1	Revealing the neural fingerprints of a missing hand
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18	
19	Abstract:
20	The hand area of the primary somatosensory cortex contains detailed finger
21	topography, thought to be shaped and maintained by daily life experience. Here we
22	utilise phantom sensations and ultra high-field neuroimaging to uncover preserved,

23 though latent, representation of amputees' missing hand. We show that representation 24 of the missing hand's individual fingers persists in the primary somatosensory cortex 25 even decades after arm amputation. By demonstrating stable topography despite 26 amputation, our finding questions the extent to which continued sensory input is 27 necessary to maintain organisation in sensory cortex, thereby reopening the question 28 what happens to a cortical territory once its main input is lost. The discovery of persistent digit topography of amputees' missing hand could be exploited for the 29 30 development of intuitive and fine-grained control of neuroprosthetics, requiring 31 neural signals of individual digits.

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33 **Impact statement:**

We show that the functional finger layout of the primary somatosensory cortex, previously thought to depend on experience, remains distinct despite the physical absence of fingers for several decades.

37 Main text:

38 The hand area of the primary somatosensory cortex (S1) contains detailed digit maps, 39 with physically adjacent digits represented next to each other. Using ultra high-field neuroimaging, it is now possible to identify these characteristic digit maps in humans, 40 41 with high inter- and intra-subject reliability (1,2). Digit topography is characterised in 42 neuroimaging by two main principles (3): digit selectivity (2) and inter-digit overlap (1). These maps are thought to be shaped and maintained by daily life experience: 43 digits used more frequently together in daily life benefit from increased 44 45 representational overlap (1), and following single digit amputation remaining digits' topography changes (4). 46

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Amputees commonly experience lingering sensations from their amputated body part 48 49 (5). Phantom sensations are not necessarily painful, and are best described as a vivid 50 sensation of the missing hand as if it is still present (5). When instructed to move their 51 phantom hand, amputees report detailed kinaesthetic sensations regarding the extent 52 of movement afforded by different phantom digits. Phantom movements are known to 53 evoke signals in the sensorimotor system (6-8), previously attributed to abnormal processing caused by the amputation (e.g. aberrant inputs (6), peripheral 54 reorganisation (7)). Here we interrogate the information content underlying activity 55 56 elicited by phantom hand movements. If this information content is unchanged despite amputation, then activity patterns should show characteristic S1 digit 57 58 topography.

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To study phantom digit topography, we used ultra high-field 7 tesla neuroimaging in two individuals who lost their left hand several decades ago (25 and 31 years post

62 amputation) and eleven right-handed controls. Both amputees reported exceptionally vivid kinaesthetic phantom sensations during individual digit movements (see Figure 63 64 1- Source data 1, 2 for clinical and demographic details). Amputees and controls were visually cued to execute individual phantom digit movements (left hand digits in 65 controls). Importantly, phantom movements are distinguishable from imagined 66 67 movements. This is supported by empirical evidence demonstrating that phantom 68 limb movements elicit both central and peripheral motor signals, that are different 69 from those found during imagined movements (6-8). To ensure adequate task 70 performance, amputees were asked to demonstrate to the experimenter outside the 71 scanner the extent of volitional movement carried out in each of their phantom digits 72 during the task, by mirroring the phantom movements with their intact hand.

73

74 To capture the first principle of topography, we employed a technique designed to 75 identify digit preference in S1 (travelling wave design, see Figure 1- figure supplement 1 for design details; see Figure 1- figure supplement 2 for other brain 76 77 areas activated by phantom hand movements). The resulting gradients of digit 78 preference (hereafter digit maps) are presented in Figure 1. As indicated by the black 79 arrows in the example control participants (Figure 1A), a characteristic digit map shows a gradient of digit preference, progressing from thumb (red, medially) to little 80 finger (pink, laterally). Similarly, both amputees showed a clear gradient of digit 81 preference in the central sulcus and postcentral gyrus (Figure 1B; see Figure 1- figure 82 83 supplement 3 for intact hand maps). Qualitatively, the position, digit order, and extent 84 of the missing hand maps were similar to those observed in controls. Analysis of 85 spatial correspondence of "same" versus "different" digit clusters between two halves of the dataset further confirmed that while reduced (compared to controls), digit 86

selectivity in amputees is consistent (Figure 1D; see materials and methods for furtherdetails).

89

90 A hallmark of a functional sensorimotor system is the distinct representation of the 91 two hands. To further confirm the existence of the missing hand map independently 92 of intact hand contributions (9), we designed an opposing bimanual travelling wave 93 task. Participants performed paired movements with digits of the phantom and intact 94 hands in reverse sequences (i.e. one hand moved D1 to D5, while the other simultaneously moved D5 to D1; Figure 2A). Both amputees showed maps in the 95 96 hemisphere contralateral to the intact hand, dissociated from a second map in the 97 missing hand hemisphere (Figure 2B, C). The latter map corresponded to the original 98 (unimanual movements) missing hand map, as confirmed by higher spatial 99 correspondence for "same" (0.49, 0.40 for above and below elbow amputees) versus 100 "different" (0.06, 0.04) digit clusters across the two (unimanual and bimanual) 101 missing hand datasets (Figure 2E). This provides a replication for the preserved 102 missing hand map. This result also demonstrates the independence of the missing 103 hand topography from the representation of the intact hand. Our group and others 104 previously showed that following unilateral arm amputation, the intact hand becomes 105 over-represented in the missing hand territory (9-11). Our results therefore confirm 106 that the digit map in the missing hand territory is not driven by an emerging 107 representation of the intact hand. Note, however, that our results do not exclude the 108 possibility for reorganisation in the missing hand territory. As demonstrated in Figure 109 2- figure supplement 1, this study's participants showed over-representation of their 110 intact hand in the missing hand territory, indicating that the preserved missing hand 111 topography can co-occur with remapping of body parts.

113 While the travelling wave maps demonstrate digit preference, they provide little information about the second principle of digit topography: inter-digit overlap. To 114 115 study inter-digit overlap, we used pairwise digit representational similarity of 116 multivoxel patterns (12). This approach fully reveals the intricate overlap pattern 117 across all digits (1), as shown for controls in Figure 3B, D. In general, both amputees 118 showed greater average overlap across digits (0.55 and 0.54 for above and below 119 elbow amputees respectively), compared to controls (95%) confidence intervals (CI) = 120 0.24-0.41). However, when examining the inter-digit overlap pattern in the missing 121 hand map, both amputees demonstrated typical patterns (Figure 3A, C; see Figure 3-122 figure supplement 1 for intact hand). This was reflected in high correlation between 123 each amputee's inter-digit overlap pattern and the controls, as assessed using a bootstrapping approach (average $r_s = 0.61$ and $r_s = 0.78$, 95% CI = 0.25-0.89 and 95% 124 CI = 0.62-0.90 for above and below elbow amputees respectively). These average 125 126 amputee-to-controls Spearman correlation values fell well within the controls-tocontrols Spearman correlation range (95% CI = 0.35-0.95; Figure 3C), providing 127 128 further evidence for characteristic missing hand representation decades after 129 amputation.

130

Finally, we also studied missing hand representation in a third amputee (31 years since amputation) whose cause of amputation involved a brachial plexus avulsion, abolishing communication between the residual arm and the central nervous system. Due to MRI safety limitations, 3 tesla neuroimaging was used (see Figure 4B for quality comparisons to ultra high-field digit maps). Using the bimanual task specified above (Figure 4A) we identified digit preference for the missing hand in the

postcentral gyrus (Figure 4C). We also identified a typical inter-digit overlap pattern (Figure 4D), as confirmed in comparison with the control population described in Figure 3 (average Spearman correlation with controls = 0.76 with 95% CI = 0.30-0.92). This result provides further evidence for the existence of preserved digit topography in the absence of peripheral input.

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143 Together we show that, although the missing hand maps were weaker and noisier than 144 the maps found in controls, the functional digit layout of S1 prevails following arm 145 amputation. Digit topography, previously thought to depend on experience (1,4), was 146 detectable despite decades without organised peripheral inputs associated with normal 147 hand function. Our findings call for a reassessment of the role of sensory input in 148 regulating brain organisation and plasticity. By demonstrating characteristic 149 topography of the missing hand decades after arm amputation, our findings reopen the 150 question of what happens to a cortical territory once its main inputs are removed.

151

152 Textbooks teach us that the cortical territory previously assigned to processing the 153 now lost input is invaded by new representations. For example, following arm 154 amputation, the missing hand territory in S1 is taken over by representations of other 155 body parts (e.g. the neighbouring representation of the face in monkeys (13), or the 156 intact hand in humans (9)). Conversely, recent research in the visual cortex suggests 157 that reorganisation in the adult brain may be restricted. For example following 158 macular degeneration, the functional representation of the intact visual field was 159 unchanged ((14), see (15) for similar results in non-human primates). Common to all 160 these previous studies aiming to characterise reorganisation or the lack thereof, is that 161 the authors probed the cortical neighbours of the area previously responsible for processing the lost input. While this approach is suitable for documenting shifted representation of the cortical neighbours, it leaves unexplored the possibility that the original function of the region deprived of sensory input may be preserved, though latent. Amputees experiencing phantom sensations provide a unique model to study what happens to the deprived cortical territory itself during sensory input loss. Here we show that reorganisation in the missing hand territory following input loss does not abolish the original functional layout in sensory cortex.

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170 How can our finding of preserved S1 topography of a missing hand be allied with the 171 wealth of evidence showing cortical reorganisation in S1 following sensory input 172 loss? (e.g. amputation and spinal cord injury (4,13,16); see Figure 2- figure 173 supplement 1 for reorganisation in the current study's volunteers cohort). Already in 174 their seminal work Merzenich et al. suggested that reorganisation following sensory 175 input loss does not exclude the possibility for simultaneous preservation of the 176 original function of that region (4). Accordingly, recent structural and functional 177 evidence shows that the capacity for S1 reorganisation is more limited than initially 178 thought, and that instead the functional changes previously observed in S1 following 179 input loss could be attributed to reorganisation in sub-cortical areas in the afferent 180 pathway, principally the brainstem (17–19). In other words, previous findings of 181 massive cortical reorganisation in S1 reflect reorganisation of inputs, rather than 182 reorganisation within S1 itself. This recent evident nicely complements our own 183 finding of SI reorganisation, overlaid on preserved structure and function.

184

Which inputs could contribute to the maintenance of the missing hand topography?The variability in the level and nature of amputations in this study's cohort allows us

187 to consider the potential contribution of the peripheral nervous system in the 188 preservation of missing hand topography. In the below elbow amputee, some forearm 189 muscles normally controlling hand movements are spared and therefore 190 proprioceptive inputs relating to phantom hand movements likely persist (20). In the 191 above elbow amputee, these inputs would be absent, though ectopic firing from the 192 injured nerve (20) or intact dorsal root ganglia could preserve some afferent inputs (as 193 previously shown following peripheral nerve injury (21), see also (22) for related 194 findings). However, in the amputee suffering from brachial plexus avulsion injury, the 195 dorsal root ganglia are damaged, meaning that no such peripheral input should be 196 available. Given the observation of preserved topography in all three cases, it is 197 highly probable that the preserved missing hand maps are not maintained by 198 peripheral input, but rather are driven by processing in the central nervous system 199 itself.

200

201 What neural signals may be triggering the brain activations subserving the missing 202 hand maps? Given the relatively unimpaired motor system in amputees, it is possible 203 that these representations are driven by motor (efferent) information. The motor 204 system is thought to provide information about its descending commands to the 205 sensory system, by means of efference copy. When efference signals reach the 206 sensory areas, they evoke activity in those areas. The pattern of this corollary 207 discharge could resemble that of the sensory feedback to be expected from the 208 movement (23). While predictive signals are fundamental components for current 209 theories of motor control (24), surprisingly little empirical evidence exists to 210 demonstrate efferent signals in the primates' S1 hand representation independently of 211 afferent processing (23). Our evidence for digit topography in S1 despite the physical

absence of a hand suggests the involvement of non-afferent processing in S1. The persistence of efference signals from the motor system could contribute to the maintenance of preserved structure and function in SI despite afferent input loss.

215

216 Our findings are based on the unique phenomenology of phantom sensations, and as such all tested amputees reported experiencing exceptionally vivid phantom 217 218 sensations, allowing them to voluntarily move each of their phantom fingers. An open 219 question remains whether a relationship exists between the experience of phantom 220 sensations and preserved missing hand topography. Our previous findings, showing 221 that phantom hand movements activate the missing hand territory in individuals 2.2.2 experiencing varying levels of phantom sensation vividness (6,25) might indicate that 223 this is a general phenomenon. However, we note that these previous studies do not 224 provide information on whether the topographic features underlying the phantom-225 evoked activity were preserved. Further research is needed to determine whether the preservation of missing hand topography depends on (or gives rise to) the experience 226 227 of phantom sensations, or whether our finding reflects a fundamental organising 228 principle of the brain that is independent of experience.

229

The notion of brain reorganisation has posed an unexpected obstacle to recent technological developments of human-technology fusion, i.e. neuroprosthetics. Neuroprosthetics allow patients with sensorimotor impairments (e.g. amputation, paralysis) to directly interface with a robotic limb using cortical signals of the hand. However, the relevant information necessary for intuitive neuroprosthesis control may not be attainable once input is lost and functional reorganisation occurs. By demonstrating persistence of topography despite input loss, our finding could be

- 237 exploited to develop intuitive fine-grained control of neuroprosthetics (26) (e.g.
- 238 requiring representation of individual digits) in disabled populations.

239 Materials and methods

240 **Participants**

241 The main inclusion criteria for amputees in the study were: (a) unilateral upper limb 242 amputation; (b) vivid kinaesthetic sensations during voluntary movements of each of 243 the five digits of the phantom hand (based on self-report); (c) compatibility with 244 magnetic resonance imaging (MRI) safety guidelines. We initially screened 63 unilateral upper-limb amputees, of which 22 met the initial criteria for MRI safety. 245 Eight amputees met our 2nd criterion for vividness of kinaesthetic sensations while 246 volitionally moving each of their phantom digits. Only two of these candidates were 247 approved for undergoing ultra high-field (7 tesla) MRI, based on institute's standard 248 249 operating procedures for MRI safety¹. These amputees were invited to take part in the 250 main study (see Figure 1- Source data 1, 2 for demographic and clinical details). This 251 involved studying movements of the phantom and intact hands, across two scanning 252 sessions (see Supplementary file 1). Two additional inclusion criteria were used for a 253 further investigation of peripheral contributions to the phantom digit topography: (d) 254 brachial plexus avulsion injury; (e) abolishment of afferent inputs and efferent outputs 255 (based on behavioural and electromyography (EMG) testing, see below for details). 256 Three amputees who met criteria a, b and c also met criterion d. Only one of these 257 candidates also met criterion e and was therefore invited to participate in the additional study (see Figure 1- Source data 1, 2). Because this individual was not 258 approved to undergo ultra high-field MRI, he was tested in a single session at 259 260 standard field MRI. All three amputees included in the study were amputated on their

¹ Ultra high-field (7T) imaging is a newly emerging technique, and as such most clinical implants (e.g. surgical clips) have not been approved for safety in these devices. Therefore, according to current local guidelines it is unsafe to scan individuals who have previously undergone major surgery (e.g. amputees) without a conclusive surgical history.

left arm due to a trauma and were right-hand dominant prior to the amputation (basedon self report).

263

264 In addition, a group of thirteen two-handed control participants with a dominant right hand were recruited to take part in the study (mean age \pm s.e.m. = 43 \pm 3; three 265 266 females). All control participants performed tasks with their left (non-dominant) hand 267 as a control for the phantom digit movements, in a single session. A subset of six age-268 and sex-matched control participants (mean age \pm s.e.m. = 50 \pm 14; all male) also completed tasks involving right (dominant) hand movements. One of these 269 270 participants was subsequently excluded from the study due to an atypical functional 271 digit layout of both hands with a reversed representation of the thumb and index 272 finger. Another participant was excluded due to excessive head motion inside the 273 scanner (greater than the functional voxel dimension (1.2mm) of absolute mean 274 displacement), leading to visible spin history artefact. The resulting subset of four 275 control participants (45 ± 13 ; all male: C1 – C4) also completed a standard field (3) 276 tesla) version of the study. Participants in this group therefore completed three 277 sessions (two ultra high-field sessions and one standard field session; see 278 Supplementary file 1). Ethical approval was granted by the NHS National Research 279 Ethics service (10/H0707/29) and written informed consent was obtained from all 280 participants prior to the study.

281

An additional standard field imaging dataset of 15 control participants and the three amputees specified above was also utilised in the current study. The purpose of this analysis was to explore inter-hemispheric (a)symmetry of the intact hand. The pertinent details relating to this dataset are highlighted in Figure 2- figure supplement 1, and will not be detailed further in the materials and methods section.

288 Behavioural testing

289 To investigate topographic mapping in the absence of peripheral input, we screened 290 participants with a brachial plexus avulsion injury. This injury involves the tearing of 291 the nerve from its attachment at the spinal cord, abolishing afferent inputs and 292 efferent outputs to the residual limb. To determine the extent of the brachial plexus 293 avulsion injury, we conducted behavioural testing to measure stump sensitivity. 294 Stimuli ranging from light touch to painful pinpricks were applied on the triceps of 295 the residual arm (stump) and a corresponding site on the intact arm. Participants were 296 blindfolded and asked to detect the stimuli, which were presented at varying inter-297 stimulus intervals in the following order: air puff, light brush, cotton swab stroke, 298 PinPrick probes (MRC systems) with weights: 8mN, 64mN and 512mN. Each 299 stimulus was presented several times. These stimuli are routinely used for qualitative 300 sensoric testing (QST), a technique used to determine sensitivity to touch and pain 301 (27).

302

To assess stump muscle activity we used surface electromyography (EMG). We targeted the biceps and triceps of the residual arm, known to show muscle activity during phantom hand movements in amputees (8,28).

306

All three amputees were able to detect each of the stimuli when presented on their intact arm. Only one of the three tested amputees reporting to suffer from brachial plexus avulsion injury was unable to detect any of the stimuli on the residual arm. This participant also did not reveal a change in the EMG channels induced by cued phantom hand movements. This amputee therefore met our requirement for

abolishment of afferent inputs and efferent outputs and was included in the standardfield (3T) study.

314

315 MRI tasks

Digit representation was probed using a visually cued active (motor) task. In an intact sensorimotor system, movement recruits a combination of peripheral receptors encoding a range of somatosensory modalities (e.g. surface and deeper mechanoreceptors; proprioceptors), as well as efference information from the motor system. Using an active task, we have previously shown high consistency of primary somatosensory (S1) digit topography across multiple scanning sessions (2).

322

323 The present study involved functional MRI (fMRI) paradigms designed to identify the 324 two principles of topographic mapping of the hand, as previously shown using 325 neuroimaging (3): digit selectivity (2) and inter-digit overlap (1). 1) To identify 326 voxels showing digit preference (digit maps) a travelling wave approach was 327 employed (also known as phase-encoding fMRI) (29). This approach has previously 328 been well-validated for sensory body mapping (2,30–34). Whereas the travelling 329 wave approach provides detailed information regarding digit preference, the winner-330 takes-all analysis (see below) makes this approach insensitive to overlapping cortical 331 digit representations. Moreover, as the paradigm involves continuous cycles of digit 332 movements with no interruption of baseline periods, regions activated by multiple 333 digits will be difficult to identify using a standard GLM (35). 2) To identify the extent 334 of overlap between representations of individual digits (inter-digit representational 335 similarity) a block design was therefore also employed. Below we describe the 336 experimental design and analysis in further detail.

338 Participants were presented with five white circles, corresponding to the five digits, 339 shown on a visual display projected into the scanner bore. To cue the participant 340 which digit should be moved, the circle corresponding to this digit changed (i.e. in 341 colour or by flashing). Participants were instructed to perform individual digit 342 movements with either their intact or phantom hand. Volitional (and not necessarily 343 painful) movement of a phantom hand elicits both central (6,8,9,36) and peripheral 344 motor signals (7,8,28,36) that are different from those found during imagined 345 movements. It was clearly stated to the amputees that they were required to perform 346 actual movements with their phantom digits (i.e. try and move the digits of the 347 missing hand), rather than motor imagery. To ensure good understanding of these 348 instructions, the amputees were asked to demonstrate to the experimenter outside the 349 scanner the extent of volitional movement carried out in each of their phantom digits, 350 by mirroring each movement onto their intact hand. For the below elbow amputee, 351 stump muscles were palpated by the experimenter outside the scanner to verify that 352 actual movements were executed during movement of the phantom digits.

353

To compare between the amputees and controls, the phantom (left) hand was matched to the non-dominant (left) hand of control participants, and the intact (right) hand was matched to the (right) dominant hand of controls. Below we describe the parameters used for the main (ultra high-field) study. Adjusted parameters used for the standard field control experiment are mentioned when relevant.

359

360 Missing hand unimanual map

The traveling wave paradigm, as detailed in Figure 1- figure supplement 1, involved unimanual digit movements in a set sequence. The task consisted of blocks of 9s in which the participant was instructed to move one digit. Each digit block was followed

by a subsequent block of a neighbouring digit, and repeated as follows: The forward sequence cycled through the movement blocks for digits: D1-D2-D3-D4-D5. The backward sequence cycled through the movement blocks in a reverse of the forward sequence (D5-D4-D3-D2-D1, Figure 1- figure supplement 1A). A sequence was repeated 8 times per run, with a duration of 6min and 25s. A forward and backward sequence was employed in separate runs. Each of the forward and backward runs was repeated twice, with a total duration of 25min and 40s.

371

372 When one of the white circles on the screen turned red, participants performed self-373 paced flexion and extension phantom (or left hand) digit movements with the digit 374 corresponding to that circle, for the duration of the block (indicated by the presence of 375 the red circle). The leftmost circle corresponded to D5 and the rightmost circle 376 corresponded to D1 of the phantom (or left) hand. Amputees were instructed to move 377 their phantom digits at a comfortable pace. Controls were instructed to perform slow 378 movements, roughly corresponding to the pace and range of movement reported by 379 the amputees (3-4s per flexion and extension movement). Participants practiced the 380 movements extensively with an experimenter outside the scanner prior to the scan.

381

382 Missing hand inter-digit overlap

To assess inter-digit representational similarity of the missing hand, a block design was also employed. This task involved individual digit movement blocks for each of the five digits, as well as a no movement (rest) condition. Each of these 6 conditions was repeated 7 times in a counterbalanced order and each block lasted 12s, with a total duration of 8min and 24s. Participants were instructed to perform self-paced individual digit movements when the circle corresponding to this digit changed in colour, as described above. All five circles remaining white, and a brief flash of the
word 'Rest' indicated the rest condition.

391

392 Bimanual digit map

393 To replicate the missing hand maps using a separate dataset and additionally test 394 whether topographic representation of the missing hand in amputees was independent 395 of the intact hand, we asked our participants to perform a bimanual travelling-wave 396 task. Participants were provided with the visual display of five white circles (as 397 described above) and were instructed to engage both hands in paired simultaneous 398 digit movements when the relevant circle turned red, resulting in reversed digit 399 movement cycles between the two hands. The leftmost circle corresponded to D5 of 400 the left hand and D1 of the right hand; the second left circle corresponded to D4 of the 401 left hand and D2 of the right hand; etc. (Figure 2A). A forward and backward 402 sequence was used in separate runs. Each of the forward and backward runs was 403 repeated twice, for a total duration of 25min and 40s.

404

405 MRI acquisition

406 Ultra high-field

Ultra high-field fMRI data was acquired using a Siemens 7 tesla (7T) Magnetom system with a 32-channel head coil. Task fMRI data was acquired using a limited field of view (FOV), with 19-22 true axial slices centred on the anatomical location of the hand knob (37) in the central sulcus bilaterally. The following acquisition parameters were used: sequence: multislice gradient echo EPI, TR: 1500ms, TE: 25ms, flip angle: 90°, GRAPPA factor: 2. The spatial resolution was 1.2mm isotropic.

To improve image registration, a whole brain and a partial field of view single volume high-saturation EPI image were acquired with the same slice positioning as the task fMRI. Anatomical T1-weighed scans, used for surface projection, were acquired using a 3 tesla (3T) system when available. For control participants 1, 5, 6, and 11 a 7T whole-brain T1-weighted image was acquired.

419

420 Standard field

421 Standard field MRI images were acquired using a 3T Verio MRI scanner (Siemens, 422 Erlangen, Germany) with a 32-channel head coil. A multiband T2*-weighted pulse 423 sequence with an acceleration factor of 6 was used (38,39). This provided the 424 opportunity to acquire data with increased spatial (2mm isotropic) and temporal (TR: 425 1300ms) resolution than available with standard EPI sequences. The following 426 acquisition parameters were used: TE: 40ms; flip angle: 66°, 72 transversal slices. A 427 high-saturation first volume of each acquired multiband run was collected for 428 registration purposes. Field maps were acquired for field unwarping.

429

430 MRI analysis

MRI analysis was implemented using tools from FSL and Connectome Workbench
software (<u>http://fsl.fmrib.ox.ac.uk/fsl; http://www.humanconnectome.org</u>) (40–42) in
combination with in house scripts developed using Matlab (version 8.4, R2014b).
Cortical surface reconstructions, used for visualisation of the fMRI results, were
produced using FreeSurfer (<u>http://freesurfer.net</u>) (43,44).

436

437 MRI preprocessing

Common pre-processing steps for fMRI data were applied to each individual run in
native (three dimensional, 3D) space, using FSL's Expert Analysis Tool FEAT

440 (v6.00; fsl.fmrib.ox.ac.uk/fsl/fslwiki). The following steps were included: Motion 441 correction using MCFLIRT (45), brain extraction using automated brain extraction 442 tool BET (46), spatial smoothing using a 1.5mm FWHM (full width at half 443 maximum) Gaussian kernel for the ultra high-field scans and 2mm FWHM for the 444 standard field scans, and high pass temporal filtering with a cut-off of 100s. All 445 BOLD EPI data were assessed for excessive motion using motion estimate outputs 446 from MCFLIRT: ultra high-field functional data from one participant exhibited 447 greater than 1.2mm (functional voxel size) of absolute mean displacement and was 448 excluded from all further analysis.

449

450 Image registration

451 Ultra high-field

Image registration was accomplished using FLIRT (FMRIB's linear image registration tool) (45,47). To ensure good registration of the partial-FOV functional data to the anatomical image, image registration was carried out in individual, visually inspected, steps.

456

457 First, the task fMRI data from each run was registered to a partial-FOV single volume 458 high-saturation EPI image, acquired at the first session. This partial-FOV image was 459 registered to a whole brain FOV single volume high-saturation EPI image. The 460 whole-brain image was then registered to the T1-weighted image; initially using the 461 mutual information cost function (6 degrees of freedom), and then optimised using 462 boundary-based-registration (48) (6 degrees of freedom, FMRIB's Automated Segmentation Tool (FAST) for white matter segmentation, no search). For two 463 464 participants (C4 and C11), manual alignment was used to register the single volume 465 partial-FOV high-saturation EPI image to the structural MEMPRAGE white matter and pial surfaces using blink comparison as implemented in Freeview.

467

468 <u>Standard field</u>

Functional and anatomical images were aligned using a similar pipeline as described above. Each individual run was first co-registered to the high-saturation first volume of the acquired multiband run, and then to the T1-weighted image. In addition, individual field maps and field map based unwarping of the multiband images were included to reduce spatial distortions and additionally improve co-registration.

474

475 Travelling wave analysis

476 The travelling wave runs were analysed for each individual participant in native (3D) 477 space, using a cross-correlation approach previously applied in retinotopy (49). The 478 approach is based on continuous presentation of stimuli in a set cycle that are 479 expected to result in neighbouring cortical representations (e.g. rotating wedge in the 480 visual field; sequential digit stimulation in a set cycle). It is designed to capture voxels 481 showing increased response to one condition, above and beyond all other conditions 482 (in our case, preference for a specific digit). Beyond preference, this technique also 483 provides a powerful tool for capturing the smooth progression of adjacent 484 representations that are typical for topographic maps. It is therefore considered a 485 preferable technique for capturing topographic representations (29). With respect to 486 somatotopy, the travelling wave approach (or the homologous phase-encoding 487 approach) has previously been validated against blocked and event-related fMRI 488 paradigms (2,31,32,35).

489

We have recently utilized the traveling wave approach, in combination with ultra high-field fMRI to demonstrate highly reproducible maps of individual digits in S1

492 (2). In the current study, we closely followed these previously validated experimental 493 procedures, as described in Figure 1- figure supplement 1. Participants moved 494 individual digits in a set cycle (see the fMRI tasks section above). A reference model 495 was generated using a gamma-HRF convolved boxcar function, while taking into 496 account the hemodynamic delay. The model was constructed using a 9s 'on' (the 497 duration of a single digit movement) and 36s 'off' (the period of movement of all other digits), accounting for a single 45s cycle (Figure 1- figure supplement 1B). This 498 499 cycle was repeated 8 times to reflect the full run duration. The reference model was 500 systematically shifted in time to model activity throughout the full movement cycle. 501 Because the runs were acquired using a TR of 1.5s, the model was shifted 30 times by 502 1 lag to account for the full 45s cycle of movement.

503

For each individual voxel, each of the 30 reference models was correlated with the preprocessed BOLD signal time course to estimate cross-correlation values. The resulting r-values were standardised using the Fisher's r-to-z transformation. By plotting these standardised r-values as a function of the lag, tuning curves can be created, and the optimal fit for each voxel can be inferred (Figure 1- figure supplement 1C).

510

The travelling wave approach uses a set cycle, and as such could be susceptible to order-related biases resulting from the sluggish hemodynamic response. For this reason, the order of the cycle was varied between forward (D1-D2-D3-D4-D5) and backward (D5-D4-D3-D2-D1) in different runs (Figure 1- figure supplement 1A). To average across the four runs, lags were initially assigned to each of the five digits (six lags per digit). Within each voxel, the r-values corresponding to each digit were averaged, resulting in five r-values, corresponding to each of the digits for a given

run. The digit-specific r-values were then averaged across the forward and backward runs on a voxel-by-voxel basis. A winner-take-all approach was applied to produce maps in which each voxel was assigned exclusively to one individual digit, providing us with digit specificity.

522

To visualise the gradient of progression across digits, lag-specific maps were also produced. For each backward run, the resulting r-values from the cross-correlation analysis were standardised and time-reversed. Forward and backward runs were averaged, on a lag-by-lag basis. To construct a gradient map, a winner-take-all approach was used across all 30 lags, in which each voxel was assigned exclusively to one individual lag.

529

530 Cortical surface projections were constructed from T1-weighted images. The digit and 531 gradient maps were registered to structural space and projected to two-dimensional 532 surface space using a cortical ribbon mapping method. To account for multiple 533 comparisons, thresholding was implemented on the surface using the false discovery 534 rate (FDR) (50), calculated for each digit individually. This approach doesn't take into 535 consideration the neighbourhood relationships of the voxels, and therefore doesn't 536 force cluster patterns. The thresholded maps were set at a false detection criterion of q 537 < 0.05 based on the native (3D) values. The FDR thresholded digit-specific clusters 538 were overlaid into a single hand map. Within the resulting five-digit map, we used a 539 30-lag colour code to visualise the gradient of progression across digits (Figure 1-540 figure supplement 1D).

541

For the standard field digit maps, the same procedures were used, with the following exceptions. Due to the difference in temporal resolution (TR = 1.3s), each digit block

lasted 9.1s, resulting in slightly longer movement cycles (45.5s). 35 HRF reference models were constructed (compatible with 35 lags), and a more conservative FDR criterion (q < 0.01) was selected, due to a reduced signal/noise ratio.

547

548 Split-half consistency

To quantify consistency in digit preference, as identified using the travelling wave task, we split the data used to construct the digit preference maps, and compared the spatial correspondence between digit selective clusters. Split-half consistency of the digit-specific clusters was calculated using the Dice coefficient (2,51). The Dice coefficient varies from 0 (no spatial correspondence between digit representations) to 1 (perfect spatial correspondence between digit representations). Where A and B are the areas of two digit representations, the Dice Coefficient is expressed as:

$$\frac{2 \times |A \cap B|}{|A| + |B|}$$

556 Each digit's winner-take-all map was minimally thresholded on the cortical surface (Z557 > 2). Spatial correspondence was calculated in S1 (as defined by FreeSurfer) between 558 each possible digit pair across the split-halves of the unimanual digit maps (Figure 1, 559 Figure 1- figure supplement 3). The first forward and backward runs were combined 560 to provide the first digit-specific clusters, and the second forward and backward runs 561 were combined to form the second digit-specific clusters. By doing so, we established 562 a split-half consistency measure for the travelling wave maps of the phantom (or left) 563 hand and intact (or right) hand separately. One control participant was discarded from 564 the subsequent analysis, as his mean spatial correspondence values (across all 565 possible digit combinations) fell outside two standard deviations from the control 566 group mean.

A benchmark for digit selectivity is greater split-half spatial correspondence across 568 569 "same" versus "different" digits. Mean "same"-digit values (across all five "same"-570 digit pairs) were greater than mean "different"-digit values (across all twenty 571 "different"-digit pairs) in each of the participants. The "same"-digit spatial 572 correspondence values in the two amputees (0.30 for the above and below elbow 573 amputees, average across all "same"-digit pairings) were smaller than those observed 574 in the control participants (0.57 on average across all control participants; Figure 1D), 575 suggesting lower consistency of digit selectivity in amputees. To further assess digit 576 selectivity in the amputees, the "different"-digit distribution of Dice spatial 577 correspondence values was estimated using a bootstrapping procedure (5,000 578 iterations) for each individual participant. "Same"-digit values were averaged for each 579 participant, and compared against the 95% confidence interval (CI) on a subject-by-580 subject basis. For all participants (amputees and controls), the "same"-digit value fell 581 well outside the range of spatial correspondence seen between "different" digits. This 582 indicates that the consistency shown for digit-selective clusters was greater than 583 expected by chance in all participants. Next, the mean "same"-digit values in the two 584 amputees were compared to the "different"-digit spatial correspondence distribution 585 of each of the controls. "Same"-digit spatial correspondence values in both amputees 586 fell outside the "different"-digit spatial correspondence distribution of each of the 587 controls (CI: 0.03-0.08 on average). This was also confirmed by comparing the mean 588 spatial correspondence between "same"-digit values for each of the amputees and 589 "different"-digit values of the controls (two-tailed Crawford and Howell t-test (52); 590 t(9) = 14.24, p < 0.001 and t(9) = 14.31, p < 0.001 for above and below elbow 591 amputees respectively). This indicated that although reduced, the digit maps of the 592 amputees were consistent, even relative to controls.

594 In addition we aimed to compare between the unimanual and bimanual digit maps 595 within the missing hand hemisphere (Figure 2E), providing us with an estimate of 596 consistency when accounting for potential contributions of the intact hand. The 597 "different"-digit distribution of Dice spatial correspondence values between the 598 unimanual and bimanual maps was estimated using a bootstrapping procedure (5,000 599 iterations) for each individual participant. "Same"-digit spatial correspondence values 600 (mean across all "same" digits) in both amputees (0.49 and 0.40 for above and below 601 elbow amputees respectively) fell outside the "different"-digit spatial correspondence 602 distribution. This was observed both compared to their own "different"-digit 603 distribution (CI: 0.03-10 for both above and below elbow amputees) and each of the 604 controls' "different"-digit distributions (CI: 0.03-0.13 on average). This was also 605 confirmed by comparing the mean spatial correspondence between "same"-digit 606 values for each of the amputees and "different"-digit values of the controls (two-tailed 607 Crawford and Howell t-test (52); t(3) = 29.14, p < 0.001 and t(3) = 22.57, p < 0.001608 for above and below elbow amputees respectively).

609

610 Inter-digit representational similarity analysis

611 We used an fMRI pattern component approach to identify the extent to which 612 representations of the different digits overlapped with each other, while considering 613 multivoxel patterns underlying the S1 digit maps. Voxels underlying the digit 614 preference map surrounding the anatomical hand knob were combined to form a 615 region of interest (ROI) in native space. The multivoxel pattern-component model is 616 described in detail in (12) and implemented in http://www.icn.ucl.ac.uk/ 617 motorcontrol/imaging/multivariate analysis.html. This method allows deriving 618 unbiased estimates of the true correlations between the underlying activation patterns 619 for each condition.

621 In brief, a generative model is created that assumes the observed patterns are 62.2 associated with a set of underlying pattern components that relate to the different 623 experimental conditions or noise. Activity patterns are decomposed within the ROI 624 into a common (noise) component that is shared between all trials and one specific 625 component for each of the five digits. By estimating the variability (or strength) of the similarity between the latter components, the inter-digit representational overlap in 626 627 the ROI is revealed. Figure 3 and Figure 3- figure supplement 1 (Figure 4D for 628 standard field) show the resulting matrices, demonstrating the level of 629 representational similarity (or overlap) between each digit pair. The topographic interdigit pattern is characterised by increased overlap between neighbouring digits. 630 631 Furthermore, digits showing increased synergies in daily life (e.g. digits 3 and 4) tend 632 to show greater representational overlap than digits that are more independent during 633 hand function (e.g. digits 1 and 2) (1). The resulting inter-digit representational overlap pattern therefore provides a detailed "fingerprint" of hand representation. 634 635

We initially assessed overall (mean) overlap within the matrix of each participant, by 636 averaging all unique inter-digit similarity r-values (across the 10 cells of the matrix, 637 while excluding the diagonal). On average, the amputees showed higher inter-digit 638 639 overlap (0.55 and 0.54 for above and below elbow amputees, respectively) than the 640 control group (CI: 0.24-0.41). To investigate whether the missing hand inter-digit 641 overlap pattern was comparable to normal hand representation, as found in controls, 642 we generated the distribution (and 95% confidence intervals) of correlation across the 643 control population. For this purpose, inter-digit overlap patterns (i.e. the cells in the 644 similarity matrices) were correlated across pairs of controls, using a bootstrapping 645 approach (10,000 iterations) and a Spearman test. One control participant was 646 discarded from the subsequent analysis, as his mean Spearman correlation value (with 647 all other controls) fell outside two standard deviations from the control group mean. 648 (Note that the exclusion of the outlier participant did not affect the outcome of this 649 analysis.) Next, the same procedure was carried out between each amputee and each 650 of the controls' inter-digit overlap patterns. By plotting the average amputee-control 651 Spearman rho values and confidence intervals against the confidence interval of the 652 controls-to-controls correlation distribution, we were able to show that the amputees' 653 inter-digit representational overlap pattern fell within the normal range of the 654 controls. There was no significant difference between the amputees-to-controls 655 average Spearman correlation and the control-to-control correlations also when using 656 more conventional statistics (two-tailed Crawford and Howell t-test (52); t(9) = -1.10, 657 p = 0.30 and t(9) = 0.31, p = 0.76 for above and below elbow amputees respectively).

658

659 Because the pattern component approach implemented here is minimally affected by 660 differences in noise amplitude resulting from different scanners and acquisition 661 parameters (12), we repeated the same analysis, using the brachial plexus amputees' 662 data and the control participants' data (ultra high-field MRI). The average correlation 663 of this amputee's inter-digit overlap pattern with each of the controls' inter-digit 664 overlap patterns (0.76), fell within the controls-to-controls correlation range. There 665 was no significant difference between the amputee-to-controls average Spearman 666 correlation and the control-to-control correlations (two-tailed Crawford and Howell t-667 test (52); t(9) = 0.14, p = 0.89)

668

669 We also calculated a Bayesian t-test to compare between the controls-to-controls

670 distribution and each of the amputee-to-controls distributions using JASP (53-55). 671 The Cauchy prior width was set at 0.707 (default). Based on the well accepted 672 criterion of Bayes factor smaller than 1/3 (56,57) our findings support the null 673 hypothesis (amputees' inter-digit similarity pattern is not different from controls) for 674 the below elbow and brachial plexus amputee (Bayes factor = 0.23 and 0.28respectively). For the above elbow amputee the evidence was inconclusive (Bayes 675 676 factor = 2.94). Note that this criterion for Bayesian factor is considered as moderate 677 (though positive) evidence (58).

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844 Figure titles and legends

845 Figure 1. Missing hand maps revealed in amputees during phantom digits 846 movement. Black arrow indicate preference for digits 1-5: thumb (red); index 847 (yellow); middle (green); ring (blue) and little finger (purple) in two-handed controls 848 (A, C) and amputees (B). Participants performed single digit flexion and extension 849 movements with their non-dominant (controls) or phantom hand (amputees) in a 850 travelling wave paradigm. Qualitatively similar digit topographies were found in each 851 amputee and controls. White arrows indicate the central sulcus. A = anterior; P =852 posterior. Multiple comparisons were adjusted using false discovery rate (FDR). (D) 853 Maps' intra-individual split-half consistency, assessed using the Dice overlap 854 coefficient. On average, "same"-digit selective clusters (dark bars) showed greater 855 consistency than "different"-digit clusters (light bars) in amputees and controls. 856 Amputees showed lower split-half consistency for "same"-digit clusters (averaged 857 across digits) compared to controls (95% confidence intervals (CI) = 0.43-0.68, as 858 assessed using a bootstrap approach). However, amputees' "same"-digit clusters were 859 more consistent than "different"-digit clusters in controls (i.e. fell outside the CI of 860 "different"-digit clusters split-half consistency in controls), indicating that although 861 reduced, the digit maps of the amputees were consistent.

863 Figure 2. Missing hand maps are replicated during dissociated bimanual hand 864 movements. (A) In the bimanual task, individuals performed paired digit movements 865 with both hands, resulting in reversed digit cycles between the hands. The colour code 866 indicates the cycle lag as shown in (A). Symmetrical colour gradients across the two 867 hemispheres would reveal the missing hand map as dependent on intact hand 868 movements (B: case 1). Colour gradient reversal across hemispheres indicates 869 dissociated hand representations (B: case 2), as was seen in both amputees and 870 controls (C, D respectively, left two panels). Maps contralateral to the missing hand 871 (left hand in controls) resembled the unimanual tasks' maps (Figure 1), both 872 qualitatively (C, D, right panel) and quantitatively, using the Dice overlap coefficient 873 (E). The digit maps were adjusted for multiple comparisons using false discovery rate 874 (FDR). Other annotations are as in Figure 1.

875 Figure 3. Amputees show characteristic inter-digit overlap patterns during 876 phantom digit movements. Inter-digit representational similarity of multivoxel 877 patterns underlying the maps shown in Figure 1, derived from a block-design 878 paradigm in amputees (A) and controls (B, D). Similarity (or overlap) is decreased 879 between non-neighbouring digits and tends to increase between digits 3-5, as shown 880 in the controls' averaged matrix. (C) Positive distribution of inter-subject correlations, 881 between controls' inter-digit overlap patterns. The grey area indicates the 95% 882 confidence interval (CI) for controls. Pink and blue lines indicate average amputee-to-883 controls correlations for above and below elbow amputees respectively. Pink and blue 884 shaded areas correspond to the 95% CIs. Both amputee-to-controls correlation 885 averages values fell within the normal controls-to-controls correlation range, 886 suggesting that the amputees exhibited a characteristic pattern of inter-digit overlap.

887 Figure 4. Topography is preserved despite the absence of peripheral input. To 888 rule out the potential contribution of peripheral inputs from the injured nerve to 889 preservation of the phantom hand map, we tested an upper-limb ampute suffering 890 from brachial plexus avulsion injury with vivid kinaesthetic phantom digit sensations. 891 This injury involves the tearing of the nerve from its attachment at the spinal cord, 892 abolishing afferent inputs and efferent outputs to the residual limb. 3 tesla MRI was 893 used here due to safety restrictions, meaning that standard field digit preference maps 894 were acquired. The bimanual travelling wave task (A, Figure 2) elicited maps in the 895 controls with reversed colour gradients (B - left 2 panels). In comparison to the ultra 896 high-field left hand maps (right panel), the standard field left hand maps were cruder 897 (e.g. less digit specificity, B - middle panel). (C) The brachial plexus amputee showed 898 two maps with reversed colour gradients, comparable to those found in controls. The 899 map in the missing hand hemisphere showed preference for digits in primary 900 somatosensory cortex. (D) Inter-digit representational similarity was assessed for 901 multivoxel patterns underlying the missing hand map. The correlation value of the 902 brachial plexus amputee's inter-digit overlap pattern with the controls exemplified in 903 Figure 3 fell within the normal controls-to-controls correlation range. Together, these 904 results suggest that preserved representation of the missing hand existed 905 independently of peripheral inputs. Other annotations are as in Figure 1.

906 Figures supplements titles and Figures supplements legends

907 Figure 1– figure supplement 1. Travelling wave task and analysis. A travelling 908 wave paradigm, involving digit movements in a set sequence, was used to capture 909 digit preference and the smooth progression of adjacent digit representations in 910 primary somatosensory cortex. (A) The travelling wave task involved 8 repeated 911 cycles of continuous individual digit movements. Each 45s cycle consisted of five 9s 912 blocks dedicated to each digit (as exemplified by the coloured squares), with each run 913 cycling through either D1-D2-D3-D4-D5 (forward, top) or D5-D4-D3-D2-D1 914 (backward, bottom). Each of the forward and backward runs was repeated twice. The 915 numbers 1-5 and colours correspond to the five digits, as visualised in the right side of 916 the panel. (B) Preprocessed BOLD time courses from individual voxels (example 917 time course of one voxel is shown in red) were cross-correlated against a reference 918 model. The model corresponded to a single-digit movement (9s 'on', 36s 'off'- the 919 period of movement of all other digits), and was systematically shifted in time to 920 model activity throughout a full cycle (30 shifts, corresponding to the 30 lags of the 921 45s cycle). The figure shows three different model lags (1, 12 and 24), resulting in 922 varying cross-correlation outcomes. Lag 24 was the optimal model lag for this 923 preprocessed BOLD time course. (C) The standardised r-values (Fisher's r-to-z, y-924 axis) resulting from the cross-correlation were plotted as a function of lag (x-axis) for 925 each individual voxel in each run. In the left panel, cross-correlation values from the 926 example voxel shown in B are plotted for each of the four runs. The optimal fit for the 927 voxel is demonstrated by the peak cross-correlation (digit 1, in the left panel; digit 2 928 in the right panel, taken from a separate voxel). Lags associated with the same digits 929 were averaged, first within and then across runs. A winner-take-all approach was used 930 to determine digit preference, based on the highest average r-value, resulting in five

931 separate digit maps. False discovery rate (FDR) was applied for each of these single 932 digit maps to adjust the threshold based on the criterion q (FDR) ≤ 0.05 . (D) Each of 933 the five digits was assigned a colour code, as demonstrated on the left. The five digit 934 maps were projected to the cortical surface, to display digit preference maps. Note 935 that no masking was applied when presenting the resulting maps. Within the resulting 936 five-digit map a gradient of progression across digits was visualised, based on the 937 voxel-wise r-values for each of the 30 lags. The resulting map was located in the central sulcus and postcentral gyrus, corresponding to the primary somatosensory 938 939 cortex, around the anatomical hand knob.

940 Figure 1- figure supplement 2. Areas activated by phantom hand movement. 941 Contrast between phantom (amputees; A) or left (controls; B) hand movements and 942 baseline (rest) in the block design, projected on cortical surfaces of the contralateral 943 hemisphere. Analysis was performed using a standard 2-level general linear model. 944 Amputees and controls showed activation in primary somatosensory and motor cortex 945 (S1 and M1, respectively; left panel), as well as in the supplementary motor area 946 (SMA, right panel). Note that more inferior areas (e.g. secondary somatosensory 947 cortex) were not covered by the partial field of view utilised for the ultra high-field 948 acquisition. White arrows indicate the central sulcus. A = anterior; P = posterior. 949 Displayed activations were thresholded at Z > 2. These results show that phantom 950 hand movements activate a network of brain areas normally associated with hand 951 movement.

952 Figure 1- figure supplement 3. Intact hand maps in amputees. In order to 953 visualise standard topography in the amputees' somatosensory cortex, topographic 954 mapping was also carried out for the intact hand. For this purpose, task demands were 955 adjusted to optimally activate the somatosensory cortex. Participants were provided 956 with tactile feedback via a MRI-compatible button-box (manufactured in house), 957 placed on the participant's torso for the duration of the scan. Participants pressed the 958 button box with the relevant digit using a travelling wave paradigm (Figure 1- figure 959 supplement 1). Since this task did not involve any difficulty for the amputees as it 960 only consisted of moving digits of the intact hand, all participants were paced to 961 perform button press movements with individual digits at a set (1Hz) frequency, as 962 indicated by the flashing of the corresponding circle at the specified rate. A forward 963 and backward sequence was employed in separate runs. Each of the forward and 964 backward runs was repeated twice, for a total duration of 25min and 40s. All other 965 procedures and analysis were as described in the main analysis for the missing hand 966 digit maps (Figure 1). The black arrows indicate preferences for digits 1-5: thumb 967 (red); index (yellow); middle (green); ring (blue) and little finger (purple) for the 968 intact hand in amputees (A) and two handed controls (B). White arrows indicate the 969 central sulcus. A = anterior; P = posterior. Multiple comparisons were adjusted using 970 false discovery rate (FDR). (C) Intra-individual split-half consistency was assessed 971 using the Dice overlap coefficient for the digit maps. "Same"-digit selective clusters 972 (dark) showed significantly greater consistency than "different"-digit clusters (light) 973 in amputees and controls. Error bars indicate 95% confidence intervals. Note that the 974 organisation, extent and consistency of the digit maps qualitatively resembled the left 975 (missing) hand maps (obtained using self-paced digit flexion and extension) found in 976 both controls and amputees (Figure 1).

977 Figure 2- figure supplement 1. Over-representation of the intact hand in the 978 missing hand territory. To investigate whether the observed missing hand maps 979 (Figure 1, Figure 4) were preserved despite reorganisation of displaced inputs (as 980 reported previously (9)), we quantified intact hand representation in the territory of 981 the missing hand in the amputees participating in the current study. For this purpose, 982 we employed an independent dataset, acquired using standard field neuroimaging 983 (acquisition parameters were identical to those described in the materials and methods 984 section, with the exception of the number of acquisition volumes). (A) Participants 985 were cued to perform movements with their intact hand (simultaneous flexion and 986 extension of all five digits), or dominant hand in controls (n = 15), as part of a block-987 design protocol, used to identify body-part representations (see (9) for details of a 988 similar protocol). This movement condition was contrasted against a baseline (rest) 989 period and standardised (z) beta values during intact/dominant hand movements were 990 extracted from the peak voxel within a postcentral gyrus (PCG) mask in the 991 contralateral hemisphere. To identify the estimated position of the missing hand 992 territory, this peak voxel was mirror flipped on the axial plane, and the standardised 993 beta value for intact hand activity was extracted from this ROI in the ipsilateral 994 hemisphere. (B) To assess asymmetry in intact hand activity across the two 995 hemisphere, the following inter-hemispheric asymmetry index was calculated for each 996 of the three amputees and 15 controls individually: (contralateral hemisphere -997 ipsilateral hemisphere) / (contralateral hemisphere + ipsilateral hemisphere). Higher 998 values of this index indicate that the activity pattern for the intact hand was greater in 999 the contralateral, compared to the ipsilateral sensorimotor hand area, as found in the 1000 control participants. Lower values in this index represent less asymmetry, resulting 1001 from (relatively) higher activity in the ipsilateral hand area during intact hand 1002 movements. Error bars indicate 95% confidence intervals. The above elbow and

brachial plexus amputees showed significantly lower asymmetry than controls (one-1003 tailed Crawford and Howell t-test (52); t(14) = -1.84, p = 0.04; and t(14) = -1.96, p = -1.961004 1005 0.04 respectively). The below elbow amputee showed a high asymmetry value, similar to controls (one-tailed Crawford and Howell t-test (52); t(14) = 1.26, p = 1006 1007 0.11). This indicates that two of the tested amputees show over-representation of their 1008 intact hand in the missing hand territory, as previously reported (9). These results 1009 suggest that the missing hand maps can be preserved while reorganisation of 1010 displaced inputs from the intact hand has taken place.

1011 Figure 3- figure supplement 1. Intact hand inter-digit overlap in amputees. In 1012 order to visualise standard topography in the amputees' somatosensory cortex, inter-1013 digit representational similarity was assessed for multivoxel patterns underlying the 1014 right (intact) hand maps shown in Figure 1- figure supplement 3 in amputees (intact 1015 hand, A) and controls (dominant hand, B). To estimate inter-digit representational 1016 similarity for the intact hand, the same blocked design as described in the materials 1017 and methods section *missing hand inter-digit overlap* was used. Tactile feedback 1018 (from the button box) was incorporated into the task to optimally activate the 1019 somatosensory cortex. Participants were instructed to perform single digit button 1020 presses (using an MRI-compatible button-box manufactured in house) with their right 1021 intact (amputees) or right dominant (controls) hand at a 1Hz frequency. To cue the 1022 participant which digit should be moved, the circle corresponding to this digit flashed 1023 at the specified 1Hz rate. Digits 1-5: thumb; index; middle; ring and little finger.

1024 Supplementary files title and legend

Supplementary file 1. Runs acquired for each participant. x = acquired; - = not
acquired.

1027 Source data files titles and legends

1028 Figure 1– Source data 1. Amputee demographic and clinical details. To measure 1029 phantom sensations, as well as other demographic and clinical details of potential 1030 relevance to the studied missing hand representation, amputees completed a range of 1031 questionnaires (e.g. amputation details, prosthesis usage etc.). Amputees rated 1032 intensities of phantom/stump pain and non-painful phantom sensations (vividness), using a 0 - 100 scale, as follows: (i) intensity of worst pain/vivid sensation 1033 1034 experienced during the last week (or in a typical week involving such sensations); (ii) 1035 intensity of phantom pain on average over the last week (or in a typical week if last week was atypical); and (iii) current intensity/vividness of phantom pain and 1036 1037 sensations, during the scanning day. Phantom limb pain magnitude was calculated by 1038 dividing worst pain intensity by pain frequency (1- all the time; 2- daily; 3- weekly). 1039 An analogous measure was obtained for vividness of non-painful phantom sensations. 1040 This approach represented the chronic aspect of the phantom pain/sensation as it 1041 combines frequency and intensity (6). We also asked amputees whether they 1042 experienced telescoping, a common phenomenon where the perceived length of the 1043 phantom limb is changed (5). Amp. = amputation; Amp. level = amputation level 1044 measured in percentages: (residual arm length/intact arm length) x 100. Intact arm 1045 length is measured from shoulder to fingertips, where 41% is equivalent to the level 1046 of the elbow and 75% is equivalent to the level of the wrist; PLS = phantom limb 1047 sensations; mag. = magnitude; scan = score of sensation phantom limb vividness/pain 1048 intensity on scanning day (scale 0 - 100); PLP = phantom limb pain; ave. = score of 1049 average phantom pain in a typical week (scale 0 - 100); prosthetics usage = prosthetic 1050 limb usage (frequency): 0- never, 1- rarely, 2- occasionally, 3- daily, 4- more than 4 1051 hours a day, 5- more than 8 hours a day; $Y_{.} = yes$; $N_{.} = no$.

1052 Figure 1- Source data 2. Phantom digits movement vividness, difficulty and 1053 quality. Amputees were asked to rate how vivid the phantom movement felt (PDS = 1054 phantom digit sensation; scale 0 - 100; ranging from unable to feel movement to 1055 movement is felt as vividly as the sensation of movement elicited by the intact hand 1056 digits). In addition, amputees were asked to rate how difficult it was to perform each 1057 phantom digit movement (diff. = difficulty; scale 0 - 100; ranging from easy to 1058 impossible). Finally, participants were asked to rate the quality and extent of 1059 movement afforded by each phantom digit. Amputees' answers were standardised as 1060 follows: 1 - full range of movements (i.e. equivalent to movements with the 1061 homologous digit on the intact hand). 2 – near to full range of movement (i.e. similar, 1062 though reduced, to movement of the intact hand). 3 - noticeably less range of 1063 movement compared to the intact hand (i.e. partial finger flexion and extension 1064 movement).

A. Example controls











A. Travelling wave task digit order

C. Cross-correlation r-values







A. Approach

1. Movement > baseline 2. Left PCG mask





3. Peak voxel



4. Swap peak voxel



B. Plasticity results



