Reorganization in cerebral and cerebellar cortices is not restricted by proximity between body-part representations

Abbreviated title: Somatotopy does not limit reorganization

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Abstract

Different terminals of the sensorimotor network show varied somatotopic arrangements of body-part representations. Since somatotopy is best characterized in the primary somatosensory (S1) and motor (M1) cortices, these terminals have been the main target for research on somatotopic alterations following loss of sensory input (e.g. arm amputation). Such reorganization is generally considered to be driven by the spatial layout of the underlying somatotopy, such that neighboring body-part representations tend to occupy the deprived brain region. For example, following arm deafferentation in non-human primates, the S1 deprived hand area becomes responsive to inputs from the chin, which has a native representation that cortically neighbors that of the (now deprived) hand region. Here we challenge the assumption of proximity-based reorganization, by comparing patterns of reorganization in people born without one hand (hereafter, one-handers, n=26, 15 female) in the cerebellum and the primary sensorimotor cortex. We first report that in the cerebellum of one-handers, the deprived hand region represents multiple body-parts, despite varying distances between the native representations of these body-parts and the deprived hand region. We further replicate our previous finding, showing a similar pattern of reorganization in the deprived hand region of the cerebral cortex in one-handers. Since the cerebellar and cerebral cortices contain different somatotopic layouts, the parallel reorganization observed in these two terminals demonstrates that the mere spatial layout of body-part representations may not exclusively dictate reorganization in the sensorimotor network. Instead, we discuss alternative drivers of reorganization, and particularly, the possible role of adaptive behavior and sub-cortical plasticity.

Significance Statement

When a hand is missing, the brain region that typically processes information from that hand may instead process information from other body-parts, a phenomenon termed reorganization. It is commonly thought that only body-parts whose information is processed in regions adjacent to the hand region, could take up the resources of this now deprived region. Here we demonstrate that information from the same body-parts is processed in the hand regions of both the cerebral cortex and cerebellum. The native brain regions of these body-parts have varied relative distances from the hand region of the cerebellum compared to the cerebral cortex, and do not necessarily neighbor these hand regions. We therefore propose that proximity between brain regions cannot fully explain brain reorganization.

Introduction

The “sensorimotor homunculus” in primary somatosensory and motor cortices is thought to reflect the lateralized and segregated neural activation patterns associated with sensations from- and movements of-distinct body parts (Penfield and Boldrey, 1937; Penfield and Rasmussen, 1950; Catani, 2017; Roux et al., 2018). Following input and output loss, e.g. following arm amputation, the sensorimotor homunculus undergoes reorganization, such that the region previously responsible for representing the hand becomes responsive to inputs from other body-parts (Flor et al., 1995; Makin et al., 2013b; Chand and Jain, 2015; Raffin et al., 2016). The organizing principles underlying such architectural change are thought to derive from the spatial layout of the homunculus: neighboring representations, which share greater cortical overlap (Merzenich et al., 1984; Pons et al., 1991; Merzenich and Jenkins, 1993; Florence et al., 1998) and/or receive stronger inhibition from now absent inputs (Faggin et al., 1997; Margolis et al., 2012) are more likely to “invade” the deprived cortical region. Subsequently, findings showing increased expression of facial inputs in the missing-hand region following arm amputation have been taken as evidence for the role of somatotopic layout in scaffolding reorganization (Jain et al., 2008; Kaas et al., 2008; MacIver et al., 2008; Foell et al., 2014; Andoh et al., 2017).

We and others have recently challenged this view, by demonstrating that reorganization may also occur between distal body-part representations. For example, the intact hand of amputees shows increased representation in the region of the missing hand in the primary sensorimotor cortex (hereafter deprived cerebral hand region) (Bogdanov et al., 2012; Makin et al., 2013b; Philip and Frey, 2014). Similarly, individuals with bilateral hand-loss show increased feet representation in their deprived cerebral hand region (Stoeckel et al., 2009; Yu et al., 2014; Striem-Amit et al., 2018). This feet-to-hand reorganization occurs despite the inherent cortical distance between feet and hand representations. Finally, in individuals born without one hand (hereafter, one-handers) multiple body parts (residual arm, lips and feet , but not the intact hand) show increased representation in the deprived cerebral hand region (Makin et al., 2013b; Hahamy et al., 2017). As the representations of the feet and lips are not the immediate neighbors of the

hand region, we have suggested that proximity between body-part representations is not a prerequisite for reorganization, though this interpretation is still debated (Striem-Amit et al., 2018). The relationship between functional somatotopy and reorganization therefore remains unclear.

Here we address this question by examining reorganization in one-handers, at multiple sensorimotor terminals with varying somatotopic arrangements. Somatotopic organization has been previously identified throughout the sensorimotor network, such as in the cerebellum (Manni and Petrosini, 2004), brainstem (Jang et al., 2011), basal ganglia and motor thalamus (Nambu, 2011). Since somatotopic representations in the human cerebellum can be reliably identified using fMRI (Grodd et al., 2001; Buckner et al., 2011; Wiestler et al., 2011; Haak et al., 2017), we use the cerebellum as a model for examining the role of somatotopic layout in shaping sensorimotor brain reorganization in one-handers. The organization of body-part representations in the cerebellum differs from that of the primary sensorimotor homunculus (Manni and Petrosini, 2004; Mottolese et al., 2013; Mottolese et al., 2015), allowing us to study whether differences in the spatial layout of representations would lead to different patterns of reorganization between the cerebral and cerebellar deprived hand regions. We find that the same body-part representations (residual arm, lips and feet) are remapped to the deprived hand region of the cerebellar and cerebral cortices, thus documenting similar large-scale reorganization across the hierarchy of the sensorimotor network. We therefore propose that reorganization is not determined by the physical proximity between the native and remapped representations, and discuss alternative factors that may underlie this extensive brain plasticity.

Materials and Methods

Participants

Recruitment was carried out in accordance with NHS national research ethics service approval (10/H0707/29, dataset1) and with Oxford University's Medical Sciences inter-divisional research ethics

committee (MS-IDREC-C2- 2015-012, dataset2). Informed consent and consent to publish was obtained in accordance with ethical standards set out by the Declaration of Helsinki.

The first dataset (hereafter, Dataset1) contains the same population recruited for a previous study, using the same scanning procedures and exclusion criteria as described before (Hahamy et al., 2015b). 25 healthy controls (15 females, age = 41.12±12.86, 8 left hand dominant) and 14 individuals with a congenital unilateral upper limb deficit (one-handers, 9 females, age = 36.64±12.02, 4 with absent right hand) were recruited for the study. The proportion of one-handers with a missing right hand (n=4) and controls who are left-hand dominant (n=8) were similar ($\chi^2_{(1)}$=0.18, p=0.67).

The second dataset (hereafter, Dataset2) was acquired as part of a larger study (the full study protocol is currently under preparation and will be made available via Open Science Framework). These data include the scanning of 12 healthy controls (5 females, age = 45.33±14.85, 5 left hand dominant), and 14 one-handers (7 females, age = 45.25±11.38, 6 with absent right hand) (see Table 1 for demographic details). The proportions of one-handers with a missing right hand (n=6) and controls who are left-hand dominant (n=5) were similar ($\chi^2_{(1)}$=0.05, p=0.82). Four one-handers participated in both studies, with data acquired some 5 years apart.

Experimental Design

Scanning protocol for both datasets included multiple scans (see protocol in https://osf.io/4vcmx/). Only an anatomical T1 scan and a task scan for body-part functional localization were used and analyzed here (these scanning procedures were described previously, see Makin et al., 2013b; Hahamy et al., 2015b).

The sensorimotor task in both datasets followed the same procedure: Participants were visually instructed to move each of their hands (finger flexion/extension), arms (elbow flexion/extension), their feet (bilateral toe movements), or lips, as paced by a visual cue. Note that none of the one-handers experienced phantom sensations. Therefore, in conditions concerning missing hand movements (and elbow movements for one participant with an above-elbow deficiency) participants were instructed to

imagine moving their missing limb. This condition was only included to match the experimental design across groups and was not submitted to statistical analyses. The protocol consisted of alternating 12-s periods of movement and rest. Each of the six conditions was repeated four times in a semi-counterbalanced order. Participants were trained before the scan on the degree and form of the movements. To confirm that appropriate movements were made at the instructed times, task performance was visually monitored online, and video recordings were made in a subset of the scans for further off-line evaluation.

MRI data acquisition

The MRI measurements of Dataset1 were obtained using a 3T Verio scanner (Siemens, Erlangen, Germany) with a 32-channel head coil. Anatomical data were acquired using a T1-weighted magnetization prepared rapid acquisition gradient echo sequence (MPRAGE) with the parameters: TR: 2040 ms; TE: 4.7 ms; flip angle: 8°, voxel size: 1 mm isotropic resolution. Functional data based on the blood oxygenation level-dependent (BOLD) signal were acquired using a multiple gradient echo-planar T2*-weighted pulse sequence, with the parameters: TR: 2000 ms; TE: 30 ms; flip angle: 90°; imaging matrix: 64 × 64; FOV: 192 mm axial slices. 46 slices with slice thickness of 3 mm and no gap were oriented in the oblique axial plane, covering the whole cortex, with partial coverage of the cerebellum.

MRI images of Dataset2 were acquired using a 3T MAGNETON Prisma MRI scanner (Siemens, Erlangen, Germany) with a 32-channel head coil. Anatomical images were acquired using a T1-weighted sequence with the parameters TR: 1900 ms, TE: 3.97 ms, flip angle: 8°, voxel size: 1 mm isotropic resolution. Functional images were collected using a multiband T2*-weighted pulse sequence with a between-slice acceleration factor of 4 and no in-slice acceleration. This allowed acquiring data with increased spatial (2mm isotropic) and temporal (TR: 1500ms) resolution, covering the entire brain. The following acquisition parameters were used - TE: 32.40ms; flip angle: 75°, 72 transversal slices. Field maps were acquired for field unwarping.

Preprocessing of functional data

All imaging data were processed using FSL 5.1 (www.fmrib.ox.ac.uk/fsl). Data collected for individuals with absent right limbs were mirror reversed across the mid-sagittal plane prior to all analyses so that the hemisphere corresponding to the missing hand was consistently aligned. Data collected for left-hand dominant controls were also flipped, in order to account for potential biases stemming from this procedure. Note that the proportion of flipped data did not differ between experimental groups in either datasets ($\chi^2_{(1)}$=0.18, p=0.67 for Dataset1; $\chi^2_{(1)}$=0.05, p=0.82 for Dataset2), and that this flipping procedure has been validated using multiple approaches (see Hahamy et al., 2017).

Functional data were analyzed using FMRIB's expert analysis tool (FEAT, version 5.98). The following pre-statistics processing was applied to each individual task-based run: motion correction using FMRIB's Linear Image Registration Tool (Jenkinson et al., 2002); brain-extraction using BET (Smith, 2002); mean-based intensity normalization; high pass temporal filtering of 100 s; and spatial smoothing using a Gaussian kernel of FWHM (full width at half maximum) 4 mm. Time-course statistical analysis was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction. Functional data were aligned to structural images (within-subject) initially using linear registration (FMRIB's Linear Image Registration Tool, FLIRT), then optimized using Boundary-Based Registration (Greve and Fischl, 2009). Structural images were transformed to standard MNI space using a non-linear registration tool (FNIRT), and the resulting warp fields were applied to the functional statistical summary images.

Statistical analyses

Meta-analysis approach

The current study makes use of three separate datasets, acquired across several years and using different magnets and scanning parameters. Multiple datasets can, in principle, be collapsed for analysis purposes, benefiting statistical power to identify weak effects that may not be noticeable in each separate dataset (Friston, 2012). However, as the current study is guided by an a-priori hypothesis that is also spatially focal (reorganization in the deprived hand region of one-handers), it calls for more stringent inference

methods rather than for exploratory ones that benefit from enhanced power. We therefore opted to analyzing each dataset separately and combining results using a meta-analysis approach (Hahamy et al., 2015a), which allows us to test inherent reproducibility of findings across datasets, and hence make more valid inferences (Ioannidis et al., 2014; Picciotto, 2018).

Our analysis pipeline for all ROI-based analyses reported below included a-parametric permutation tests performed within each separate dataset. Note that permutation tests are statistically stringent as they make no assumptions regarding the finite sample distribution of the data, but rather derives it given the data observed (Holmes et al., 1996; Nichols and Holmes, 2002), and are also less sensitive to outlier effects (Masyn et al., 2013), thus contributing to the robustness of findings. The dataset–specific p-values resulting from each of the below-described permutation tests were then combined across datasets and meta-analyzed using Fisher's method (Fisher, 1925; Fisher, 1948) to test the reproducibility of results across datasets. To establish the robustness of the reported effects, p-values were additionally tested using Stouffer's test (Stouffer et al., 1949) and the weighted Z-test (weights set to the square root of each sample size, Liptak, 1958). To correct for multiple hypotheses testing across the 3 experimental conditions of interest (movements of the residual arm, lips and feet), the alpha level was adjusted to 0.017 based on the highly conservative Bonferroni correction.

Spatial layout of body-part representations

To measure cortical proximity between native body-part representations, the level of overlap between activations of the hand, lips and feet in the "intact" hemisphere (cerebral hemisphere contralateral to the dominant/intact hand and cerebellar hemisphere ipsilateral to the dominant/intact hand) were measured in all participants using the Dice coefficient (Dice, 1945; Kikkert et al., 2016):

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

Where A and B represent activation evoked by movements of specific body-parts (intact hand and lips or intact hand and feet) within a sensorimotor mask. To that end, for each participant, the activation maps of intact hand, lips and feet conditions were minimally thresholded at z=2 to allow a relatively wide spread

of activation. The few participants who had particularly low spread of activation (<25 voxels) in the intact hemisphere in either condition, despite the lenient threshold, were excluded from this analysis (Dataset1: 3 control participants; Dataset2: one congenital and one control participant). In the cerebral cortex, the level of overlapping activations between the hand condition and each of the lips and feet conditions were assessed within a mask of the left pre-central gyrus, taken from the Harvard-Oxford probabilistic atlas (this mask was used unthresholded to contain the central sulcus and both the pre- and post-central gyri, see Figure 1-1A). In the cerebellar cortex, the level of overlapping activations between the hand condition and each of the lips and feet conditions were assessed within a mask of right lobules I-IV,V and VI, taken from FSL's cerebellar probabilistic atlas. Each of these three cerebellar masks were thresholded at 50 prior to their unification in order to restrict the unified mask to the sensorimotor sections of the cerebellar anterior lobe (see Figure 1-1A).

For each participant, 4 Dice coefficients were calculated: overlap between intact hand and feet activations, and overlap between intact hand and lips activations, in each of the cerebrum/cerebellum masks separately. We next aimed to verify that the overlap relationship of body-part representations differs between the cerebrum and the cerebellum, as previously reported (see Introduction). However, a direct comparison between overlap in representations in the cerebrum vs. cerebellum may be confounded by the different spatial scales of these two structures. We therefore targeted a comparison between intra-structure overlap relations, which we will refer to as "neighborhood relationship" of each of the cerebral or cerebellar cortices. This neighborhood relationship was defined as the ratio of lips-hand overlap to feet-hand overlap in each brain structure (cerebrum/cerebellum, Figure 1-1C). Note that as neighborhood relationships are devised as ratios within each brain structure, they normalize the Dice coefficients and enable a comparison between the cerebrum and cerebellum. To evaluate whether these neighborhood relationships are different between the cerebrum and cerebellum, a permutation test was employed within each dataset. The test's statistic was defined as the cross-participants mean ratio between cerebellar neighborhood relationship and cerebral neighborhood relationship (a ratio that significantly deviates from 1would suggest a difference in topographies between the cerebrum and cerebellum). To this end, for each

participant, the cerebral neighborhood relationship was divided by the cerebellar neighborhood relationship. Under the null hypothesis of no difference between cerebral and cerebellar neighborhood relationships, the cerebral and cerebellar neighborhood relationships were shuffled within participants and then averaged across participants, a procedure which was repeated 10,000 times to create the null distribution. The position of the true (unshuffled) test statistic within this distribution was then used to obtain a two-sided p-value. Finally, the resulting dataset-specific p-values were tested using Fisher's method to assess the consistency of affects across datasets.

Whole-brain analysis

All statistical analyses were designed to follow the procedures described in our original report (Hahamy et al., 2017). Statistical analyses were conducted using FSL and in-house Matlab code. To compute task-based statistical parametric maps, we applied a voxel-based general linear model (GLM), as implemented in FEAT, using a double-gamma hemodynamic response function and its temporal derivative convolved with the experimental model. The 6 motion parameters and their derivatives were also included in the GLM as nuisance regressors. Our main comparisons contrasted intact/dominant hand, residual/nondominant arm, lips and feet conditions against a baseline (rest) condition.

Group level analysis of statistical maps was carried out using FMRIB's Local Analysis of Mixed Effects (FLAME). Intact hand movements in the one-handed group were compared with dominant hand movements in controls, and residual arm movements were compared with non-dominant arm movements. The cross-subject GLM included planned comparisons between the two groups. Z (Gaussianized T/F) statistic images were thresholded using clusters determined by $Z>2.6$ ($p<0.01$), and a family-wise-error corrected cluster significance threshold of $p<0.01$ was applied to the suprathreshold clusters. Note that this whole-brain analysis tests the specificity of plasticity to the deprived hand region of one-handers, hence a lenient statistical threshold ($p<0.05$) is typically used in such procedures (Makin et al., 2013b; Hahamy et al., 2017). Nevertheless, as we test several whole-brain comparisons (residual arm, lips, feet conditions), we chose a more strict threshold of 0.01 across our tests to correct for any alpha inflation.

For presentation purposes, statistical parametric activation maps of the cerebellum were projected onto a flat cerebellar surface using SUIT (Diedrichsen and Zotow, 2015), and parametric activations in the cerebral cortex were projected onto an inflated cortical surface of a representative participant's cortex using workbench.

Cerebellar Region Of Interest (ROI) definition

In order to ascertain that the observed increased cerebellar activation in one-handers falls within the hand region, and to measure its extent, single-subject activation values were extracted from independently defined hand-region ROIs and compared between experimental groups. To define the dominant/intact and nondominant/deprived cerebellar hand regions, the 100 cerebellar voxels of highest activation evoked by either dominant or nondominant hand movements in the control group of one dataset were used as ROIs in the second dataset, and vice-versa (see Table 2, Figure 1, Figure 1-2). Percent signal change activation values from the individual statistical parametric maps were extracted for the intact and deprived hand ROIs for each participant in the residual/nondominant arm, lips, feet and intact hand conditions. Since the functional data of one control participant in Dataset1 did not cover the cerebellum, data from this individual were excluded from the cerebellar ROI analysis. The same method was used to define the cerebellar representation of the lips and feet for visualization purposes.

Statistical analysis of cerebellar ROIs

To a-parametrically assess each planned group-contrast (experimental conditions involving movements of different body parts), permutation tests were employed within each dataset separately (Holmes et al., 1996; Nichols and Holmes, 2002). In each experimental condition separately, the test statistic was set as the difference between mean group activations in a certain ROI. Next, participants' labels (one-handers or control) were permuted under the null hypothesis of no group differences in the levels of ROI activation under each experimental condition. Thus, two random experimental groups were created for each condition, and the difference between the groups' mean activation in a given ROI was calculated. This procedure was repeated 10,000 times, creating 10,000 random differences that constructed the null

distribution. For each experimental condition, the position of the true (unshuffled) group-difference relative to the null distribution was used to obtain a two-sided p-values. Using the same pipeline, under the null hypothesis of no 2-way interactions between groups and hemispheres (ipsilateral and contralateral to the missing/nondominant hand), both participants' labels and within-participant hemisphere-labels were permuted in each dataset and experimental condition separately. The differences between hemisphere-scores were calculated per participant, averaged across participants of the same experimental group, and mean group differences were derived. The position of the true group-difference relative to the null distribution (resulting from 10,000 such iterations) in each experimental condition was used to derive a two-sided p-value.

Assessing reorganization in the cerebral cortex

The overlap in participants between Datasets1 and Datase2 is relatively small (4 out of 24 participants), which allowed us to perform the above described cross-dataset replication analyses for the cerebellum. However, we also aimed to test the reproducibility of our previously reported findings of reorganization in the cerebral cortex (Hahamy et al., 2017) using the current and previously published datasets, and these contained larger overlap in participants. Dataset2 included only 5 participants who also participated in the Hahamy et al., 2017 study (with data acquired some 2 years apart). However Dataset1 and the data used in Hahamy et al., 2017 greatly overlapped (12 out of 14 participants, with data acquired some 3 years apart). Hence, cerebral-related results obtained from Dataset1 should be taken as a measure of test-retest reliability with regards to Hahamy et al., 2017, rather than an independent replication.

Analyses performed on the cerebral cortex are identical to those described for the cerebellum, except for the following differences: 1) For cerebral hand ROIs (in both hemispheres) we used independent regions previously defined based on the original sample of one-handers and controls of our previous study (Hahamy et al., 2017; see Table 2; this ROI will be made freely available via open science framework), to standardize the analysis across the three datasets (the two current ones and the previously published one). ROIs for lips and feet sensorimotor representations were also adopted from the same

previous work to visualize the sensorimotor homunculus. Note that limitations in both MRI acquisition and analysis resolution precluded reliable separation between the two cerebral foot regions. For this reason, a bilateral ROI was defined for the feet. For the same technical reasons we were unable to dissociate the somatosensory and motor primary cortices (SI and M1, respectively). Therefore, all body part ROIs are labelled 'sensorimotor'. 2) Since the cerebral-focused ROI analyses were guided by a predefined hypothesis (over-representation of the residual arm, lips and feet in the deprived hand region of one-handers) based on our previous study, one-tailed statistical tests within each of Dataset1 and Dataset2 were used. Since our previous study did not find a significant interaction between groups and hemispheres for the feet condition, the tests of this effect in the two current datasets were performed in a two-tailed form. For each experimental condition, sets of 3 dataset-specific p-values (resulting from each of the two current datasets, as well as the previously reported p-values of the original dataset) were combined and tested using the same methods described for the cerebellum analyses, including correction for multiple comparisons.

Results

Neighborhood relationship between body-part representations differ between the cerebrum and cerebellum

In both the cerebral and cerebellar cortices, the hand area resides between the foot and lip representations, however, the level of overlap between these representations was previously reported to differ between the two brain structures (see introduction). To verify this difference in overlap between body-part representations (hereafter, neighborhood relationship) in the cerebrum and cerebellum, we used brain activations as a proxy for representations. To this end, here and in all other reported cerebellar analyses, we compared results across two independently acquired datasets of one-handers and controls who underwent a functional MRI scan, involving simple movements of hands, arms, lips and feet.

To measure the extent of overlap between representations, we confined activations to the sensorimotor parts of the cerebellum and cerebrum (Figure 1-1A), and employed the Dice coefficients (Dice, 1945; Kikkert et al., 2016; see Materials and Methods). As demonstrated in the intact/dominant hemispheres of controls and one-handers in Figure 1-1B, some degree of overlap was indeed observed between the peripheral aspects of the hand region and the lip and feet activations of both cerebrum and cerebellum, allowing us to quantify overlapping activations within each brain structure. This level of overlap was evaluated using permutation tests on each of the dataset-specific Dice coefficients. Results of these tests were then combined across the two datasets (See Materials and Methods). This analysis demonstrated differences in neighborhood relationships between the cerebrum and cerebellum (Dataset1 $p=0.02$, Dataset2 $p=0.048$ permutation tests; Meta-analysis: $\chi^2_{(4)}=13.59$, $p=0.009$ Fisher's test, $\alpha=0.05$), reflecting that the representations of lips and feet in the cerebral cortex show more similar levels of overlap with the hand representation (Makin et al., 2015) relative to the cerebellum (see Figure 1-1C).

Cerebellar reorganization is not restricted by somatotopic layout

To test whether somatotopy restricts reorganization in the cerebellum, we assessed functional reorganization of the residual arm (overlapping the hand area, Figure1- 2), as well as the lips and feet (whose representations have differing levels of overlap with the hand area, as demonstrated above) in one-handers compared to controls. Whole-brain activations evoked by movements of the residual/nondominant arm, lips and feet (body-part representations previously shown to reorganize in the cerebral cortex, Hahamy et al., 2017), and of the intact hand (whose representation did not show such reorganization, Hahamy et al., 2017) was compared between experimental groups within each dataset. These analyses revealed that movements of the lips and feet, but not movements of the intact hand, excessively activated a region in Lobules V \ VI of the cerebellar hemisphere ipsilateral to the missing hand in one-handers (see Figure 1, Table 3). These clusters overlapped with the independently defined ROI of the deprived hand region of the anterior cerebellum (see Materials and Methods, Figure 1). Unlike

our previous findings in the cerebral cortex, this whole-brain group contrast did not reveal increased activation in one-handers for residual arm movements compared to controls. This could potentially stem from near complete overlap between the arm and hand representations found in the cerebellum (Mottolese et al., 2013, and see Figure 1-2). Specifically, if the arm representation naturally overlaps with the hand representation, additional remapping between these representations in one-handers may be too subtle to be detected using a whole-brain analysis. Additional clusters showing increased activation in one-handers compared to controls were also found within specific datasets, but unlike the deprived hand region, these clusters were not consistent across all datasets and task-conditions (see Figure 1-3). Group activation maps of each condition vs. rest are presented in Figure 1-4.

We next aimed to measure the degree of remapped activation in the deprived cerebellar hand region during movements of different body parts, and assess its consistency across datasets. To this end, within each dataset and movement condition separately, between-group permutation tests were used to compare mean fMRI activation values (percent signal change) obtained from two independent hand ROIs (deprived and intact hand regions of the cerebellar hemispheres' anterior lobe, ROIs depicted in Figure 1). These ROIs were obtained from the control group of one dataset and tested on the other dataset, and are completely independent of the group-contrast activations reported above. Results of these tests were combined across the two datasets (See Materials and Methods; Dataset-specific p-values for all experimental conditions are presented in Figure 1-5). These analyses confirmed increased activation in the deprived cerebellar hand ROI when one-handers moved their lips ($\chi^2_{(4)}$=23.21, p<0.001, α=0.017 Bonferroni corrected, Fisher's test) and feet ($\chi^2_{(4)}$=19.91, p<0.001, α=0.017 Bonferroni corrected, Fisher's test), as well as their residual arm ($\chi^2_{(4)}$=15.29, p=0.004, α=0.017 Bonferroni corrected, Fisher's test), compared with controls. Movements of the intact hand (whose representation does not reorganize in the cerebral cortex, Hahamy et al., 2017) did not result in increased representation in the deprived cerebellar hand region of one-handers ($\chi^2_{(4)}$=3.33, p=0.51, Fisher's test). In addition, two-way interactions were consistently revealed between hemispheres and groups (non-dominant/residual arm: $\chi^2_{(4)}$=20.61, p<0.001; lips: $\chi^2_{(4)}$=19.39, p<0.001; feet: $\chi^2_{(4)}$=19.23, p<0.001, Fisher's test, α=0.017 Bonferroni corrected for all

tests). These interactions reflect dissociated recruitment of the deprived cerebellar hand region by movements of various body parts in one-handers, in comparison with the intact cerebellar hand region and with the control group, as shown in the Figure 2. These findings echo the pattern of reorganization we previously reported in the cerebral cortex of one-handers, and reflect sensorimotor reorganization which is not restricted by the topographic arrangement of representations in the cerebellar homunculus.

Similar pattern of reorganization seen in the cerebellar and cerebral deprived hand regions of one-handers

We next used these two datasets to test the reproducibility of our previous findings of reorganization in the cerebral cortex of one-handers. As shown in Figure 3, movements of the residual arm, lips or feet, but not movements of the intact hand, activated the sensorimotor cerebral cortex to a greater extent in one-handers compared to controls, as shown using whole-brain contrast maps. For all three body parts, increased activation in one-handers was centered on the hand knob of the central sulcus in the deprived hemisphere, spanning both the pre- and post-central gyrus of the hemisphere contralateral to the missing hand (see Table 3). These clusters overlapped with an ROI of the putative region of the missing hand in the cerebral cortex (see Materials and Methods, Figure 3). Additional clusters showing increased activation in one-handers compared to controls were also found within specific datasets, but unlike the deprived hand region, these clusters were not consistent across all datasets and task-conditions (see Figure 1-3).

Next, an ROI analysis of the cerebral hand regions was performed using independently defined ROIs (see Figure 3). These group-based ROIs, defined based on our previously analyzed dataset of participants (Hahamy et al., 2017), were used to preserve consistency with our previous study and allowed us to combine results across the two current datasets as well as the dataset used in our previous study to assess reproducibility (dataset-specific p-values for all experimental conditions are presented in Figure 1-5). As depicted in Figure 4, these tests confirmed increased activation in the deprived cerebral

hand ROI when one-handers moved their residual arm ($\chi^2_{(6)}$=47.73, p<0.001, α=0.017 Bonferroni corrected, Fisher's test), lips ($\chi^2_{(6)}$=43.8, p<0.001, α=0.017 Bonferroni corrected, Fisher's test) and feet ($\chi^2_{(6)}$=37.08, p<0.001, α=0.017 Bonferroni corrected, Fisher's test) compared with controls. In addition, two-way interactions between hemispheres and groups consistently revealed dissociated recruitment of the deprived cerebral hand region (compared to the intact hand region) by movements of various body parts between one-handers and controls (non-dominant/residual arm: $\chi^2_{(6)}$=32.54, p<0.001; lips: $\chi^2_{(6)}$=28.07, p<0.001; feet: $\chi^2_{(6)}$=23.7, p<0.001, Fisher's test, α=0.017 Bonferroni corrected in all tests). Similar group differences were not found when participants were moving their intact/dominant hand ($\chi^2_{(6)}$=8.31, p=0.22, Fisher's test).

Figure 1-6 presents the results of integration across datasets using additional meta-analysis measures for both the cerebral and cerebellar hand regions.

Discussion

Here we report large scale neural reorganization of body-part representations in both the cerebellar and cerebral homunculi of individuals born without one hand. We previously demonstrated reorganization in the primary sensorimotor cortex, such that the residual arm, lips and feet, but not the intact hand, are simultaneously represented in the deprived cerebral hand region (Hahamy et al., 2017). Here we replicate these findings and extend them to the deprived hand region of the cerebellum. Of importance, in both the cerebellum and cerebrum, the representations of multiple body parts excessively expand into the deprived hand region (Figures 1 & 3). This expansion occurs despite differences in the somatotopic layout in these two sensorimotor terminals (Manni and Petrosini, 2004; Mottolese et al., 2013; Makin et al., 2015; Mottolese et al., 2015). Our findings therefore challenge the view that sensorimotor reorganization is restricted to the immediate neighbors of the deprived region (Merzenich et al., 1984; Pons et al., 1991; Merzenich and Jenkins, 1993; Faggin et al., 1997; Florence et al., 1998; Margolis et al., 2012).

Previous studies of similar sensorimotor-deprived populations have produced mixed evidence for the extent and drivers of reorganization. As such studies of reorganization rely on rare clinical populations, published results tend to be based on relatively small sample sizes, and replication of results is scarce. Here we constructed the largest imaging database to date of one-handers (n=26), and demonstrated the reproducibility of our results across independently acquired datasets as a means for establishing statistical validity (Ioannidis et al., 2014; Picciotto, 2018). Indeed, the meta-analysis approach we have taken here allowed us to confirm the robust and reproducible nature of the reported effects. Our findings therefore provide solid evidence to the ongoing debate regarding the role of neighborhood relationships in driving cortical reorganization, and emphasize the need to consider sensorimotor reorganization as a more complex phenomenon than has previously been discussed.

If the extent of overlap between body-part representations does not account for reorganization, what could drive the large-scale sensorimotor reorganization reported by us and others? One possibility is that reorganization is shaped by altered inputs to the deprived cortex, which are associated with compensatory behavior. We have previously characterized the behavioral repertoire of one-handers, and found that these individuals use their residual arm, lips and feet to compensate for the absence of a hand (Hahamy et al., 2017). As previously reported and further extended here, the same body-parts used for compensatory purposes also remap onto the deprived hand regions of both the cerebellum and cerebrum. Importantly, the intact hand, which is not over-used for compensatory purposes (Makin et al., 2013b; Philip and Frey, 2014; Hahamy et al., 2017), does not show remapping into either the cerebellar or cerebral deprived hand regions. While these findings suggest compensatory behavior as a driver for reorganization, it is important to note that so far we were unable to find a correlation between individuals' idiosyncratic compensatory strategies and brain reorganization. Indeed, other studies reported large-scale reorganization that did not reflect compensatory behavioral profiles (Yu et al., 2014; Striem-Amit et al., 2018), though compensatory daily behavior is difficult to quantify comprehensively and reliably.

Similar controversy also exist in reports of reorganization in adulthood following limb amputation, which is most commonly reported as remapping of the residual arm (Kew et al., 1994;

Irlbacher et al., 2002; Raffin et al., 2016) (but see Gagne et al., 2011; Makin et al., 2013b) and mouth (Flor et al., 1995; Elbert et al., 1997; Karl et al., 2001; Lotze et al., 2001; MacIver et al., 2008; Foell et al., 2014), (though see Makin et al., 2013a; Makin et al., 2015; Raffin et al., 2016), both thought to neighbor the hand region (though see Zeharia et al., 2015; Roux et al., 2018; as well as hand and lip regions in Figure 3). Nevertheless, more recent findings reveal remapping of the intact hand representation (which resides in the opposite hemisphere) onto the deprived hand region in adult human amputees (Bogdanov et al., 2012; Makin et al., 2013b; Philip and Frey, 2014). These more recent findings have ascribed intact hand remapping to adaptive reorganization, reflecting the compensatory use of the intact hand by amputees. Thus, findings across varied sensorimotor-deprived populations raise the possibility that body-part representations that have very little overlap, if any, with the hand region (lips and feet in one-handers, feet in individuals with bilateral congenital limb loss or childhood amputation, and intact hand in amputated adults) can remap onto the deprived hand region, provided that this remapping corresponds with behavioral change. Conversely, representations of body-parts which neighbor the deprived hand region can also show reorganization, even under reduced behavioral pressure (Striem-Amit et al., 2018).

It should also be noted that the absence of intact hand representation in the missing hand region of one-handers could stem from reasons other than lack of compensatory use of the intact hand. For example, in the current experimental design, the intact hand is the only body-part contralateral to the deprived cerebral hemisphere/ipsilateral to the deprived cerebellar hemisphere (note that movements of the lips and feet were performed bilaterally in this experiment). We therefore cannot exclude the possibility that reorganization in the missing hand area of one-handers is restricted to body-part representations that reside within the deprived hemisphere.

We also cannot exclude the possibility that behavior and brain reorganization may not be directly related. For example, the observed reorganization in one-handers may merely reflect weak normal inputs from different body-parts to the hand area, which are typically supressed by the main inputs from the hand. These inputs may be simply unmasked in the absence of a hand, and may not necessarily causally support compensatory behaviour (for further discussion see Krakauer and Carmichael, 2017; Makin and

Bensmaia, 2017). Taken together, further research is needed to validate the causal origins and consequences of behaviour on the large scale reorganization reported here.

Finally, it may also be conjectured that the reported reorganization is indeed constrained by proximity between body-part representations - not in the cerebellum/cerebrum, but rather in subcortical terminals of the sensorimotor pathway. For example, it has been demonstrated that representations of the hand, lip and foot, which are distal in the cerebral cortex, neighbor each other in the putamen of the human basal ganglia (Gerardin et al., 2003; Staempfli et al., 2008). These neighboring representations may thus more easily remap to the deprived hand region, and consequently, projections from the putamen hand representation to its cerebral/cerebellar counterparts would appear as reorganization that is independent of the cerebral/cerebellar somatotopic layout. This hypothesis is particularly appealing since the primary sensorimotor cortex, cerebellum and basal ganglia form a closed-loop, reciprocal circuit, such that each terminal projects to and receives inputs from each other terminal, with varying layouts of somatotopy within each terminal (Nambu, 2011; Dum et al., 2014; Zeharia et al., 2015). As our findings reveal reorganization in two of these three interconnected terminals (cerebral and cerebellar cortices), it is plausible that a similar reorganization also exists in, and is perhaps initiated by, the basal ganglia. Consistent with this conjecture, while it has originally been suggested that somatosensory reorganization occurs at the level of the cerebral cortex (Pons et al., 1991; Florence et al., 1998), recent studies in monkeys emphasize the role of subcortical structures, such as the brainstem, in which the layout of somatotopic representations differs from that seen in the cerebral cortex (Jain et al., 2000; Kambi et al., 2014; Chand and Jain, 2015; Liao et al., 2016). For example, Kambi and colleagues (2014) have recently demonstrated that facial reorganization in the deprived cerebral hand region of spinal-cord-injured monkeys is abolished upon inactivation of the deprived cuneate nucleus. The fact that the cuneate nucleus does not normally receive inputs from the face suggests that reorganization seen in the cerebral cortex is likely driven by reorganization at the level of the brainstem (see also Herbert et al., 2015 for a related example of reorganization in the motor cortex). Taken together with our current findings, it is particularly tempting to speculate that the upstream sensorimotor terminal at which reorganization may be initiated

would contain a somatotopic layout specifically suitable for supporting the emerging repertoire of compensatory behaviors. However, since our results demonstrate remapping in both primary somatosensory and motor cortices (Figure 3), which have differing upstream hierarchies, this interesting interpretation awaits further confirmation.

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Tables

Table 1. Demographic details of individuals with congenital limb-absence included in Dataset2 (Full details of the participants of Dataset1 are available in Hahamy et al., 2015b).

Participant	Age	Gender	Level of limb deficiency	Affected limb	participated in both studies
1	39	Male	below elbow	Left	yes
2	35	Male	below elbow	Right	
3	29	Female	below elbow	Left	
4	58	Male	below elbow	Left	yes
5	37	Female	below elbow	Right	yes
6	52	Female	below elbow	Left	
7	32	Male	below elbow	Left	
8	61	Male	below elbow	Left	
9	42	Female	below elbow	Left	

10	53	Female	below elbow	Right	yes
11	44	Male	below elbow	Left	
12	35	Male	below elbow	Right	
13	51	Female	below elbow	Left	
14	63	Female	below elbow	Right	

Table 2. Number of voxels ($(2mm)^3$) and center-of-gravity coordinates of regions of interest.

	# voxels	Coordinates
Intact/dominant hand (cerebellum, Dataset1)	100	21,-51,-21
Deprived/nondominant hand (cerebellum, Dataset1)	100	-21,-52,-21
Intact/dominant hand (cerebellum, Dataset2)	100	21,-51,-22
Deprived/nondominant hand (cerebellum, Dataset2)	100	-20,-51,-24
Intact/dominant hand (cerebrum)	388	64, 52, 64
Deprived/nondominant hand (cerebrum)	388	-64, 52, 64

Table 3. Group-contrast statistics of activation in the hand regions. The number of voxels (#vox), peak intensity (z_{max}) and coordinates of the center of gravity of hand-region activations in the cerebellum and cerebrum are presented for each dataset (rows) and task-condition (columns). Coordinates are based on the MNI 152 brain template.

Brain region	Dataset		Residual arm	Lips	Feet
Cerebellum	1	# vox	-	329	246
		Z_{max}	-	5	3.94
		Coordinates	-	-13,-55,-17	-21,-58,-16
	2	# vox	-	594	623
		Z_{max}	-	4.45	4.03
		Coordinates	-	-17,-59,-21	-21,-57,-22
Cerebrum	1	# vox	244	439	268
		Z_{max}	3.82	4.5	4.11
		Coordinates	46,-20,58	45,-17,54	44,-20,58
	2	# vox	422	411	450
		Z_{max}	5.79	4.24	4.14
		coordinates	49,-20,56	48,-16,54	52,-22,50

Figures

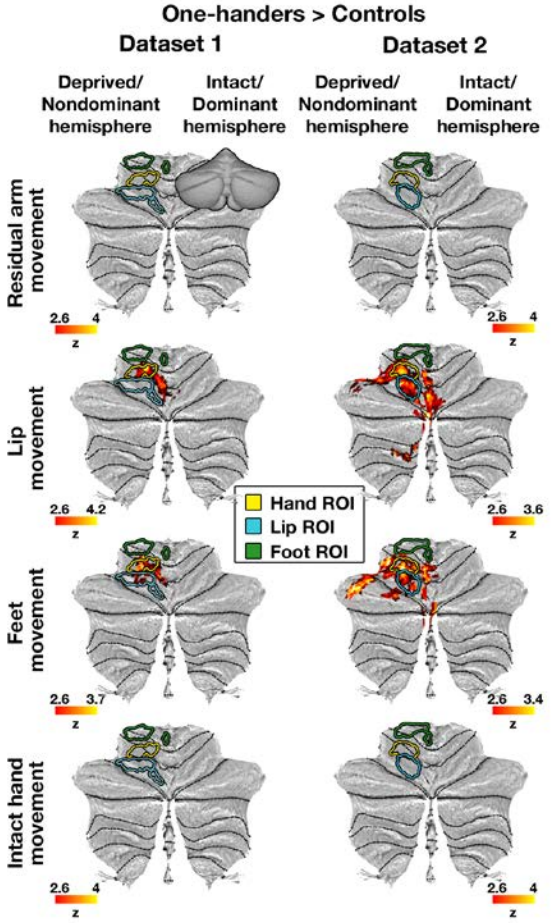

Figure 1. Representation of multiple body-parts in the deprived cerebellar hand region of one-handers: whole-brain analysis. The left/right panels show group contrast maps of Dataset1/Dataset2, respectively, during residual/nondominant arm (one-handers/controls), lips, feet and intact/dominant hand movements, projected onto a flat surface of the cerebellum (see example of an inflated surface on the top left). In the lips and feet (but not residual arm or intact hand) conditions, one-handers showed increased activation compared to controls, centred on the deprived cerebellar hand region. Yellow/blue/green contours indicate the hand/lips/feet ROIs, respectively. Intact/dominant hemisphere, ipsilateral to the intact/dominant hand; deprived/nondominant hemisphere, ipsilateral to missing/nondominant hand. Results of residual arm movements in a subset of participants from Dataset1 were previously reported (Makin et al., 2013b). See also Figures 1-1, 1-2, 1-3, 1-4.

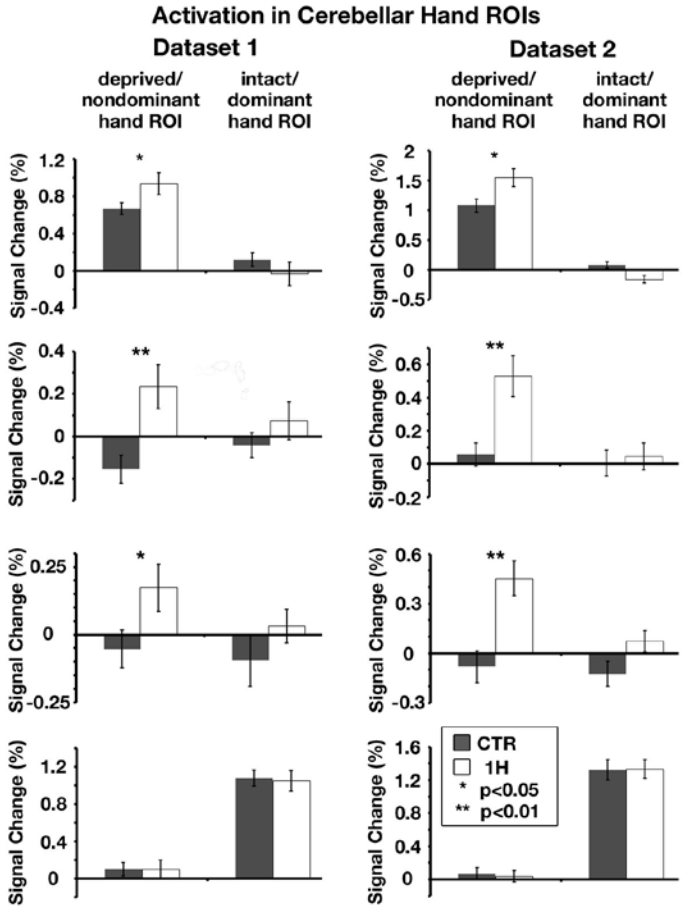

Figure 2. Representation of multiple body-parts in the deprived cerebellar hand region of one-handers: ROI analysis. The left/right panels show activation levels in Dataset1/Dataset2 (respectively) in the bilateral cerebellar hand regions (independently defined for each dataset, ROIs depicted in Figure 1, Figure 1-1), during residual/nondominant arm (one-handers/controls), lips, feet and intact/dominant hand movements. Activation levels in the deprived cerebellar hand region of one-handers (white bars) were greater than activations in the nondominant-hand region of controls (grey bars) in all but the intact hand condition. 1H, one-handers; CTR, controls; intact/dominant hand ROI, ipsilateral to the intact/dominant hand; deprived/nondominant hand ROI, ipsilateral to missing/nondominant hand. Error bars depict SEMs. Results of residual arm movements in a subset of participants from Dataset1 were previously reported (Makin et al., 2013b). See also Figures 1-5, 1-6.

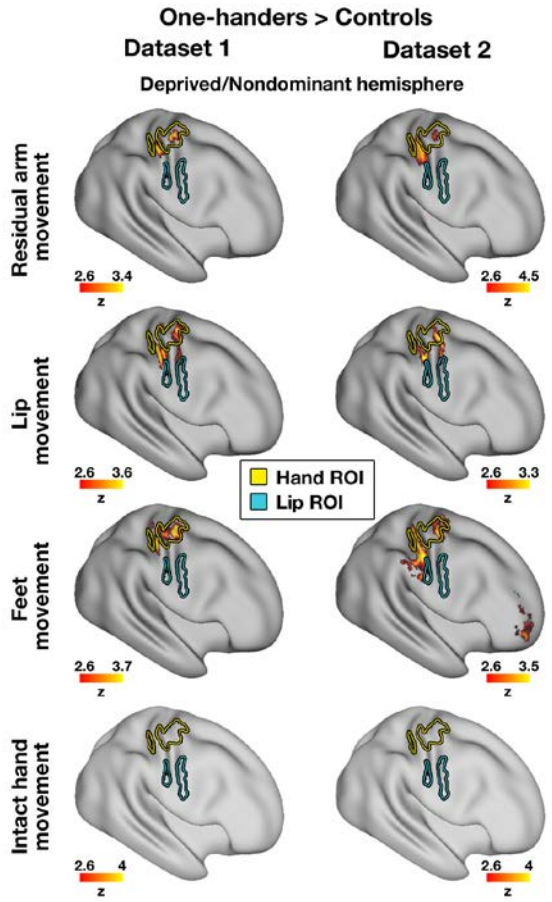

Figure 3. Representation of multiple body-parts in the deprived cerebral hand-region of one-handers: whole-brain analysis. The left/right panels show group contrast maps of Dataset1/Dataset2, respectively, during residual/nondominant arm (one-handers/controls), lips, feet and intact/dominant hand movements, projected onto an inflated surface of a template brain. In each of the arm, lip and feet (but not intact hand) conditions, one-handers showed increased activation compared to controls, centred on the deprived cerebral hand region. Yellow/blue contours indicate the hand/lip ROIs, respectively. Deprived/Nondominant hemisphere, contralateral to missing/nondominant hand. Results of residual arm movements in a subset of participants from Dataset1 were previously reported (Makin et al., 2013b). See also Figures 1-1, 1-3.

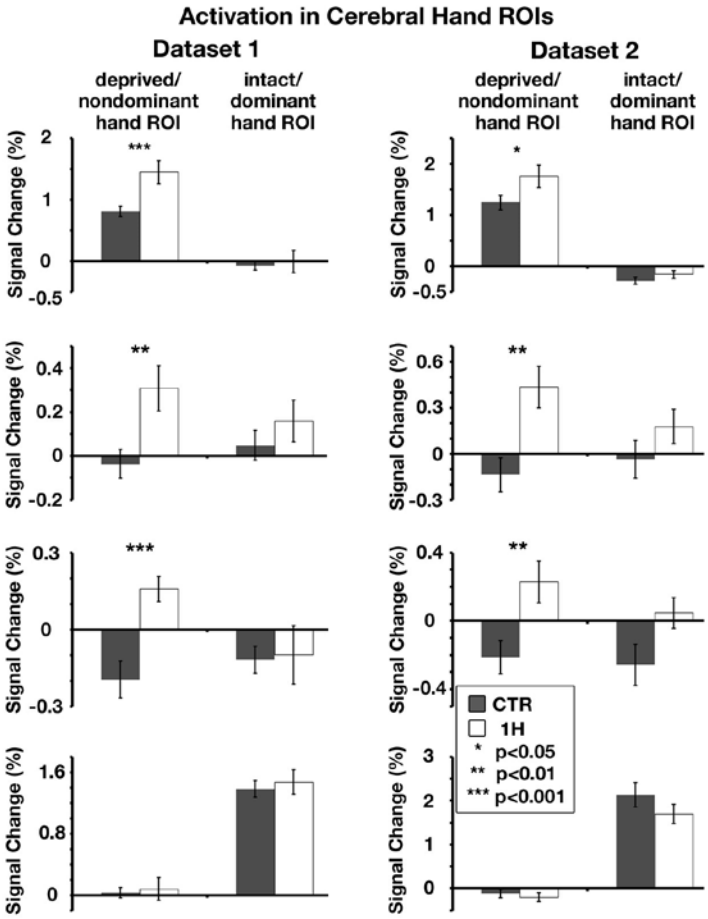

Figure 4. Representation of multiple body-parts in the deprived cerebral hand-region of one-handers: ROI analysis. The left/right panels show activation levels in Dataset1/Dataset2 (respectively) in the bilateral cerebral hand regions (independently defined, ROIs depicted in Figure 3, Figure 1-1), during residual/nondominant arm (one-handers/controls), lips, feet and intact/dominant hand movements. Activation levels in the deprived cerebral hand region of one-handers (white bars) were greater than in the nondominant-hand region of controls (grey bars) in all but the intact hand condition. 1H, one-handers; CTR, controls; intact/dominant hand ROI, contralateral to the intact/dominant hand; deprived/nondominant hand ROI, contralateral to missing/nondominant hand. Error bars depict SEMs. Results of residual arm movements in a subset of participants from Dataset1 were previously reported (Makin et al., 2013b). See also Figures 1-5, 1-6.