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Effect of interstimulus interval on cortical proprioceptive responses to passive finger movements

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Abstract

Shortening of the interstimulus interval (ISI) generally leads to attenuation of cortical sensory responses. For proprioception, however, this ISI effect is still poorly known. Our aim was to characterize the ISI dependence of movement-evoked proprioceptive cortical responses and to find the optimum ISI for proprioceptive stimulation. We measured, from 15 healthy adults, magnetoencephalographic responses to passive flexion and extension movements of the right index finger. The movements were generated by a movement actuator at fixed ISIs of 0.5, 1, 2, 4, 8, and 16 s, in separate blocks. The responses peaked at ~70 ms (extension) and ~90 ms (flexion) in the contralateral primary somatosensory cortex. The strength of the cortical source increased with the ISI, plateauing at the 8-s ISI. Modeling the ISI dependence with an exponential saturation function revealed response lifetimes of 1.3 s (extension) and 2.2 s (flexion), implying that the maximum signal-to-noise ratio (SNR) in a given measurement time is achieved with ISIs of 1.7 s and 2.8 s, respectively. **We conclude that ISIs of 1.5–3 s should be used to maximize SNR in recordings of proprioceptive cortical responses to passive finger movements.** Our findings can benefit the assessment of proprioceptive afference in both clinical and research settings.

Introduction

Proprioceptors in muscles, tendons, and joints sense positions, movements, and forces of body parts (for a review, see Proske & Gandevia, 2012), thus being crucial for proper motor control (Scott, 2012). Previous studies have assessed proprioceptive afference by recording cortical responses to passive movements with electroencephalography (EEG; Rodin *et al.*, 1969; Papakostopoulos *et al.*, 1974; Shibasaki *et al.*, 1980) and magnetoencephalography (MEG; Xiang *et al.*, 1997; Lange *et al.*, 2001; Alary *et al.*, 2002; Druschky *et al.*, 2003; Piitulainen *et al.*, 2013, 2015; Bourguignon *et al.*, 2015). These responses indeed seem to represent proprioceptive rather than tactile afference, as they can be measured even during cutaneous anesthesia (Starr *et al.*, 1981; Abbruzzese *et al.*, 1985; Mima *et al.*, 1996) and regardless of the level of tactile component in the stimulation (Piitulainen *et al.*, 2013; Bourguignon *et al.*, 2015).

Transient passive movements elicit prominent responses peaking at ~70–80 ms. According to MEG studies, these responses originate in the primary sensorimotor cortex (Xiang *et al.*, 1997; Piitulainen *et al.*, 2013), with some results highlighting the primary motor (MI) cortex (Lange *et al.*, 2001) and others the primary somatosensory (SI) cortex (Alary *et al.*, 2002).

In previous studies on proprioceptive afference, the dependence of response amplitude on interstimulus interval (ISI) has received little attention. Yet, this information would be highly relevant for understanding the temporal operating scales of cortical proprioceptive processing. Furthermore, it would help develop reliable neurophysiological tests to quantify proprioceptive afference. Such robust tests would benefit both basic research and clinical evaluation of proprioceptive processing; the current clinical tests are quite poorly controlled as the examiner typically manually moves the patient's toes or fingers and asks the patient to report, eyes closed, the perceived changes in position.

As a general feature of sensory processing, shortening the ISI leads to attenuation of cortical responses. Maximum responses may be elicited only at ISIs of several seconds or even tens of seconds, depending on the response latency, cortical area, and sensory modality. Thus, previous stimuli can leave to the cortex long-lasting traces, the durations of which can be characterized with the "lifetime" of the response in question. The lifetime is the exponential decay constant of the exponential saturation function fitted to the peak response amplitudes as a function of ISI (Lu *et al.*, 1992a). Lifetimes are typically shorter for short- than long-latency responses and shorter at lower than higher stages of the cortical processing stream.

The ISI effects have been scrutinized by means of MEG, e.g., in the auditory, visual, and somatosensory modalities. The supratemporal 100-ms auditory evoked field is attenuated at ISIs below 8–16 s and has a lifetime of ~1.5 s (Hari *et al.*, 1982; Lu *et al.*, 1992a; Mäkelä *et al.*, 1993). Occipital visual evoked fields peaking at 90–180 ms have lifetimes from 0.2 s to 0.6 s whereas the lifetimes for later extra-occipital responses at 180–440 ms range from 7 to 30 s (Uusitalo *et al.*, 1996). In the SI cortex, shortening the ISI of median nerve stimulation from 0.5 to 0.2 s suppresses the 27-ms deflection P27m of the somatosensory evoked field (SEF) much more than the 20-ms deflection N20m (Tiihonen *et al.*, 1989). Furthermore, the 35- and 60-ms SEFs attenuate monotonously when the ISI is reduced from 5 to 0.3 s (Wikström *et al.*, 1996). In the secondary somatosensory (SII) cortex, the ISI dependence of the ~100-ms response resembles that of the auditory 100-ms response (Hari *et al.*, 1993). These observations support the existence of multiple hierarchically organized timescales both within brain areas and within cortical processing streams (for a review, see Hari *et al.*, 2010).

In previous studies on proprioceptive input, the 80-ms EEG responses to wrist extension decreased by ~50% when the ISI was reduced from 6.7 to 1 s (Abbruzzese *et al.*, 1985), and the 90-ms EEG response to ankle flexion decreased by more than 50% when the ISI was reduced from 5 to 0.5 s (Starr *et al.*, 1981). **Also in a recent MEG study (Piitulainen *et al.*, 2015), the 90-ms response to finger flexion–extension movements decreased by 80% when intermittent stimulation at an ISI of 3.2–4 s was replaced with continuous movements at 12 Hz (ISI = 0.083 s). More detailed characterization of the ISI effect would help design time-efficient stimulation protocols for both basic and clinical research by making it possible to determine the ISI yielding the maximum signal-to-noise ratio (SNR) within a given recording time. Furthermore, knowledge of the ISI dependence of proprioceptive responses in healthy individuals could be utilized to study potential abnormalities in this dependence in various diseases (Andrade *et al.*, 2016).**

Here we recorded cortical responses to passive index-finger movements at various ISIs. Our aim was to accurately model the ISI dependence of the responses to learn about the temporal operating scales of the underlying cortical processes and to estimate the optimum ISI for assessing proprioceptive afference.

Materials and methods

Subjects

We studied 15 healthy adults (9 men, 6 women; ages 22–38 yrs, mean 29.9 yrs). All subjects were right-handed (score range 60–100, mean score 87) according to the Edinburgh handedness inventory (Oldfield, 1971). All had signed a written informed consent prior to participation. Participants received compensation for travel expenses and lost working hours. The study **conformed to the Declaration of Helsinki and** had a prior approval by the ethics committee of Aalto University.

Experimental protocol

Fig. 1 shows the MEG setup. The subjects were instructed to sit relaxed, resting their left hand on their thigh and their pronated right hand on the upper surface of a pneumatic artificial-muscle (PAM) stimulator (Piitulainen *et al.*, 2015), placed on a table in front of them. The tip of the right index finger was taped (Leukoplast® medical tape) to the extremity of the vertically-oriented actuator of the PAM stimulator that generated transient flexion and extension movements primarily at the metacarpophalangeal joint of the finger. The subjects were not able to see the finger nor the PAM stimulator as their vision for that part of the visual field was blocked with a sheet of paper (Fig. 1).

Fig. 2A illustrates the timecourse of the movement stimuli at an ISI of 8 s. Extension and flexion movements occurred alternately every 4 s (half of the ISI), meaning that one ISI included two movements and two volleys of proprioceptive afference. Fig. 2B shows the enlarged profiles of an extension and flexion movement. The movements were rapid in both directions, with about 100 ms from movement onset to the midpoint of the trajectory (Fig. 2B, dashed horizontal line).

The experiment comprised seven movement blocks, and one rest block. Movements were elicited at fixed ISIs of 0.5, 1, 2, 4, 8, and 16 s for 2 min ($ISI_{0.5}$, ISI_1 and ISI_2), 4 min (ISI_4), 6 min (ISI_8), or 2 x 6 min (ISI_{16}), corresponding to 45–240 movements depending on the ISI. The time between consecutive blocks was ~2 min. The order of the different movement blocks was randomized for each subject. In the rest block, which was always the last of the session, the subjects were sitting relaxed and eyes open for 5 min, resting the right hand on the PAM stimulator as in the movement blocks. During the rest block no stimuli were applied and the data were used only to estimate the electromyogram (EMG) level during rest.

During the MEG recordings, the subjects fixated their gaze on a fixation cross displayed on a screen in front of them. They wore earplugs to avoid hearing any acoustic noise from the PAM stimulator.

Recordings

Magnetoencephalographic (MEG) signals were recorded with a 306-channel whole-scalp neuromagnetometer (Elekta Neuromag™, Elekta Oy, Helsinki, Finland) in a magnetically shielded room (Imedco AG, Hägendorf, Switzerland) at the MEG Core of Aalto NeuroImaging, Aalto University. Recording passband was 0.1–330 Hz and the sampling rate 1000 Hz. The position of the subject's head inside the MEG helmet was continuously monitored by feeding current through five head-position coils attached to the scalp at frequencies well above the physiological frequencies of interest.

Acceleration signals from passive finger movements were recorded with a 3-axis accelerometer (ADXL335 iMEMS Accelerometer, Analog Devices Inc., Norwood, MA, USA) attached to the nail of the right index finger. The filtered (passband 0.1–330 Hz) acceleration signals were sampled at 1000 Hz, time-locked to the MEG signals.

Surface EMG was monitored from right antebrachial muscles, with one active electrode on the flexor and another on the extensor side; the reference electrode was over the distal radial bone. EMG signals were filtered (passband 10–330 Hz) and sampled at 1000 Hz time-locked to the MEG signals.

Anatomical magnetic resonance images (MRIs) were acquired with a General Electric Signa® 3T (Signa VH/i, General Electric, Milwaukee, WI, USA) or a MAGNETOM Skyra® 3T (Siemens Healthcare, Erlangen, Germany) whole-body MRI scanner at the AMI Centre of Aalto NeuroImaging, Aalto University.

Data preprocessing

A temporal extension of the signal space separation method (Taulu & Simola, 2006) was applied off-line to the MEG signals (segment length 16 s; correlation limit 0.9) to reduce artifacts and to correct for head movements. For each subject, signals representing different ISIs were transformed to the same head-coordinate system (mean over ISIs) to allow comparison between responses obtained at different ISIs.

Wide-band MEG signals were averaged with custom-made MATLAB® (MathWorks, Natick, MA, USA) scripts separately for each ISI and both movement directions, thus yielding 12 averaged responses for each subject. To find the proper triggering moment, we first filtered (passband 1–195 Hz) the 3 orthogonal acceleration signals and combined them into a single signal by computing their Euclidean norm. Movement onset was determined, for all single movements, as the time point preceding the rise of acceleration above 10% of its maximum during that movement. To avoid any bias caused by rarely occurring artifacts in the acceleration signal, we finally defined the movement onset on the basis of the typical (most frequent) trigger-to-movement-onset latency, separately for each subject, ISI, and movement direction. We then averaged 39–244 (depending on the ISI) MEG epochs spanning from −500 to 1000 ms with respect to movement onset. All stimuli occurring during the first 4 s were discarded from the analysis to avoid inclusion of nonstationary responses. We also omitted epochs during which any of the MEG signals exceeded 3 pT (magnetometers) or 0.7 pT/cm (gradiometers). Finally, the averaged MEG signals were low-pass filtered at 40 Hz and adjusted to a baseline from −150 to 0 ms.

Data analysis

Source-space analysis

We modeled, separately for each subject's extension and flexion movements, the source of the most prominent deflection of the ISI_8 response with an equivalent current dipole (ECD) using Elekta Neuromag™ software (Elekta Oy, Helsinki, Finland). The most prominent response peaked ~70 ms after extension and ~90 ms after flexion movements. The ECD was fitted to best explain the signals obtained from a fixed selection of 18 planar gradiometers (from nine sensor units) above the left sensorimotor cortex. The ECD obtained at ISI_8 was then employed as a reference source also for

the other ISIs. Subject 10 lacked a clear cortical response in the typical time window for ISI_8 flexion, and in his case, the ECD for flexion movements was obtained from the ISI_4 response.

The source waveform was plotted for each ISI, and its maximum value between 30 and 130 ms was extracted. These maximum source-strength values were individually normalized by dividing them by the individual's mean across ISIs. Then, the ISI dependence of the source strength, was modeled with the exponential saturation function

$$A(ISI) = A_{\text{max}}\left(1 - e^{-\frac{ISI}{\tau}}\right),$$

where A is the normalized source strength, A_{max} is the highest A that can be reached for arbitrarily long ISIs, and τ is the lifetime of the response. A(ISI) was fitted to the group-mean data with the unconstrained nonlinear optimization function "*fminsearch*" implemented in MATLAB.

Within a fixed measurement time, prolongation of the ISI increases the response amplitude but simultaneously increases the noise level since a smaller number of stimuli (n) can be collected in the given time. For stationary noise, the noise level decreases by a factor of $\sqrt{n} \sim \frac{1}{\sqrt{ISI}}$ with response averaging, and consequently, the SNR of the averaged response is proportional to $\left(1 - e^{-\frac{ISI}{\tau}}\right)/\sqrt{ISI}$. This SNR value is at maximum when ISI ≈ 1.26 τ. In other words, 1.26 τ is the optimum ISI within a fixed measurement time. **To verify that noise was indeed stationary across ISIs, which would justify the use of this estimate, we evaluated the noise level separately for each subject and ISI. Noise was evaluated as the standard deviation of the differences at each time point between all single trials and the averaged response.**

We performed a similar source-space analysis also with stimulus-number-matched data to control for the effect of the number of stimulus repetitions that also affects the SNR of the averaged response. The number of stimuli was higher at ISIs of 0.5, 1, and 2 s (with ~60, ~120, and ~240 responses averaged, respectively) compared with ISIs of 4, 8, and 16 s (~45). Here, when computing the averaged responses at $ISI_{0.5}$–ISI_2, we matched the number of single trials with that at ISI_8. Otherwise the analysis was identical to the description above.

Sensor-space analysis

To confirm that the results we obtain are not related to the method used to estimate source activity, we analyzed the responses to extension movements also in sensor-space. To do so, we first combined the signals from gradiometer pairs by calculating their vector sums. For each subject, we then selected the sensor (among the nine pairs used in the source analysis) showing the highest peak vector sum between 30 and 130 ms for most of the ISIs. We extracted the peak values in this sensor and time window (30–130 ms) and normalized them within subjects. The ISI dependence of these sensor-space-response amplitudes was then modeled with an exponential saturation function as described above for source strengths. We performed this analysis only for extension (and not flexion) movements due to the smaller variability of the responses across ISIs and subjects.

EMG analysis

EMG signals from the flexor and extensor muscles were bandpass-filtered from 20 to 295 Hz, and root-mean-square (rms) values of whole-length EMG signals were computed for all movement and rest conditions. Similarly as in the averaging procedure, we discarded the data during the first

stimuli and started the analysis from the onset of the first flexion movement included in the average. Additionally, we averaged rectified EMG signals from the same epochs as the MEG signals.

Statistical analysis

To compare the cortical sources for extension and flexion movements, we tested the effect of movement direction on (a) source location with a repeated-measures one-way multivariate analysis of variance (MANOVA; x, y, and z coordinates as 3 individual dependent factors), (b) source orientation on a tangential plane with a two-tailed paired t-test, and (c) source strength at each ISI with two-tailed paired t-tests and Bonferroni correction for multiple comparisons. The effect of ISI on source strength was modeled with an exponential saturation function fitted to the data, as described in *Source-space analysis* section. Furthermore, we assessed the effects of movement direction and ISI on the peak latency of the source waveform with a repeated-measures two-way analysis of variance (ANOVA). Here we also tested the interaction between movement direction and ISI, but as no statistically significant interaction was found, we removed the interaction term from the final model. In the case of violations of the sphericity assumption in the ANOVAs, the degrees of freedom were corrected with the Greenhouse–Geisser procedure. For such ANOVAs we report uncorrected degrees of freedom together with the correction factor ε, as well as the corrected F and P values. The alpha level was set at 0.05 in all statistical tests. All statistical analyses were performed with IBM SPSS Statistics 22.

Results

We successfully recorded cortical responses to passive finger extension and flexion movements from all 15 subjects, at least at the 5 longest ISIs (1–16 s). At the shortest ISI of 0.5 s, responses to extensions and flexions were not detected in 2 and 4 subjects, respectively.

Movement characteristics

Fig. 2B shows the displacement of the movement actuator for both extension and flexion movements at ISI_8. **During the first 50 ms after movement onset—the part of the stimulus that is relevant for the studied cortical responses peaking at ~70 and ~90 ms—the displacement was very similar across all ISIs: 1.2–1.3 mm for extensions and 0.7–0.9 mm for flexions, corresponding to mean velocities of 25–26 and 13–18 mm/s, respectively. The total range of movement was 3.9 mm at $ISI_{0.5}$ and increased with the ISI up to 5.5 mm at ISI_{16}. At $ISI_{0.5}$, the movements lasted through the entire extension and flexion phases (250 ms each), whereas at longer ISIs, the movements reached a plateau at ~300 ms.**

Figs. 2C–E show the averaged finger acceleration and surface EMG signals for one representative subject (S1) at ISI_8. The peak acceleration was obtained already at ~5 ms, and it was higher for extension than flexion movements (Fig. 2C); similar acceleration profiles were obtained across subjects. EMG activity was negligible (~1 μV) in both extensor and flexor muscles during the movements (Figs. 2D and E). In group analysis, the rms EMG level during the movement conditions did not exceed the rest level, indicating that subjects were able to remain relaxed during the stimulation as instructed. However, tiny stimulus-locked EMG responses were visible in 14/15 subjects after the flexions (Fig. 2D and E) but only in 1/15 subjects after the extensions. These EMG

responses peaked between 100 and 200 ms after the movement onset, and thus clearly later than the observed cortical responses.

MEG responses, cortical sources, and effect of ISI

Fig. 3 shows the spatial distribution (A) and ISI dependence (B) of the averaged responses, as well as the corresponding cortical sources (C) and source waveforms (D) of a representative subject (S1). Prominent deflections occurred in the left contralateral sensorimotor cortex, peaking at 78–85 ms for extensions and 92–96 ms for flexions (Fig. 3B). In this subject, as well as in the whole group **(Fig. S1)**, the responses to extensions were earlier, stronger, narrower, and more consistent than those to flexions. For both movement directions, the response amplitudes increased as a function of ISI, from practically no visible responses at $ISI_{0.5}$ to prominent responses at the longest ISIs.

The sources of the responses to both extensions and flexions (Fig. 3C) were located in the posterior bank of the central sulcus, in the "hand knob" area (Yousry *et al.*, 1997). Also in the other subjects, sources were located in the SI cortex with the intracellular current during the main peak always pointing posteriorly. At group level, the source location and orientation did not differ between extension and flexion movements (main effect of movement direction on source location in repeated-measures one-way MANOVA: $F_{3,12} = 0.18$, $P = 0.91$; effect of movement direction on source orientation in two-tailed paired *t*-test: $t_{14} = 1.8$, $P = 0.097$). **The group-median goodness-of-fit values and confidence volumes for the ECDs at ISI_8 were 99.1% (range 96.4–99.7%) and 0.2 cm^3 (0.04–1.6 cm^3) for extensions and 96.5% (91.6–99.7%) and 1.4 (0.1–7.3 cm^3) for flexions, respectively.**

The ISI dependence was similar for source strengths (Fig. 3D) as for the original averaged responses (Fig. 3B). The source strengths peaked, at group level, on average 19 ms earlier for extensions (mean ± SD 75 ± 17 ms; range 47–125 ms) than flexions (94 ± 23 ms; 32–130 ms), whereas the peak latencies did not differ across ISIs (main effects of movement direction and ISI, respectively, on peak latency in repeated-measures two-way ANOVA: $F_{1,14} = 26$, $P = 0.00017$ and $F_{5,70} = 2.0$, $\varepsilon = 0.58$, $P = 0.13$). Furthermore, the sources were 46–163% stronger for extension than flexion movements at ISI_1–ISI_{16}, whereas at $ISI_{0.5}$ no difference was observed between movement directions ($P_{corrected} < 0.05$ for ISI_1–ISI_{16} and $P_{uncorrected} = 0.51$ for $ISI_{0.5}$; two-tailed paired *t*-tests with Bonferroni correction).

Fig. 4 shows the group-level ISI effect on source strengths for both extensions and flexions. The source strengths increased approximately 3-fold for extensions and 6-fold for flexions when the ISI was prolonged from 0.5 to 8 s, and then the responses plateaued. **This relationship was closely modeled with an exponential saturation function (see *Source-space analysis* section of Materials and methods), with response lifetimes (τ) and maximum source strengths (A_{max}; normalized within subjects) of 1.3 s and 1.3 for extension and 2.2 s and 1.5 for flexion movements. As the noise levels in the single-trial MEG signals were very similar across ISIs (group averages between 62 and 64 fT/cm at all ISIs), the optimum ISI, maximizing SNR in a given measurement time, could be estimated simply as 1.26 τ yielding 1.7 s for extension and 2.8 s for flexion movements.** Based on these results, the optimum ISI (1.26 τ) that maximizes SNR in a given measurement time is 1.7 s for extension and 2.8 s for flexion movements. **The optimum ISIs were similar (1.5 s for extensions and 2.0 s for flexions) also when estimated from the stimulus-number-matched data.** For sensor-space-response amplitudes (shown for extension movements in Fig. 4, left, dashed curve), the ISI dependence was closely similar to that for the source-space data (solid curve).

Discussion

In the current study, passive finger movements elicited prominent responses in the contralateral sensorimotor cortex in accordance with earlier findings (Xiang *et al.*, 1997; Lange *et al.*, 2001; Alary *et al.*, 2002; Druschky *et al.*, 2003; Woldag *et al.*, 2003; Onishi *et al.*, 2013; Piitulainen *et al.*, 2013, 2015). The corresponding cortical sources peaked ~70 ms after extension movements and ~90 ms after flexion movements. Source strengths were markedly increased when the ISI was prolonged from 0.5 to 8 s. Increasing the ISI further to 16 s did not any more increase the source strengths.

Origin of passive-movement-evoked responses

According to prior evidence, EEG/MEG responses to passive movements are primarily triggered by proprioceptive afference, whereas the role of tactile afference from cutaneous receptors is minor (Starr *et al.*, 1981; Abbruzzese *et al.*, 1985; Druschky *et al.*, 2003; Piitulainen *et al.*, 2013; Bourguignon *et al.*, 2015). For example, EEG responses to passive plantar flexions of the ankle are not affected by cutaneous anesthesia of the foot (induced with a cuff above the ankle) but are attenuated by blocking the flow of proprioceptive afference in the peroneal nerve either with pressure or a local anesthetic (Starr *et al.*, 1981). Similarly, cutaneous anesthesia has no effect on EEG responses to passive wrist extensions (Abbruzzese *et al.*, 1985) or finger flexions (Mima *et al.*, 1996). Furthermore, MEG responses to passive finger movements differ from tactile responses in their longer peak latencies and higher amplitudes (Druschky *et al.*, 2003). Finally, decisive evidence for the dominance of proprioceptive afference in movement-evoked cortical responses has come from recent MEG studies (Piitulainen *et al.*, 2013; Bourguignon *et al.*, 2015) exploring the coupling between peripheral movements and cortical activity by means of corticokinematic coherence (CKC; Bourguignon *et al.*, 2011). CKC represents the steady-state counterpart of the movement-evoked cortical responses. It peaks in the primary sensorimotor cortex contralateral to the movements and is considerably (2.7–15.5 times) stronger in the afferent than in the efferent direction (Bourguignon *et al.*, 2015). Concomitant tactile stimulation (by letting the moving index finger touch the table), increased the afferent coherence by up to 40% (Bourguignon *et al.*, 2015) but even in this case, the proprioceptive signaling was the leading contributor to the coherent cortical activity. The dominant role of proprioceptive afferents to the elicited cortical signals is also strongly supported by the similarity of CKC for both active and passive finger movements (Piitulainen *et al.*, 2013).

Muscle receptors are the most important proprioceptors for kinesthesia, the sense of movement (for a review, see Proske & Gandevia, 2012). However, also cutaneous stretch receptors—that are located around joints, especially in fingers, but distinct from the cutaneous pressure receptors that mediate tactile sensations (Gardner & Johnson, 2013)—contribute to kinesthetic percepts (Collins *et al.*, 2005). The present data do not allow to determine the relative contributions of these different proprioceptor types on the observed cortical responses.

In monkeys, muscle receptors project to areas 3a and 2 of the SI cortex (Burchfiel & Duffy, 1972; Schwarz *et al.*, 1973), whereas areas 3b and 1 receive mostly tactile input (for a review, see Kaas, 1993). Some MI neurons are activated by passive movements as well, both in monkeys (Lucier *et al.*, 1975) and humans (Goldring & Ratcheson, 1972). In the current study, the responses to passive finger movements were adequately modeled with posteriorly-pointing dipoles in the contralateral primary sensorimotor cortex, in good agreement with previous MEG studies (Xiang *et al.*, 1997; Lange *et al.*, 2001; Alary *et al.*, 2002; Druschky *et al.*, 2003). More specifically, our results

indicated that the sources were located in the posterior bank of the central sulcus, corresponding to areas 3a/3b of the SI cortex. The current study did not allow differentiation between these areas, although earlier knowledge about the functional organization of the SI cortex would suggest area 3a rather than 3b as the origin of proprioceptive responses (Kaas, 1993). In the posterior bank of the central sulcus, a posterior source orientation implies intracellular currents flowing from the cortical surface towards deeper layers. Such currents are best explained by excitation in the superficial cortical layers.

ISI dependence of cortical reactivity

The observed ISI dependence of the passive-movement-elicited cortical responses is in line with previous EEG studies using transient passive movements (Starr *et al.*, 1981; Abbruzzese *et al.*, 1985) and with a recent MEG study using both transient and continuous passive movements (Piitulainen *et al.*, 2015). To our knowledge, however, ours is the first study to characterize this ISI dependence in detail and quantify the lifetime of these cortical responses. Our exponential saturation function that modeled well the ISI dependence of the response strength is similar to the models proposed previously for auditory (Lu *et al.*, 1992a), visual (Uusitalo *et al.*, 1996), and nociceptive (Raij *et al.*, 2003) cortical responses. **Based on our model, we were also able to estimate the optimum ISI for proprioceptive stimulation to reach maximum SNR in a given measurement time. This optimization was a compromise between response amplitude, which increases, and the number of stimuli, which decreases as a function of ISI. A similar ISI-optimization approach has been previously applied to visual (Ahlfors *et al.*, 1993) and nociceptive (Raij *et al.*, 2003) MEG responses.**

Cortical activation by external sensory stimulation is typically followed by a recovery period of lowered reactivity and decreased response amplitudes to subsequent stimuli. These changes cannot be explained by neuronal fatigue, but might instead be the result of active inhibition of a subset of the neuronal populations contributing to the response (Loveless *et al.*, 1989). The duration of the recovery period is characterized by the response lifetime τ, and it varies depending on the cortical area as well as the specific neuronal population within that area (for a review, see Hari *et al.*, 2010). Responses to visual stimuli in early occipital areas recover faster than those in higher-order temporal, parietal and frontal areas (Uusitalo *et al.*, 1996), and a similar division is observed between the auditory cortices (Lu *et al.*, 1992b; Sams *et al.*, 1993).

Late EEG responses at ~460 ms after painful stimulation of the nasal mucosa are attenuated at ISIs as long as 60 s, as are also subjects' ratings of stimulus intensity (Hummel & Kobal, 1999). However, this attenuation largely depends on peripheral adaptation mechanisms (Hummel *et al.*, 1996).

The current results indicate that at ISIs up to 4 s, and even longer, the SI cortex does not fully recover between consecutive volleys of proprioceptive afference, whereas ISIs of 8 s and 16 s yield practically the maximum responses. Closer analysis revealed response lifetimes of 1.3 s for extension and 2.2 s for flexion movements. Thus, the recovery rate of proprioceptive SI responses was comparable to those reported previously for the supratemporal auditory N100m response (Hari *et al.*, 1982; Lu *et al.*, 1992a) and the ~100-ms SII response to electrical median nerve stimulation (Hari *et al.*, 1993). However, due to the biphasic stimulation—containing one extension and one flexion movement per cycle—the interval between consecutive movements was in our study only half of the applied ISI. Thus our quantification of the response lifetimes is based on the assumption of independence of proprioceptive afference from extensor and flexor muscles. If, however, these

afferent volleys would interact in the cortex, or along the afferent pathways, then proprioceptive SI responses would in fact recover by a factor of 2 faster than estimated by the current study. Further investigations of such interactions are required to elucidate this question.

The observed response lifetimes indicate that an ISI of 1.5–3 s for passive-finger-movement stimulation maximizes the SNR of the averaged cortical responses in a given measurement time. This information may be utilized in future clinical and research protocols to save recording time and ensure repeatability between measurements. The optimization is an important step when aiming at a standardized clinical tool to diagnose impairments of proprioceptive function, which may occur for example due to stroke, cerebellar degeneration, myelopathies, cerebral palsy, and neuropsychiatric conditions. Our results can also be valuable for future developmental studies (Uppal *et al.*, 2016) as well as for exploring the link between electrophysiological markers and genetic susceptibility to diseases that affect the sensorimotor system.

Differences in cortical activations for extension and flexion movements

Although both extension and flexion movements strongly activated the contralateral SI cortex, the evoked responses differed; the extensions evoked earlier, stronger, and more consistent cortical responses with more clearly dipolar field patterns. One possible explanation for these differences is that the extensions were slightly faster than the flexions, due to mechanical properties of the PAM stimulator. Furthermore, because of the initial extended position of the finger, the extensions caused stronger stretch in the flexor muscles than the flexions in the extensors, better activating the proprioceptors (for a review, see Proske & Gandevia, 2012).

Conclusions

Proprioceptive stimulation using passive index-finger movements strongly activated the contralateral primary somatosensory cortex with the most prominent response peaking at ~70 ms for extension and ~90 ms for flexion movements. The strength of the cortical sources was markedly enhanced when the ISI was prolonged as observed previously for other sensory modalities. **Detailed characterization of this ISI effect indicated an optimum ISI of 1.5–3 s for proprioceptive stimulation to maximize the SNR in a fixed measurement time. Our results can be vastly utilized in future studies exploring proprioceptive processing in different subject groups, as well as in designing standardized stimulation protocols for both basic and translational research.**

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Conflict of interest

None of the authors have potential conflicts of interest to be disclosed.

Author contributions

ES, HP, MB, and RH designed the study. ES, HP, and MB collected the data. All authors participated in analyzing and interpreting the data and in writing the manuscript.

Abbreviations

A, strength (amplitude) of cortical source normalized within subjects; A_{max}, maximum A that can be reached for arbitralily long ISIs; ANOVA, analysis of variance; CKC, corticokinematic coherence; ECD, equivalent current dipole; EEG, electroencephalography; EMG, electromyography; ISI, interstimulus interval (subscript number indicates length of ISI in seconds, e.g. ISI_8); MANOVA, multivariate analysis of variance; MEG, magnetoencephalography; MI, primary motor; MRI, magnetic resonance image/imaging; n, number of stimulus repetitions in an experiment; PAM, pneumatic artificial muscle; rms, root mean square; SEF, somatosensory evoked field; SI, primary somatosensory; SII, secondary somatosensory; SNR, signal-to-noise ratio; τ, response lifetime.

Data accessibility

The ethical approval for our study by the ethics committee of Aalto University does not allow public sharing of the original data.

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Figure captions

Figure 1. Measurement setting. (A) Subject sitting with his head inside the MEG helmet and his right index finger attached to the actuator of the PAM stimulator. (B) Close-up of a subject's hand resting on the stimulator, with the index finger in the extended position.

Figure 2. Passive movements and EMG signals. (A) Displacement of the movement actuator during 1 min of stimulation at the 8-s ISI. (B) One movement cycle in focus. (C) Averaged finger acceleration (Euclidean norm of 3 signals) for extension (N = 45) and flexion (N = 46) movements for Subject 1 (ISI 8 s). (D, E) Averaged rectified EMG signals from antebrachial extensor and flexor muscles of Subject 1 (ISI 8 s). A small stimulus-locked EMG response peaks in both muscles ~130 ms after the flexion movement (right traces), whereas no response is visible after the extension movement (left traces).

Figure 3. MEG responses and cortical sources evoked by passive extension and flexion movements of the right index finger in Subject 1. (A) Distribution of planar-gradiometer responses to extension movements of the right index finger at the 8-s ISI. (B) Responses, from the channel marked in (A) for both extension and flexion movements at all ISIs. (C) ECDs, fitted to the peak responses to extension (white) and flexion (black) movements at the 8-s ISI, superimposed to the subject's MRI. (D) Source waveforms following extension and flexion movements at all ISIs. **In (B) and (D), solid vertical lines indicate movement onset, and dashed vertical lines at $ISI_{0.5}$ indicate the onset of the opposite movement occurring at 250 ms.**

Figure 4. ISI dependence of proprioceptive cortical responses. Normalized source strength (dots and error bars; mean ± SEM over subjects) plotted as a function of ISI, separately for extension and flexion movements. The solid curves illustrate the function $A(ISI) = A_{\max}\left(1 - e^{-\frac{ISI}{\tau}}\right)$, fitted to the group-mean values, where A_{max} is the highest A reached for arbitrarily long ISIs, and τ is the lifetime of the response. The estimated lifetimes τ are 1.3 s (extension) and 2.2 s (flexion). Note that the y-axes are scaled to A_{max}. The vertical lines indicate the optimum ISIs maximizing the SNR in a fixed measurement time. The dashed curve in the left panel, plotted on top of the solid curve, illustrates the shape of the ISI effect estimated from sensor-space responses to extension movements.