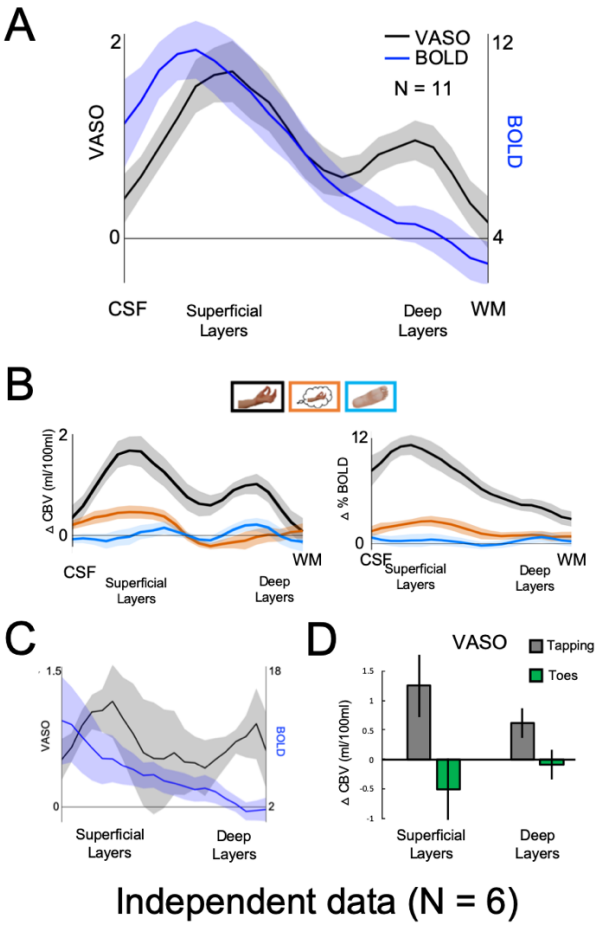

Figure S1. Comparison of VASO and BOLD responses during the experimental runs and in an independent set of data. Related to STAR Methods & Main Results. (A) Comparison of the VASO and BOLD responses across cortical layers during finger tapping. Note the double-peak response in the VASO signal, while the BOLD response peaks in the superficial layers and monotonically decreases across the deep layers to the white matter. We used a functional ROI analysis approach, in which we first used the VASO response to Finger tapping to functionally define the superficial and deep layers of M1. We were able to use this approach because prior work has shown that the VASO response to tapping produces two distinct peaks in M1 – one in the superficial layers that correspond to layers II/III and another peak in deep layers corresponding to layers Vb/VI. Since the BOLD response does not produce a distinct peak of activation in the deep layers of M1, we cannot functionally define an ROI in the deep layers to then interrogate with the Imagined tapping data. Note, since we defined the hand ROI based on the tapping condition in the experimental runs, we could not conduct statistical tests on those data. (B) The Finger tapping response plotted with responses to Imagined Tapping and Toe wiggling across layers of the functionally-defined hand ROI – VASO responses are on the left and BOLD responses on the right. (C) Independent data from a subset of six participants who completed an additional "blocked" functional run at the end of the scanner session that contained 15 second blocks of the Finger tapping and Toe wiggling conditions to replicate the layer-specific responses to finger tapping in the hand ROI. Note the expected double-peak response during finger tapping in the VASO, but not BOLD signal. (D) Significant increase in the VASO response in these independent data during the Tapping condition relative to Toe wiggling in both the superficial and deep layers of the Hand ROI (both p-values<0.05). Error bars on the line plots represent +/- 1 SEM. Error bars on the histogram are 95% within-subject confidence intervals.

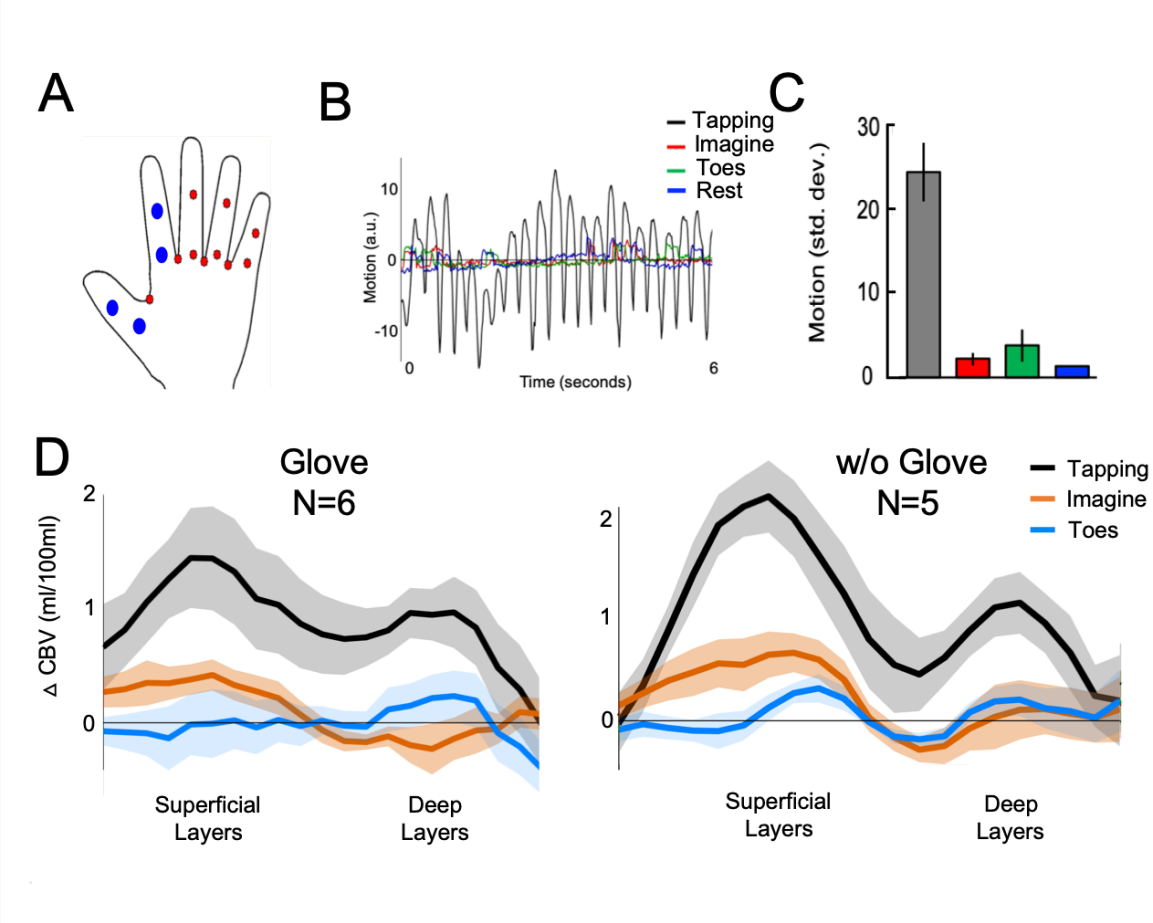

Figure S2. Hand Motion and VASO responses across conditions. Related to STAR Methods & Main Results. To ensure that the neural responses to imagined tapping were not due to participants inadvertently making small finger movements during imagery trials, we measured hand movements with a motion-detecting glove in six out of eleven participants. (A) An illustration of the sensor placement in the motion-detecting glove. We averaged the data from the four sensors with the largest fluctuations in motion (the blue dots) for further quantification of the motion data. (B) Averaged motion data from one participant during each condition. The motion data were averaged across trials. The data are plotted across the first six seconds of each trial. (C) The standard deviation of the average motion across trials for each condition. We found no significant differences in motion across imagined tapping, toe wiggling, and rest trials (all p-values>0.22), and significantly more motion during actual tapping relative to all other conditions (all p-values<0.001). (D) Layer-specific VASO responses to the conditions of interest in the participants that wore the motion-detecting glove (left) and those that did not wear the glove (right). A 2 (Glove, No Glove) x 2 (Superficial layers, Deep layers) x 3 (Finger tapping, Imagined tapping, Toe wiggling) between-groups ANOVA did not reveal a significant interaction ($F_{(2,54)}$=0.09, p=0.91, η_P^2=0.003), thus confirming that there were no significant differences in the pattern of VASO responses between the participants that wore the motion detecting glove and those who did not, allowing us to combine the data from the two groups for further analyses. Error bars on the line plots represent +/- 1 SEM. Error bars on the histogram are 95% within-subject confidence intervals.

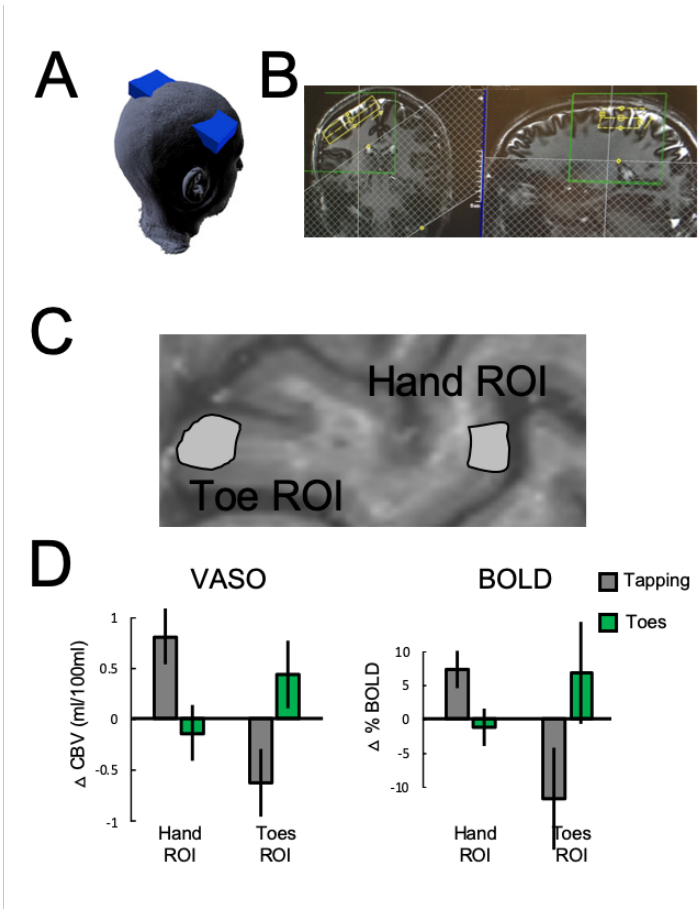

Figure S3. VASO & BOLD responses to finger tapping and toe wiggling in functionally defined hand and toe ROIs. Related to STAR Methods. (A) The imaging slice position and slice angle were adjusted individually for every participant to be perpendicular to the forefinger region of M1. (B) An online depiction of an example participant in the vendor-provided 3D-viewer. Eight slices (0.75x0.75x1.8mm) were acquired during each run. In addition to the Hand region of M1, our field of view also covered the portion of M1 in the paracentral lobule that represent the foot and toes. Therefore, in each participant, we functionally defined a toe ROI by identifying a region in the paracentral lobule that responded more to toe wiggling relative to finger tapping in the experimental runs (C). We were unable to measure layer-specific responses in the toe ROI due to its position relative to the angle of our field of view. Therefore, we made an average hand ROI in each participant by averaging across the layers in the original hand ROI, so that we could compare the average response across the hand ROI with the average response across the toe ROI in the six participants that completed the "blocked" run at the end of the scan session. (D) We found a double dissociation between the ROIs in both the VASO and BOLD signals. Specifically, we found a significant interaction between the ROIs (VASO: $F_{(1,5)}$=22.25, p=0.005, η_P^2=0.82; BOLD: $F_{(1,5)}$=12.27, p=0.02, η_P^2=0.71), with a significantly greater response to the Tapping relative to Toe wiggling condition in the hand ROI (VASO: $t_{(5)}$=3.71, p=0.01, Cohen's d=1.51; BOLD: $t_{(5)}$=3.29, p=0.02, Cohen's d=1.34) and the opposite pattern of results in the toe ROI (VASO: $t_{(5)}$=-3.47, p=0.02, Cohen's d=1.41; BOLD: $t_{(5)}$=-2.67, p=0.04, Cohen's d=1.09). These independent data confirm that the hand ROI was indeed Tapping selective, and that the Toe wiggling condition was an appropriate motor control condition for our experiment (i.e., it evoked the expected response from a region of M1 that should be involved in toe movements). Error bars are 95% within-subject confidence intervals.