

# Evidence for fusion and segregation induced by 21 Hz multiple-digit stimulation in humans

Li Chan Liu, William C. Gaetz, Daniel J. Bosnyak and Larry E. Roberts<sup>CA</sup>

Department of Psychology, McMaster University, Hamilton, Ontario L8S 4K1, Canada

<sup>CA</sup>Corresponding Author

Received 18 April 2000; accepted 5 May 2000

Subjects were trained to detect changes in the frequency of 21 Hz tactile stimulation applied to digits 2+3+4 (fusion group) or 2+4 (segregation group) of the right hand. The 21 Hz steady-state response for digit 3 was measured by 64 channel EEG on mapping trials before and after training. Discrimination improved over 3 days, confirming that subjects attended to the training stimuli. The 21 Hz response was larger on training than on mapping trials, indicating sensitivity of the

response to the strength of cortical activation. Under these conditions the 21 Hz response for digit 3 decreased after training in both groups on day 1. On day 3 this effect reversed in a subset of fusion subjects while segregation continued to yield decreases. The findings suggest that somatosensory representations are dynamically modified by the sensory input experienced on a task. *NeuroReport* 11:2313–2318 © 2000 Lippincott Williams & Wilkins.

**Key words:** Electroencephalography (EEG); Hebbian learning; Somatosensory cortex; Steady-state responses; Use-dependent plasticity

## INTRODUCTION

Recent animal studies have shown that the receptive fields (RFs) of neurons in somatosensory cortex can be altered in adult organisms by behavioural training. For example, Recanzone *et al.* [1,2] found that training owl monkeys on a tactile frequency discrimination task enlarged the neural representation for the trained skin site by a factor of 1.5–3 in cortical area 3b. An enhancement of similar magnitude was observed by Jenkins *et al.* [3] when monkeys were trained for 50–100 sessions to touch a rotating disk with the correct pressure in order to earn food reward. Remodelling of sensory representations by behavioural procedures appears to be gated by neuromodulatory systems in the thalamus and basal forebrain that condition rapidly to cues signalling task relevant events. Remodelling does not occur unless the animal is required to process the task stimuli by discriminative training (see [4] for review).

Fusion and segregation of somatosensory representations for different skin sites has also been demonstrated by animal studies. Wang *et al.* [5] applied a series of brief, phase-coherent tactile stimuli alternatively to the distal or proximal phalanges of digits 2+3+4 of owl monkeys. The task of the monkey was to detect when pulses were delivered twice in succession to either phalange. Invasive mapping of area 3b carried out after 4–6 weeks of training revealed a high proportion of neurons (44–56%) with multiple-digit RFs that were rarely seen in untrained animals. At the same time, a band representing dorsal skin emerged between the zones representing the non-simultaneously excited distal and proximal skin surfaces, suggesting a

concurrent segregation effect. Experiments on rat barrel fields indicate that lengthy training may not be necessary for the occurrence of fusion effects. Whisker-pairing leads within 24 h to fused representations which appear initially in the superficial (II, III) and deep (V, VI) layers of the barrel cortex and 10–30 days later in the input layer (IV), signifying reorganization at the level of the thalamus [6].

Wang *et al.*'s report that multiple-digit RFs were induced in ~50% of sampled neurons suggests that fusion and segregation may be detected in electrical (EEG) or magnetic (MEG) fields which can be recorded non-invasively in human subjects. In the present study we used EEG to study short-term plasticity induced by training for tactile frequency discrimination in humans. Subjects were trained to discriminate small changes in the frequency of a 21 Hz standard stimulus applied for 1 s simultaneously to the finger tips of digits 2+3+4 (fusion condition) or digits 2+4 (segregation condition) of the right hand. Based on previous animal research we hypothesised that synapses conveying input from the fingertips receiving multiple digit stimulation would be strengthened by simultaneous depolarisation of their postsynaptic targets, through a process of Hebbian learning. If so, stimulating digit 3 after training for fusion was expected to elicit a response from neurons tuned previously to digits 2 and 4, thereby augmenting the 21 Hz response. In contrast, segregation training was expected to decrease the response for digit 3 (segregated from 2 and 4) through anti-Hebbian mechanisms. Subjects were trained for 3 days to allow for the development of remodelling effects.

## MATERIALS AND METHODS

**Subjects:** Thirteen healthy right-handed male subjects (age 24–40 years) were paid for their participation. Seven subjects were trained in the fusion condition and six in the segregation condition. Informed consent was obtained in accordance with the Declaration of Helsinki.

**Procedure:** Mechanical stimulation of the fingertips was generated by a five-digit stimulator constructed of solenoids connected to tactile probes (rods of 2 mm diameter). The solenoids were housed in a sound-attenuating and electrically shielded case to reduce acoustic and electromagnetic artefact. Subjects wore headphones through which low-level white noise was presented to mask residual acoustic artefact. Tactile stimulation was presented at 21 Hz (the standard frequency) or one of eight higher frequencies up to 26 Hz (the comparison frequencies). This frequency range provided the sensation of flutter and is within the resonant frequency region for the somatosensory system [7]. Stimuli were of 1 s duration and were separated by an interstimulus interval of 1 s. To ensure that somatosensory stimulation was confined to the fingertips, the subject's forearm was positioned on a platform isolated from the stimulator. Circular foam cups were placed around the perimeter of the probe tips to achieve a stable and stereotyped finger position and to maintain stimulation to the same skin area between measurements.

The experiment was carried out on 3 consecutive days. The EEG was measured on the first and last day, but not on day 2 which consisted of behavioural training only (this day was omitted for one subject). At the outset of day 1, psychophysical calibration of the 21 Hz stimulus was performed for each subject on digits 2, 3, 4 and 5, in order to achieve a detectable light flutter sensation of comparable intensity across all digits. The level of stimulation for each digit was then fixed at this intensity level throughout the experiment, individually for each subject. After stimulus calibration, the following procedures were administered in the order stated below. These procedures were repeated on day 3.

1. *Noise measurement 1 (15 min):* In order to evaluate the artefact generated by the stimulator alone, the EEG was recorded while the stimulator was operated at 21 Hz (1 s duration) but the subject's fingers were not placed on the probes. The noise estimate consisted of all combinations of stimuli used in the experiment (specifically, digits 3 and 5 alone, digits 2 + 3 + 4, or digits 2 + 4). A total of 105 trials was presented for each digit condition, with the digit conditions sequentially intermixed in a random order.

2. *Mapping (30 min):* In the mapping phase, 21 Hz stimulation (1 s) was applied separately to digits 3 and 5 for 210 trials each, in an intermixed order. Trials were grouped into three blocks consisting of 140 trials (420 trials overall) with a rest in between to allow for subject queries and eye blinks. Digit 3 was designated as the test digit because digits 2 and 4 during the fusion or segregation training spanned it. Fusion and segregation effects were predicted for this digit. We also recorded digit 5 as a control digit for the stability of mapping measurements. However, because lateral connections in area 3b could in principle extend to digit representations distal to the stimulated site, digit 5

may not be comparable to an unstimulated control condition. Data from digit 5 were excluded from the analysis.

3. *Training (60 min):* Subjects were trained to make a same or different judgement between a standard stimulus (S1) and a comparison stimulus (S2) presented to digits 2 + 3 + 4 (fusion) or 2 + 4 (segregation). S1 was always 21 Hz, while S2 was either 21 Hz (50% of training trials) or varied from 22 to 26 Hz in steps of 0.5 Hz. S1 and S2 were 1 s duration and were separated by an interval of 1 s. Subjects indicated their discriminative choice after each S1/S2 trial by pressing an appropriate button using the left hand. Response latency was recorded. Feedback was given after each trial by illuminating a green (correct) or red (incorrect) LED placed in 1 m in front of the subject at eye level. The next trial commenced 1 s after the button press. There were three training blocks and 192 trials in each block. At the end of each block, subjects were told the ratio of the correct to incorrect responses to help them assess their progress. In order to ensure that S2 was discriminated for its frequency and not its perceived intensity, the duration of the square wave pulse applied to the probe solenoids (10 ms) was reduced by 0.1 ms for every frequency increase of 0.5 Hz. This adjustment was found in a separate psychophysical study to yield equal intensity functions for S2 stimuli ranging between 21 and 26 Hz.

4. *Mapping 2 (30 min):* Mapping 2 was performed upon the conclusion of discrimination training. The procedure was identical to Mapping 1.

5. *Noise measurement 2 (15 min):* Noise measurement 2 was identical to the first noise measurement. The two noise measurements were collapsed to give the same number of trials (210) per digit condition as was used during the mapping sessions.

The fusion and segregation conditions of this study were designed to produce opposite learned changes in the cortical representation of digit 3. Comparison of the two conditions therefore provided an assessment of whether cortical remodelling by Hebbian mechanisms had occurred. However, an unavoidable feature of the fusion and segregation manipulation is that digit 3 is stimulated during training for fusion but not during training for segregation. Hence, in principle, differences between the fusion and segregation conditions could be attributed to adaptation of the 21 Hz steady-state response by repeated stimulation of the test digit as well as to remodelling of the cortical representation for this digit. Because the expected direction of adaptation and remodelling effects was opposite, a choice between the competing interpretations was possible. We trained subjects for three days in order to allow changes consequent on Hebbian remodelling to be expressed.

**EEG recording and analysis:** A 64-channel EEG system (NeuroScan Inc) was used to measure brain responses to tactile stimulation. The electrode cap (Neuromedical Quik-Cap) covered the whole head with a symmetrical array that included the international 10–20 system. The EEG was recorded continuously DC to 100 Hz and sampled at 500 Hz. Electrodes Cz and AFz were selected respectively as the reference and the ground. During the recording,

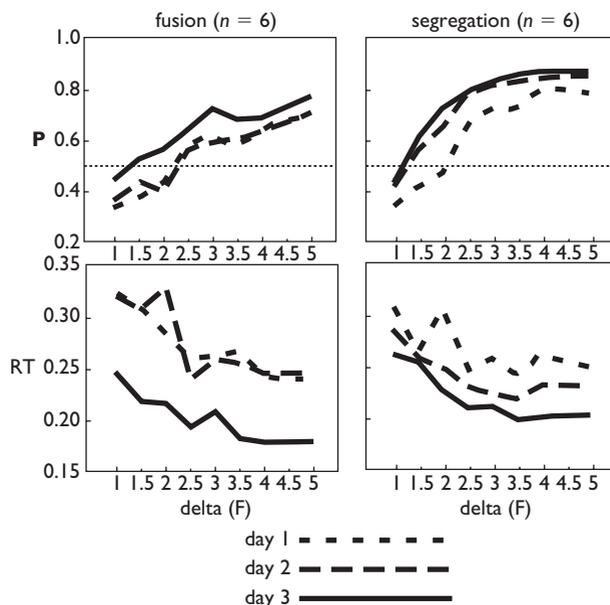
subjects were instructed to keep their eyes open and blink as little as possible.

The recorded EEG was first cut into 2 s segments from 500 ms before to 1500 ms after stimulus onset and baseline corrected for each channel. Epochs contaminated by eye blinks and movement artefact (changes  $>100\ \mu\text{V}$  at any electrode) were rejected from further analysis. EEG data for one subject in the fusion group and two in the segregation group could not be analysed owing to artefacts generated by defective lead/electrode junctions (the faulty electrode cap was subsequently replaced by Neuromedical with a modified version). Epochs for the remaining 10 subjects were averaged separately for each digit condition (3, 2 + 3 + 4 or 2 + 4) in each mapping and training session (for training, S1 only) and re-referenced to a common average reference.

Signal analysis was carried out in the frequency domain. Spectral power at 21 Hz was determined using Welch's periodogram method [8]. In addition, spectrograms were calculated depicting spectral power from 15 to 70 Hz over the EEG epoch ( $-500$  to 1500 ms) for each electrode. The effect of training for fusion and segregation was evaluated by subtracting 21 Hz spectral responses recorded for digit 3 on the first mapping session (before) from the same measure determined in the second mapping session (after), for days 1 and 3 separately. Pre-planned *t*-tests were applied to these measurements in the fusion and segregation groups. In addition, before/after changes in the 21 Hz response were evaluated on days 1 and 3 by Monte Carlo simulations which modelled the distribution of map2-map1 differences under the null hypothesis separately for each subject [9]. The area above the before/after difference obtained for each subject was calculated to determine whether statistically significant changes had occurred on days 1 or 3 of discrimination training for that subject.

## RESULTS

**Behavioural results:** The probability of a hit ( $P(H)$ ) was calculated as the proportion of trials on which the subjects responded 'different' when S1 and S2 were of different frequencies (comparison frequencies of 22–26 Hz, corresponding to a  $\Delta f$  of 1–5 Hz). The probability of a false alarm ( $P(FA)$ ) was calculated as the proportion of trials on which the subjects responded 'different' when the frequency of S2 was the same as S1 (21 Hz,  $\Delta f = 0$ ). From these two measures a performance score  $P$  was calculated for each comparison frequency  $>21$  Hz according to the formula  $P = P(H) \times [1 - P(FA)]$ , following Recanzone *et al.* [1]. Psychophysical functions were constructed for each subject and training session by plotting  $P$  against all comparison frequencies  $>21$  Hz ( $\Delta f > 0$ ). These functions are averaged and shown in the upper panel of Fig. 1 for the fusion and segregation groups, separately. Discrimination improved over the three days in each group, particularly at frequencies adjacent to the discrimination threshold ( $P = 0.5$ ,  $\Delta f \sim 2$  Hz). Response latency (shown in the lower panel of Fig. 1) also decreased over successive days in the two conditions. Overall, differences in discrimination performance between fusion and segregation were not pronounced. When the number of correct responses was contrasted between days on an individual basis, 12 of

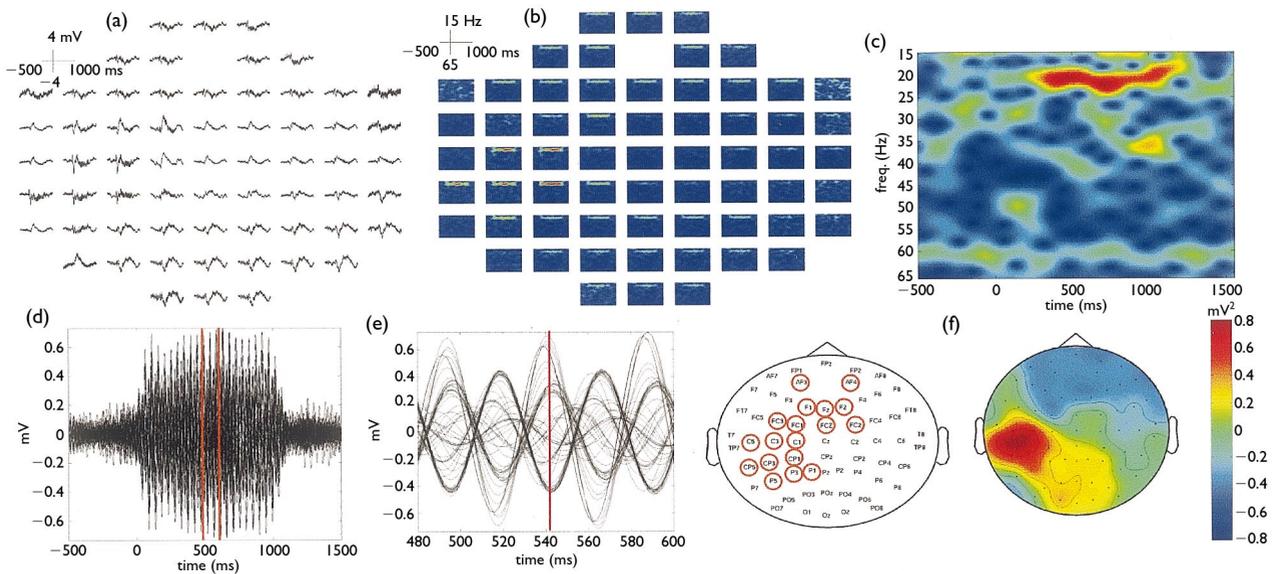


**Fig. 1.** Psychophysical functions over 3 days for subjects receiving 3 days of training ( $n = 12$ ). Values of  $P$  and response time (RT, in s) are shown at the top and bottom panels, respectively, averaged separately for the fusion and segregation conditions. The lines in the top panels indicate the threshold of discrimination ( $P = 0.5$ ).

13 subjects showed improvement on last day compared to the first day of training for fusion or segregation ( $p = 0.002$ , sign test).

**EEG results:** Figure 2 presents EEG data from a typical subject for digit 3 in a mapping session. Panel A shows the unfiltered data where oscillatory activity appears stronger at electrodes in the left hemisphere, contralateral to the site of stimulation. Spectral plots (15–65 Hz; Fig. 2b) confirmed 21 Hz activity in a cluster of electrodes over the left scalp. Although not apparent here, harmonics at 42 and 63 Hz were also noted in some subjects, but with lower amplitude than at 21 Hz. Spectral density is summed over all electrodes in Fig. 2c), where it can be seen that the 21 Hz response reached its peak amplitude about 300 ms after the stimulus onset. In Fig. 2d the time-domain average is given for all electrodes, bandpass filtered between 18 and 24 Hz. The scalp topography at the time point indicated in the time domain enlargement of Fig. 2e is shown in Fig. 2f, where a polarity reversal is seen over the left Rolandic fissure, consistent with a source of activation in primary somatosensory cortex. We also contrasted, for all subjects for whom EEG data were available ( $n = 10$ ), 21 Hz spectral power during mapping measurements for digit 3 with noise measurements taken when the stimulator was operating but the finger was not in contact with the tactile probe. On average, the peak of the 21 Hz response was found to be about eight times stronger during mapping runs than during noise runs for the fusion and segregation conditions combined.

In order to evaluate whether the 21 Hz response was sensitive to the strength of cortical activation, we investigated whether the response varied as a function of the



**Fig. 2.** EEG data from a typical subject for digit 3 in a mapping session. (a) Unfiltered EEG (–500 to 1500 ms) over 64 electrodes. (b) Spectral plots (15–65 Hz). (c) Sum of spectral density over all electrodes in (b). (d) 64-channel EEG time domain responses, filtered 18–24 Hz. (e) Time scale expanded between 480 and 600 ms in (d). (f) Right: scalp topography at 542 ms in (e); left: 64-electrode array, with 18 electrodes highlighted that gave a strong 21 Hz response.

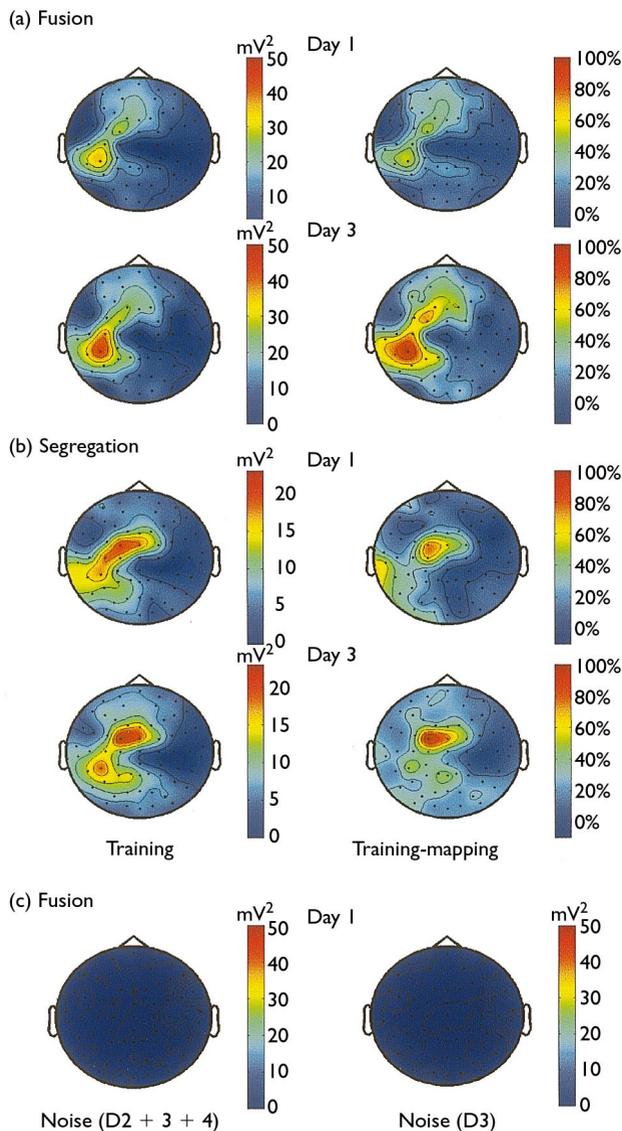
number of digits simultaneously stimulated. For this purpose we compared the response on training trials with the response on mapping trials, separately for the fusion and segregation conditions. The results are shown in Fig. 3a (grand average for fusion subjects) and Fig. 3b (segregation subjects, days 1 and 3 shown separately). Response topography (21 Hz spectral power) on training trials is depicted on the left in each panel. In the right panel, response topography on mapping trials (digit 3 stimulated) has been subtracted from the training topography (digits 2 + 3 + 4 in fusion, 2 + 4 in segregation), to show the normalized difference between them. Subtraction revealed a prominent region of increased power over the contralateral Rolandic fissure for fusion and segregation, indicating stronger activation on training trials than on mapping trials in each group. This effect was quantified using a fixed montage of 18 electrodes anterior and posterior to the left Rolandic fissure (constant for all subjects; see Fig. 2f, left). Multiple digit stimulation increased the 21 Hz response by a factor of 2.18 in the fusion group compared to a factor of 1.45 in the segregation group. The difference between the groups was consistent with a near-linear increase of the 21 Hz response as a function of the number of digits stimulated on fusion and segregation training trials. This increase represents a brain response and not stimulator artefact, because noise measurements shown in Fig. 3c did not differ between 2 + 3 + 4 and 3 conditions when the digits were removed from the tactile probes. When the fusion and segregation groups were collapsed, training activations were observed to be stronger than mapping activations in every subject ( $n = 10$ ,  $p = 0.001$ , sign test). Inspection of the right panels in Fig. 3a,b shows further that the peak of spectral power on training trials tended to be larger on day 3 than on day 1 in both conditions. When power on training trials was contrasted directly between Day 1 and Day 3 for the 18 electrode montage, a 24% increase was

observed in the fusion group and 6% in the segregation group, but these increments did not reach significance.

Fusion and segregation effects were evaluated by calculating the difference in spectral power at 21 Hz between the two mappings of digit 3 (map 2–map 1, days 1 and 3 separately). These differences were determined for the 18-electrode montage of Fig. 2f and are averaged separately for the fusion and segregation groups in Fig. 4a. Inspection of the fusion group shows that the 21 Hz response for digit 3 decreased after fusion training on day 1, but increased after fusion training on day 3. The increase on day 3 and the reversal between the days were in the direction of a fusion hypothesis, but did not reach significance at the group level. Inspection of the segregation group shows that the digit 3 response decreased after training on both days. This effect was significant for the segregation group on day 3 and when the two days were averaged ( $p = 0.026$  and  $p = 0.04$  respectively, one-tailed tests). Comparison of the fusion and segregation groups overall (days 1 and 3 averaged) yielded  $t(8) = 1.65$ ,  $p < 0.10$  (one-tailed test). The performance of individual subjects is shown in Fig. 4b,c for fusion and segregation, respectively. Performance over days was more consistent in the segregation condition, where all subjects showed decreases in the digit 3 response in each measurement. In three of these cases (indicated by an asterisk) the decreases were statistically significant when assessed by Monte Carlo simulations. In the fusion group significant increases were detected in the 21 Hz response for digit 3 following training on day 3 in two subjects, and a significant reversal between days for one of these cases. However, one fusion subject showed a significant decrease in the 21 Hz response after training on day 3.

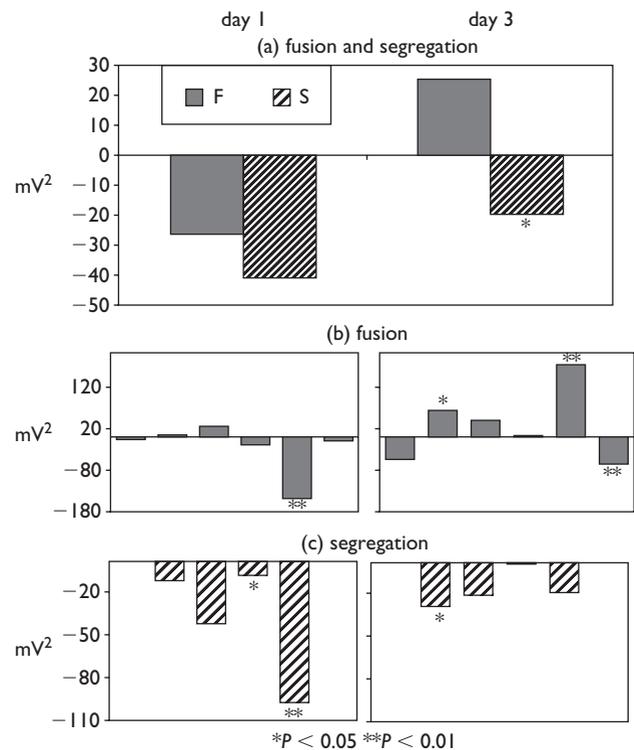
**DISCUSSION**

Several conditions were met for a test of fusion and segregation induced by cooperative 21 Hz stimulation of



**Fig. 3.** Comparison of the 21 Hz response between mapping and training trials for the fusion (a) and segregation (b) groups on day 1 (top row) and day 3 (bottom row). The left column shows the topography of the averaged 21 Hz response on training trials, while the right column is the normalized difference (percentage of peak activation) obtained when the mean of the mapping trials was subtracted from the training trials. Scaling is identical for days 1 and 3 but differs between the fusion and segregation groups. (c) Noise measurements presented for the 2 + 3 + 4 condition and the 3 condition, scaled identically to the fusion group in (a, left).

the digits. First, 12 of 13 subjects improved at the tactile frequency discrimination task over the 3 days of training. Clearly, subjects worked at the task and attended to the training stimuli. Second, the amplitude of the 21 Hz response at electrodes spanning the contralateral Rolandic fissure was found to be larger on training trials on which multiple digits were stimulated, than on mapping trials where only digit 3 was stimulated. This effect was proportionately larger on fusion training trials where three digits were stimulated compared to two digits during segrega-



**Fig. 4.** (a) Change in the 21 Hz response between mapping blocks (map2-map1) separately for the fusion and segregation groups on days 1 and 3. Grand averages are shown. (b, c) Performance of individual subjects in the fusion (b) and segregation (c) conditions. Asterisks denote changes that are significant at the group level (a) and in individual subjects assessed by Monte Carlo simulation (b, c).

tion. These findings establish that the 21 Hz steady-state response was sensitive to the strength of the somatosensory cortical activation, and that the multiple digit stimuli were reliably delivered to the fingers during training for fusion and segregation.

Under these conditions we observed a decrease in the mean response for digit 3 in both groups after the first day of training. Neural adaptation consequent upon repeated stimulation of digit 3 on training trials may have been responsible for decrements observed in the fusion group, since a hypothesis of cortical remodelling predicted the opposite effect. However, adaptation appears unlikely to account for the decrement seen in the segregation group, because digit 3 was not stimulated during training for segregation. On day 3, mean changes in the response for digit 3 were in accord with a fusion/segregation hypothesis in both groups. On this day statistical evidence for fusion was found for a subset of fusion subjects, although the reversal between days 1 and 3 did not reach significance for the fusion group as a whole. The performance of the segregation subjects was more consistent than that of the fusion subjects. All segregation subjects showed a decreased response for digit 3 following segregation training, and this effect reached significance at the group level on day 3 and when both days were combined. According to a segregation hypothesis, these changes occurred because phase coherent stimulation of digits 2 and 4 during

training for segregation was sufficient to depolarise area 3b neurons tuned to the intermediate digit 3, while synapses mediating input from this digit were silent. Under these circumstances digit 3 neurons switched their tuning to digits 2 and 4 by an anti-Hebbian mechanism. It may be noteworthy that the cortical response to the training stimulation increased (albeit nonsignificantly) on day 3 compared to day 1, particularly in the fusion group, as would be expected if multiple-digit receptive fields were established by cooperative stimulation on training trials.

The present results obtained non-invasively in humans are consistent with an earlier report of fusion and segregation obtained by Wang *et al.* in an animal study [5]. However, Spengler *et al.* reported different results for human subjects [10]. These investigators trained subjects on the task of Wang *et al.* for 3–4 weeks, before and after which single digit representations were imaged neuromagnetically (MEG) using an air-driven tactile probe. Representations for the trained digits measured as dipole moment decreased after training, although an increase was predicted. There are several procedural differences between the present study and that of Spengler *et al.* that may account for the different findings. We trained subjects with a high frequency flutter stimulus (21 Hz) which was within the resonant frequency of the human somatosensory system [7]. This stimulus was used for mapping as well as for training trials. In the Spengler *et al.* study the mapping and training stimuli were of lower frequency (~3 Hz) and differed between training and mapping in order to accommodate the requirements of MEG measurement. We also examined changes over a brief time period (three daily sessions, *vs* ~10–20 sessions by Spengler *et al.*) and contrasted a fusion group with a segregation group for evidence of remodelling. Additional research will be required to corroborate present findings and resolve the different outcomes obtained by these two studies of fusion and segregation in humans.

Behavioural studies employing EEG and MEG measurement appear to provide useful information on how the brain supports the development of tactile frequency discrimination. Mechanical vibrations applied to the skin in the range 5–50 Hz produce the sensation of flutter [11] and activate neurons in area 3b of the primary somatosensory cortex (SI) which map somatotopically to the site of stimulation [12]. Our spectral EEG topographies are consistent with neural activation of area 3b, and spectral responses arising from this region appeared to change during 3 days of training for digit fusion and segregation. However, behavioural data reported by Spengler *et al.* [10] indicate that tactile discrimination transfers robustly to the untrained hand in human subjects. Because callosal projections from SI to the somatosensory cortices of the opposite

hemisphere appear to be restricted largely to representations of the medial body surfaces and not the hand area [13–15], behavioural transfer points to the additional participation of higher levels of the somatosensory projection pathway in frequency discrimination. Callosal transfer from SII may be implicated, because there was no evidence in our EEG data for activation of ipsilateral SI during training for fusion or segregation.

## CONCLUSION

Conditions for a test of fusion and segregation induced by multiple digit tactile stimulation in humans were established in the present study. Tactile frequency discrimination improved over three days of training, confirming that subjects attended to the training stimuli. In addition, the 21 Hz steady-state response increased with the number of digits stimulated on training trials, which confirmed that the multiple-digit stimuli were reliably delivered and that the 21 Hz response was sensitive to the strength of cortical activation. Under these conditions, changes in the 21 Hz response of digit 3 were obtained on the third day of training for a subset of subjects in the fusion condition and for the segregation group as a whole that were consistent with a fusion/segregation hypothesis. The findings support the view that somatosensory responses are dynamically modified to represent the pattern of sensory input experienced on behavioural tasks.

## REFERENCES

1. Recanzone GH, Jenkins WM, Hradek GT and Merzenich MM. *J Neurophysiol* **67**, 1015–1030 (1992).
2. Recanzone GH, Merzenich MM, Jenkins WM *et al.* *J Neurophysiol* **67**, 1031–1056 (1992).
3. Jenkins WM, Merzenich MM, Ochs MT *et al.* *J Neurophysiol* **63**, 82–104 (1990).
4. Buonomano DV and Merzenich MM. *Annu Rev Neurosci* **21**, 149–186 (1998).
5. Wang X, Merzenich MM, Sameshima K and Jenkins WM. *Nature* **378**, 71–75 (1995).
6. Diamond ME, Huang W and Ebner FF. *Science*, **265**, 1885–1886 (1994).
7. Tobimatsu S, Zhang YM and Kato M. *Clin Neurophysiol* **110**, 1953–1958 (1999).
8. Welch PD. *IEEE Trans Audio Electroacoustics* **15**, 70–73 (1967).
9. Manly BFJ. *Randomisation and Monte Carlo Methods in Biology*. London: Chapman and Hall; 1991.
10. Spengler F, Roberts TPL, Poeppel D *et al.* *Neurosci Lett* **232**, 151–154 (1997).
11. Mountcastle VB, Steinmetz MA and Romo RJ. *Neuroscience* **10**, 3032–3044 (1990).
12. Sur M, Wall JT and Kaas JHJ. *Neurophysiology* **51**, 724–744 (1984).
13. Jones EG and Hendry SHC. *Neurosci Lett* **19**, 167–172 (1980).
14. Jones EG and Powell TPS. *Brain* **92**, 37–56 (1969).
15. Killackey HP, Gould HJ, Cusick CG *et al.* *J Comp Neurol* **219**, 384–419 (1983).

Acknowledgements: This study was supported by MRC and NSERC of Canada. LCL acknowledges support from the H.G. Thode Fellowship at McMaster University. We thank Mark Hahn for technical assistance and A.A. Ioannides and K.J. Jantzen for their helpful comments.