comparing MN SEF 65 ms and RN SEF 67 ms within 10 ms analysis window around mean peak time points with highest active cluster in contralateral postcentral gyrus. RN condition showed stronger activation in long latency bilateral components when comparing contralateral (RN SEF 112 ms and MN SEF 112 ms) and ipsilateral (RN SEF 130 ms and MN SEF 130 ms) activations within corresponding 10 ms time windows around mean peak time points, which were identified in RN condition with highest active clusters in bilateral posterior operculo-insular area. See table 4 and figure 3 for significantly different clusters and mean peak coordinates within clusters.

(Table 4 and figure 3 about here)

Additional experiments with 5 subjects first replicated short latency SEF results in MN and RN stimulation, with short latency component peaking earlier after MN (20 ±2.2 ms) than RN (26.6 ±5.6 ms) stimulation ($p = 0.05$, $t=-2.8$, $df = 4$). Also, NOCI short latency component at 25.2 (±2.6) ms occurred later than that of MN ($p = 0.039$, $t = -3.03$, $df = 4$). Furthermore, additional experiments produced evidence that long latency components can be obtained with MN stimulation, when ISI is longer. This can be observed in source activations in figure 4 B and C. NOCI stimulation produced similar brain activations than RN stimulation with corresponding short latency component (NOCI 26.6 ±5.6, RN 25.2 ±2.6 ms, $p = 0.544$, $t = -0.66$, $df = 4$). Interestingly, nociceptive stimulation produced long latency bilateral activations, which resembled those elicited by RN in contra- and ipsilateral hemispheres both in original and additional experiments. Those responses in ipsilateral

hemisphere after NOCI stimulation in additional experiments differed from MN in timing, NOCI 118.4 ±14.4 ms and MN 103.6 ±12.6 ms ($p = 0.01$, $t = -4.6$, $df = 4$). ROI analysis with 5 subjects among additional experiments did not reveal differences in source strengths between conditions. Additionally, we compared long latency source strengths of 5 subjects from original study with the data of additional experiments. This comparison revealed stronger cluster-level activation in contralateral operculo-insular area after long ISI MN stimulation (height threshold $p = 0.001$ (unc.), FDR-corr. $p = 0.021$, voxels per cluster = 10, $t = 6.74$, $df = 8$, peak coordinate within cluster = -46, -20, 16) compared to short ISI MN stimulation. Furthermore, NOCI stimulation revealed stronger activation in contra- (height threshold $p = 0.005$ (unc.), FDR-corr. $p = 0.006$, voxels per cluster = 39, $t = 6.64$, $df = 8$, peak coordinate within cluster = -38, -24, 20) and in ipsilateral (height threshold $p = 0.005$ (unc.), FDR-corr. $p = 0.002$, voxels per cluster = 44, $t = 6.07$, $df = 8$, peak coordinate within cluster = 32, -24, 20) operculo-insular areas compared to short ISI MN stimulation.

(Figure 4 about here)

DISCUSSION

Our results show that peripheral stimulation to only slightly diverging hand areas produces spatial and temporal dissociation in brain activations. We demonstrated that first SEF activation occurred earlier in postcentral gyrus after MN stimulation than after RN stimulation, even though middle latency SEF occurred at similar time points, also in postcentral gyrus. Interestingly, RN stimulation activated posterior operculo-insular area bilaterally with temporal differences at onset times and at mean peak activation times between contra- and ipsilateral hemispheres while MN stimulation with shorter ISI did not elicit long latency activations. However, this discrepancy corresponds well with previous

research showing that long-latency responses in SII areas decrease with shorter ISI (Wikström et al., 1996). With additional experiment using similar ISI we were able to replicate the short latency SEF results and confirm that with longer ISI long-latency responses are obtained after MN stimulation.

First SEF components in our experiments corresponded well with previous research, especially in case of median nerve stimulation where the first component peaked at 20 ms (Hari et al., 1993; Kakigi, 1994; Tiihonen et al., 1989) and the first component after radial nerve stimulation peaked at 32 ms (Inui et al., 2003). Nociceptive stimulation in our additional experiments produced short latency response corresponding to RN response. Furthermore, obtained source locations of the early SEF components corresponded well with previous research (Kakigi et al., 2000a; Mauguière et al., 1997b) locating in the contralateral postcentral gyrus. Previous research has provided evidence for short latency bilateral activation in postcentral gyrus indicating possible transcallosal interhemispheric transfer between contra- and ipsilateral primary somatosensory cortices (Schnitzler et al., 1995). However, this was not seen in the present study probably due to different stimulus paradigms. In addition to contralateral activation in postcentral gyrus, regional analysis showed also contralateral SII activation during short latency component, the finding corresponding with Karhu and Tesche (1999), who also observed contralateral SII area activation 20-30 ms after MN stimulation. Using radial nerve stimulation for eliciting somatosensory evoked fields is rather less common, however, the possible advantage in studying sensory systems could be that the radial nerve area on the dorsum of the hand only activates sensory afferents (Kimura, 2001), when median nerve at the wrist is a mixed nerve and its stimulation thus activates also motor fibers (Kimura, 2001). Previous invasive (Barba et al., 2008) and non-invasive (Srisa-an et al., 1996) research has, similarly to our present results, located the generator of middle

latency components at around 60 ms in the postcentral gyrus. It is interesting to note, although first components differed in latency between MN and RN conditions (12 ms later for RN stimulation), the middle latency components occurred at similar latencies.

We found long-latency bilateral SEF sources after radial nerve stimulation and proposed that these activations originate in posterior insular area based on latencies (Inui et al., 2003; Liberati et al., 2016) and MNI location coordinates. The present data indicated mean MNI coordinates for long-latency activation at 112 ms in the contralateral (left) hemisphere in (-44.4, -22.8, 15.7) and at 130 ms in the ipsilateral (right) hemisphere in (47.0, -17.6, 13.5) according to scouts. These peak MNI coordinates within clusters located in left hemisphere in (-44, -19, 15) and in right hemisphere in (44, -19, -15) according to SPM analysis (see tables 3 and 4). These coordinates correspond rather well to the center of gravity coordinates in posterior insula cytoarchitectonic areas Ig1 (left: -34, -28, 14 and right: 35, -27, 11) or Ig2 (left: -38, -22, 11 and right: 38, -21, 10) defined by Kurth et al. (2010), compared to operculum 1 area (OP1) where center of gravity mean coordinates were in the left hemisphere in (-52.0, -26.5, 26.8) and in right hemisphere in (52.7, -26.0, 25.6) (Eickhoff et al., 2006a). The aforementioned OP1 location Eickhoff et al. (2006a) proposed to indicate most likely SII area in humans.

Still, overlapping posterior insular and secondary somatosensory cortex (SII) activation is possible (Inui et al., 2003), although we were not able to distinguish separate brain areas contributing to these bilateral activations. One reason may be the close proximity of locations and activations reported by Inui et al. (2003) in SII and posterior insula. In the present study, activated areas were localized in the general area often labeled as parietal operculum and usually considered as the SII region (zu Eulenburg et al., 2013). Eickhoff et al. (2006a;

2006b) were able to dissociate the parietal operculum to four different cytoarchitectonic areas and they raised the question if SII region is an appropriate term for this brain area while it seems that it includes a number of anatomically and physiologically distinct areas. Furthermore, previous research has shown that posterior insular area is connected reciprocally to the neighboring SII area and receives projections also from the SI cortex (Augustine, 1985; Friedman et al., 1986) highlighting the complexity of this brain area involved in somatosensory processing.

In our present study, we found 15-18 ms latency difference between contra- and ipsilateral peak activations both in RN and in NOCI in the posterior operculo-insular areas where contralateral side peaked earlier. This result corresponds well with previous studies reporting similar latency differences between hemispheres in posterior insular area, in e.g. Inui et al. (2003) using non-nociceptive transcutaneous electrical stimulation to radial nerve area and in Liberati et al. (2016) using vibrotactile stimulation with intracerebral recording and, furthermore, in SII e.g. in Karhu and Tesche (1999) using electrical median nerve stimulation. This interhemispheric delay has been attributed to callosal transmission between contra- and ipsilateral SII areas (Frot & Mauguiere, 1999; Karhu & Tesche, 1999) but also direct thalamo-cortical connection to ipsilateral hemisphere has been suggested (Forss et al., 1999). Somatosensory cortices seem to process information in parallel (Liang et al., 2011) or serial (Khosnejad et al., 2014) manner between brain areas. However, this is still under debate and recent combined fMRI and MEG study demonstrated that early neural activity, first 100 ms after somatosensory stimulus, is best explained by parallel and subsequent activity by serial processing route (Klingner et al., 2016). It is suggested that insula shares connections with dorsal thalamus (Augustine, 1996; Dum et al., 2009; Friedman & Murray, 1986) but rather little is known about connections to and from insula. Previous evidence

suggests that insula plays a role in multisensory integration processing multimodal stimuli (Kurth et al., 2010; Liberati et al., 2016; zu Eulenburg et al., 2013). This multimodal activation in posterior insular cortex may encompass convergence of afferent somatosensory information in this area (zu Eulenburg et al., 2013) and maybe posterior insula was activated due to pure sensory input in the present experiment.

Our original study included differences in stimulus parameters, which limited our interpretation. The differences in interstimulus intervals (shorter in MN stimulation) contributed to differences in long latency components (Mauguière et al., 1997a; Wikström et al., 1996). Additional experiments with fully comparable interstimulus intervals and pain-specific electrical stimulation to fingertip provided support for bilateral long latency activations elicited by all electrical stimuli and RN and NOCI dissociation from MN in long latency activation. We recognize the lack of individual MRIs in our data. This may render some inaccuracy in specific locations however, digitization of individual head shapes can be utilized with MRI template for localization of distinct activity according to MEG recording and reporting guidelines by Gross et al. (2013, see Materials and Methods section). Furthermore, we cannot completely rule out possible effects from preparation phase to motor task before RN stimulation, although no active movement was observed during our analysis window.

CONCLUSION

Spatial and temporal dissociation was observed in brain activations following slightly diverging hand stimulation areas. Early and middle latency activations located in contralateral postcentral gyrus showing differences in source strengths however, only early component peak latencies of RN and NOCI stimulations differed from MN stimulation while

middle latency components occurred at similar latencies. Interestingly, RN stimulation showed long latency activation in bilateral operculo-insular areas. Insular area is implicated to process multimodal sensory information and function as a multisensory integration node. It is also known to participate in pain network and part of the activity after RN stimulation may be owed to possible discomfort experienced from stimulus. This suggestion is supported by the similarity of source activations we obtained in additional experiments with novel nociceptive stimulation. We showed that specific peripheral stimulation activates, in addition to primary sensory areas in contralateral postcentral gyrus, bilateral operculo-insular areas with distinct latency difference between contra- and ipsilateral hemispheres.

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CONFLICT OF INTEREST

Authors have nothing to report.

AUTHOR CONTRIBUTIONS

Study concept and design: PH, HS, IMT

Data collection and analysis or revision of manuscript: All authors

Interpretation of data and writing of manuscript: PH, IMT

DATA ACCESSIBILITY

Data of this paper is available upon request via corresponding author from University of Jyväskylä servers.

ABBREVIATIONS

CC, corpus callosum; dSPM, dynamic statistical parametric mapping; EOG, electro-oculogram; FDR, false discovery rate; HPI, head position indicator; ISI, interstimulus interval; MEG, magnetoencephalography; MN, median nerve; MRI, magnetic resonance image; NOCI, intracutaneous nociceptive stimuli; OP1, operculum 1 area; RN, radial nerve; ROI, region of interest; SEF, somatosensory evoked field; SI, primary somatosensory cortex; SII, secondary somatosensory cortex; SPM12, statistical parametric mapping v. 12; SSP, signal-space separation; SSS, signal space separation; VAS, visual analog pain scale

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FIGURE CAPTIONS

Figure 1. Grand average waveforms of (A) radial SEF and (B) median SEF from gradiometer channels covering left parietal area from the original experiments with 17 subjects. Zero denotes onset of stimulation. Whole head topographies at designated time points are illustrated. Note different topographic mapping scales for 20 and 32 ms and for 66, 112 and 130 ms.

Figure 2. Initial components 20 ms (MN) and 32 ms (RN), following components at 65 ms (MN) and 67 ms (RN) (A) and bilateral components at 112 ms and 130 ms (B) are shown for median and radial nerve stimulations from the original experiments. dSPM maps illustrate the activations at mean peak time points in contralateral postcentral gyrus (A) and bilateral operculo-insular areas (B). Temporal differences in source activities between MN SEF (cyan) and RN SEF (green) are illustrated in the middle (A) corresponding to postcentral gyrus and (B) to bilateral operculo-insular area activation time courses. Contralateral side to stimulated hand is shown in solid circle superimposed on activation on dSPM maps and the corresponding time course with solid line (A and B) and ipsilateral side is similarly shown with dashed circles and lines (B).

Figure 3. Activation maps (SPM) showing voxel-based cluster-level differences from the original experiments between (A) MN SEF at 20 (± 2) ms time window and RN SEF at 32 (± 2) ms time window. (B) shows differences between MN SEF and RN SEF at 66 (± 5) ms time window and (C) between RN SEF and MN SEF at 112 (±5) ms time window. Lastly (D) shows differences between RN SEF and MN SEF at 130 (± 5) ms time window. MNI coordinates with corresponding red arrow for peak activation within a cluster are shown

above activation maps (A-D). Short latency comparison (A) showed also a more caudal secondary cluster with peak MNI coordinates within cluster in (-54, -19, 19). Atlas based ROI masks were used to include bilateral postcentral gyrus (A and B) and bilateral insula (C and D). Examples of ROI masks are shown in three different horizontal planes (Z-coordinates given above figures).

Figure 4. Short- and long-latency components from MN, RN and NOCI stimulations are illustrated in one representative subject from the additional experiments. dSPM maps illustrate the activations at mean peak time points (MN in cyan, RN in green and NOCI in blue) in contralateral postcentral gyrus (A) and contra- (B) and ipsilateral (C) operculo-insular areas. Temporal differences in source activities between MN SEF (cyan), RN SEF (green) and NOCI SEF (blue) are illustrated with superimposed waveforms corresponding to postcentral gyrus (A) and to bilateral operculo-insular area (B and C) activation time courses.

TABLE 1. Participant characteristics, 17 individuals (10 men, 7 women) means, (±SD) and range.

	Mean	SD	Range
Age, year	30.7	6.2	18-41
Height, cm	177	11.2	159-203
Weight, kg	74	13.1	59-95
BMI	23.6	3.0	20.3-30.7
Stimulus intensity on median nerve, mA	5.8	1.4	3.5-9.0
Stimulus intensity on radial nerve, mA	7.7	2.2	5.0-12.0
Reaction time, ms	221	51	146-326
Radial nerve (RN) stimulations, n	77	3.3	68-80
Median nerve (MN) stimulations, n	299	3.5	286-300
Scout size on cortical surface, cm^2	2.97	0.42	1.77-3.77

TABLE 2 Additional experiment: 5 individuals (3 men, 2 women) means, (±SD) and range.

	Mean	SD	Range
Age, year	34.4	4.4	28-39
Height, cm	171	11.8	158-183
Weight, kg	68	14.4	49-86
BMI	23.2	2.5	19.6-25.7
Stimulus intensity on median nerve, mA	5.8	1.0	4.5-7.0
Stimulus intensity on radial nerve, mA	6.6	1.6	5.0-8.5
Stimulus intensity on intracutaneous, mA	4.6	2.2	2.0-7.5
Median nerve (MN) stimulations, n	79	1.2	77-80
Radial nerve (RN) stimulations, n	80	0.4	79-80
Intracutaneous (NOCI) stimulations, n	79	1.3	77-80

TABLE 3. MNI location coordinates of component mean peak activations identified with scouts and spatial differences between early and middle latency components.

	MNI coordinates			Spatial difference between coordinates	
	X	Y	Z		
MN 20 ms	-38.4	-21.0	51.2	MN 20 ms – RN 32 ms	4.5 mm
RN 32 ms	-35.1	-23.1	53.4	MN 20 ms – MN 65 ms	1.8 mm
MN 65 ms	-37.9	-22.5	50.3	MN 65 ms – RN 67 ms	9.3 mm
RN 67 ms	-31.7	-28.9	52.8	RN 32 ms – RN 67 ms	6.8 mm
RN 112 ms	-44.4	-22.8	15.7		
RN 130 ms	47.0	-17.6	13.5		

TABLE 4. Cluster-level statistical differences and peak MNI coordinates within clusters.

		Cluster-level p-value[d]		Peak-level p-value[d]			Peak MNI coordinates within cluster
	Height threshold	FDR-corr.	voxels per cluster	FDR-corr.	T	Z	
MN 20 ms - RN 32 ms [a,b]	T = 3.37, p = 0.001 (unc.)	< 0.001	66	0.128	5.06	4.30	-44, -25, 43
		0.017	14	0.099	5.45	4.55	-54, -19, 19
MN 66 ms - RN 66 ms [a,b]	T = 3.37, p = 0.001 (unc.)	< 0.001	53	0.050	5.41	4.53	-48, -25, 41
RN 112 ms - MN 112 ms [a,c]	T = 3.37, p = 0.001 (unc.)	0.007	24	0.224	4.76	4.11	-44, -19, 15
RN 130 ms - MN 130 ms [a,c]	T = 2.74, p = 0.005 (unc.)	0.002	59	0.309	4.47	3.91	-44, -19, 15
		0.021	28	0.555	3.46	3.16	44, -19, 15

[a] Degrees of freedom = 32, Voxel size 2.0 x 2.0 x 2.0 mm

[b] Volume 6046 voxels

[c] Volume 3861 voxels

[d] p-values adjusted for search volume