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Reversible large-scale modification of cortical networks during neuroprosthetic control

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Brain-machine interfaces (BMIs) provide a framework for studying cortical dynamics and the neural correlates of learning. Neuroprosthetic control has been associated with tuning changes in specific neurons directly projecting to the BMI (hereafter referred to as direct neurons). However, little is known about the larger network dynamics. By monitoring ensembles of neurons that were either causally linked to BMI control or indirectly involved, we found that proficient neuroprosthetic control is associated with large-scale modifications to the cortical network in macaque monkeys. Specifically, there were changes in the preferred direction of both direct and indirect neurons. Notably, with learning, there was a relative decrease in the net modulation of indirect neural activity in comparison with direct activity. These widespread differential changes in the direct and indirect population activity were markedly stable from one day to the next and readily coexisted with the long-standing cortical network for upper limb control. Thus, the process of learning BMI control is associated with differential modification of neural populations based on their specific relation to movement control.

Multiple studies have shown that, during natural motor control, neurons in motor areas experience a change in their firing properties after visuomotor adaptation[1–3] or adaptation to a new dynamical environment[4–8]. Although the observed changes in neural activity are closely linked to improvements in performance, it remains difficult to place such modifications in the context of the large cortical network for motor control. For instance, the specific neural correlates of learning and memory formation may depend on a neuron's causal role in movement control.

BMIs[9–23] offer the possibility of understanding the cortical network dynamics associated with learning to move a novel actuator in awake-behaving primates. During 'brain control', actuator movements are causally linked to an ensemble of neurons (direct neurons), typically from the primary motor cortex (M1). Several studies have shown that such direct neurons experience a change in their tuning properties during the process of learning neuroprosthetic control[12,13,21,23].

In contrast, the vast majority of neurons embedded in the larger M1 cortical network do not have a direct projection to the BMI. Although little is known about the function of such 'indirect' neurons during ensemble control, they are hypothesized to have a supportive role during the process of learning and recalling proficient brain control[24]. To characterize the large-scale cortical dynamics associated with learning neuroprosthetic control, we recorded ensembles of M1 neurons while only a subset were assigned to have a causal role during control as direct neurons. We characterized the differential plasticity of neural properties depending on the specific link to movement control.

RESULTS

We trained two macaque monkeys to perform center-out reaching movements using a robotic exoskeleton that constrained movements to the horizontal plane (that is, manual control). Following implantation of microelectrodes, a small ensemble of neurons, typically from the contralateral M1, was randomly selected to be directly linked to BMI control. The remaining neurons were recorded, but were not linked to the BMI (that is, indirect neurons). The spiking activity of the direct ensemble was transformed to motor commands with a linear decoder optimized to predict upper limb movements[11,13,18,23,25]. The animals learned brain control using stable recordings of the direct ensemble across days and a decoder that was held constant after the initial training[23,26,27]. Stability of recordings across days was assessed by measuring the stationarity of spike waveforms and the interspike interval (ISI) distribution[23,28–31]. As an additional measure, we frequently monitored the directional modulation of each unit during manual control sessions.

The monkeys were trained to perform two tasks in brain control during separate experiments. Task 1 was structured to equate initial conditions for manual and brain control and to minimize changes in posture and workspace (**Fig. 1a**)[32,33]. The right upper limb remained in the exoskeleton under both conditions (**Fig. 1a**). During manual control, the animal made physical movements to initiate and complete trials. During brain control, however, the animal first made physical movements to the center target. After a variable hold period, a brain control trial started. During brain control of the computer cursor, the animal was required to hold its arm stationary with the hand in the

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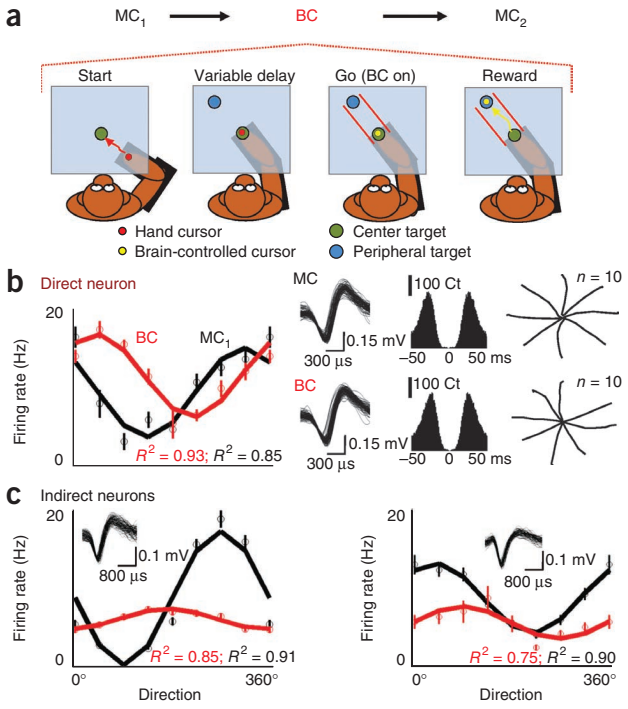

Figure 1 Modification of neural firing properties during brain control. (**a**) For each daily session, subjects were required to serially perform a delayed center-out task in manual control (MC_1), brain control and then manual control again (MC_2). In the brain control task shown, visual guides (that is, lines shown in red) enforced straight trajectories. Trials were started by the animal physically moving to the center target. After a hold period, brain control (absence of any movements) was initiated. (**b**) Changes in the preferred direction of a direct neuron ($P < 0.05$). Solid lines are the cosine fit (R^2 is the percent of variance accounted for by the fit). Circles and bars (s.e.m.) indicate the directional modulation of the firing rate. Right, waveform, crosscorrelograms (0.1% of spikes in a window < 1.5 ms) and the mean trajectories during manual control and brain control. Statistics were performed with bootstrap analysis. (**c**) Changes in the preferred direction of indirect neurons ($P < 0.05$). The directional modulation relationships are arranged similar to **b**. Insets, waveforms of the respective indirect neurons.

center target. Arm kinematics was monitored continuously and the trial was aborted if any motion occurred. We also performed electromyogram (EMG) recordings to rule out muscle contractions during brain control (**Supplementary Fig. 1**). We ensured that the trajectories were comparable using guide lines (**Fig. 1a**). If the cursor moved outside of the lines, the trial was aborted. In contrast with task 1, the second task was similar to past experiments[11–13,19,23] in which the animal's arm was taken out of the exoskeleton and restrained during brain control.

The animals typically developed proficient brain control over time (usually ≥3 d in each experiment; **Supplementary Fig. 2**). It is important to note that although both of these animals had extensive experience with brain control, they required practice to achieve skilled control with a new set of neurons and a given decoder. Task performance during 'late' sessions (that is, ≥3 d of practice) was 86 ± 2% in monkey P and 83 ± 2% in monkey R (mean ± s.e.m.), with a mean time to target of 2.4 ± 0.3 s and 2.8 ± 0.25 s in monkey P (16 late sessions from four experiments) and monkey R (nine late sessions from three experiments), respectively.

Modification of preferred directions

We first analyzed changes in the preferred direction of direct neurons during task 1 (**Fig. 1b**). We found that a significant proportion experienced a change in preferred direction during brain control in comparison with manual control (56 ± 8%, mean ± s.e.m. with a significant change; three sessions with ten neurons each from one experiment in monkey P and three sessions with 15 neurons each from one experiment in monkey P; bootstrap analysis with $P < 0.05$ and a correction for multiple comparisons). When the animals were further trained to rapidly switch between brain control and manual control on a single trial basis (**Supplementary Fig. 3**), there were still substantial shifts in preferred directions (11 of 20 neurons modified with $P < 0.05$, two sessions in monkey R). Moreover, consistent with past experiments[12–14], similar modifications were present during task 2 (61 ± 5%, mean ± s.e.m., eight sessions from four experiments, 10–45 neurons per session, $P < 0.05$ bootstrap analysis). Thus, changes in limb posture and workspace do not exclusively account for the changes in preferred direction after transition to brain control. For subsequent analysis, we combined the datasets from the two tasks.

We next analyzed the indirect neurons (**Fig. 1c**). Notably, we found that indirect neurons also experienced a similar change in their preferred direction in both animals (monkey P, $n = 6$ sessions with 18–25 units per session, 60 ± 6%, mean ± s.e.m.; monkey R, $n = 4$ sessions, 63 ± 10%, mean ± s.e.m. with 10–18 units; $P < 0.05$ bootstrap analysis). To assess specific differences among the population of neurons, we subdivided the indirect neurons (**Fig. 2a**). Indirect neurons recorded on a BMI channel (that is, microwire with a direct neuron) were labeled as 'near' (**Supplementary Fig. 4**). The remaining

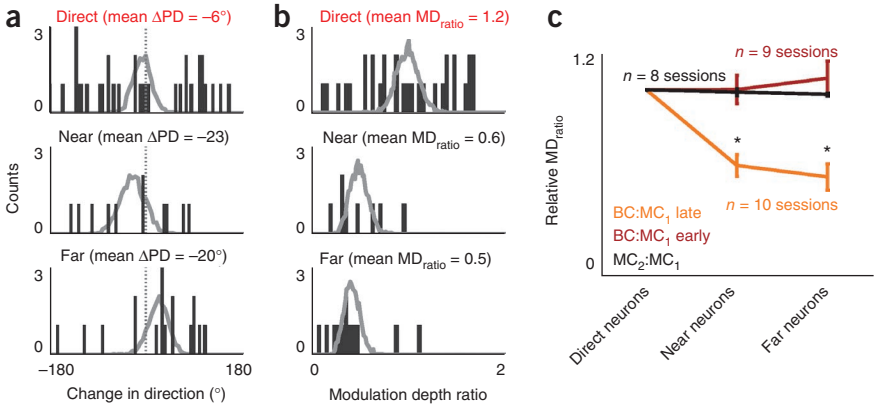

Figure 2 Differential modulation of neuronal populations during brain control. (**a**) Distribution of shifts in preferred directions (ΔPD) between manual control and brain control. Each bar shows the number of neurons (counts) with a corresponding ΔPD. The labels above indicate the mean ΔPD for each population. Superimposed in gray is the bootstrap distribution. (**b**) Distribution of changes in the BC:MC MD_{ratio} for the three neural populations. Data are presented as in **a**. (**c**) Ratio of relative modulation depths. To compare multiple experiments and experimental conditions, we normalized each session to the mean modulation depth ratio for direct neurons. Early and late represent brain control sessions from days 1 and 2 and day 3 and after training, respectively. MC_2:MC_1 is the ratio of modulation depths of the manual control sessions before and after brain control. Error bars indicate s.e.m. $^*P < 0.05$.

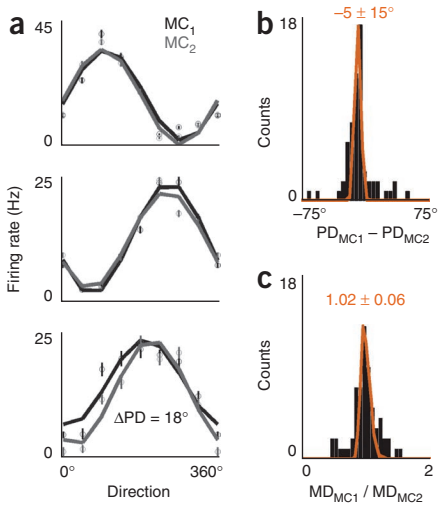

Figure 3 Stability of neural properties. (**a**) Average directional modulation relationship during MC_1 (black) and MC_2 (gray) for three neurons. The neuron in the lower panel experienced a significant change (bootstrap analysis, $P < 0.05$). Error bars show s.e.m. (**b**) Actual (solid bars) and bootstrap (orange, mean ± s.d.) distributions of changes in preferred direction during MC_1 and MC_2. All three neural populations were combined, as they behaved similarly. (**c**) Distributions of modulation depth changes. Data are presented as in **b**.

indirect neurons were labeled as 'far' (that is, recorded on a microwire ~500–700 μm from a BMI channel). We did not find a significant difference between the percentage and the extent of changes in the preferred direction of these two groups ($P > 0.05$, bootstrap analysis). Together, our results indicate that there were large-scale changes in the preferred direction of both direct and indirect neurons after the transition to brain control.

Although the analysis described above focused on individual units, we also examined changes at the population level. For the direct group, although a majority of the individual shifts in preferred direction were significant, the sum of the positive and negative shifts resulted in a nonsignificant net shift, $P > 0.05$ (**Fig. 2a**). There were no significant differences between the direct, near and far populations ($P > 0.05$, bootstrap analysis; **Fig. 2a**). A similar finding was also evident when considering all neurons in both animals (**Supplementary Fig. 5**). Thus, it appears that there is a relative remapping of the preferred directions without any substantial systematic rotational shifts for each neural population.

Differential modification of modulation depths

We next examined changes in modulation depth. For each neuron, we calculated the ratio of modulation depths between brain control and the first manual control session (BC:MC_1 MD_{ratio}). We initially focused on sessions with proficient task performance (that is, late sessions). During these brain control sessions, indirect neurons were less modulated than they were during manual control (**Figs. 1c** and **2b**,**c**). Consistent with previous findings[22,23,34], there was some heterogeneity in the direct population responses. In contrast, both of the indirect populations experienced a consistent net relative reduction in the modulation depth ratio (**Fig. 2b**).

We compared population means across multiple experiments. The mean BC:MC_1 modulation depth ratios were 1.2, 0.6 and 0.5 for the direct, near and far populations, respectively (**Fig. 2b**). The median values were 1.2, 0.5 and 0.5, respectively. Only the near and far groups showed a significant decrease ($P < 0.05$). In addition, when we varied the time window for measurement of directional tuning, we reached the same conclusion (**Supplementary Figs. 6** and **7**).

Across six experiments in both animals, we observed a consistent difference between the relative mean modulation depths of the direct and indirect neuronal populations (BC:MC_1 late, ten sessions from six experiments in monkey P and monkey R; **Fig. 2c**). To our surprise, the units with close proximity to direct neurons behaved similarly to more distant neurons. These differences emerged on stabilization of task performance (BC:MC_1 early versus BC:MC_1 late, $P < 0.05$ for near and far populations, nine sessions taken from six experiments in both monkey P and monkey R; **Fig. 2c**). Together, our results indicate that differential modulation of the neuronal populations was specifically present during proficient neuroprosthetic control and not during the initial learning period.

In addition, there were changes in the mean firing rate of individual neurons when comparing manual control with brain control (**Fig. 1** and **Supplementary Fig. 4**). These changes appeared to be independent of the changes in modulation depth (**Supplementary Fig. 4**). Although some neurons experienced a combined decrease in the mean firing rate and the modulation depth (**Fig. 1c**), other neurons experienced a change in the modulation depth while the mean firing rate remained unchanged (**Supplementary Fig. 4**). At the population level, however, there were no significant systematic differences in the mean firing rate between manual control and brain control for either the direct or the indirect populations ($n = 6$ experiments, $P > 0.05$, bootstrap analysis).

State-dependent modification of neural properties

As described above, the subjects performed manual control both before and after brain control. Thus, comparison of modulation depth during MC_1 and the second manual control session (MC_2) could reveal any lasting effects of the modifications during brain control. For example, studies of motor learning have documented the neural correlates of a memory trace after motor learning[4]. Notably, there was no significant difference between the direct, near and far groups for this comparison ($P > 0.05$, bootstrap analysis, MC_1:MC_2 MD_{ratio}; **Fig. 2c**). This indicates that the population modulation depth during manual

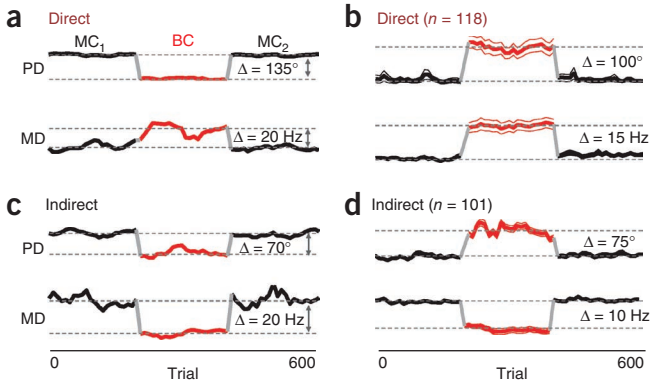

Figure 4 Stability of state-dependent changes in neural properties during a session. (**a**) Traces show the preferred direction and modulation depth for a moving window of trials (window of 16 trials) for a direct unit. Each segment is color coded and labeled (MC_1, BC, MC_2). (**b**) Average of multiple direct units from both animals. To illustrate the time course at the population level, the respective mean MC_1 value was subtracted from each individual trace and the absolute value was used for the average. n = number of units included in the average. Each plot shows the mean (thick line) ± 2 s.e.m. (thin line). (**c**,**d**) Individual example (**c**) and average (**d**) responses of indirect units. Data are presented as in **a** and **b**.

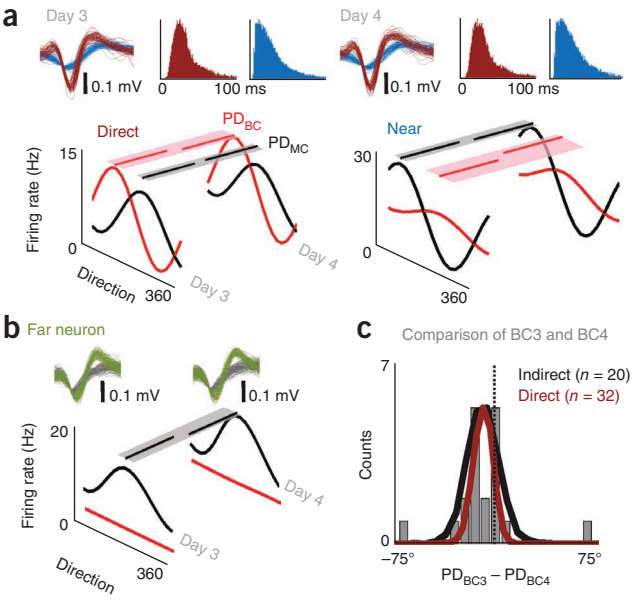

Figure 5 Stability of neural properties across consecutive days of brain control. (**a**) Average directional modulation relationship for a direct and near unit during manual control and brain control on 2 consecutive days. Partial lines above each tuning curve represent the respective preferred direction for each daily brain control (PD_{BC}) and manual control (PD_{MC}) session. The shaded region is the respective variance of the bootstrap distributions of PD_{BC} and PD_{MC}. Waveforms and interspike interval distributions from a direct (red) and near (blue) unit on consecutive days are also shown. (**b**) Directional modulation of a far unit on 2 consecutive days. PD_{BC} could not be estimated because of a lack of modulation. (**c**) Population distribution of preferred direction changes for indirect and direct neurons (PD_{BC3}–PD_{BC4}). For indirect units, the actual (gray bars) and bootstrap (black line) distributions are shown. The dark red line is the bootstrap distribution for direct units. Dotted vertical line represents a ΔPD of 0.

control, both before and after the brain control, was very similar. Moreover, the MC_2:MC_1 MD_{ratio} was significantly different from the BC:MC_1 late relationship for both the near and far neurons ($P < 0.05$, bootstrap analysis, eight sessions taken from six experiments). This further implies that the population modulation depth reverts back to its original properties during the manual control task.

We subsequently assessed for differences at the level of individual units. The vast majority of units did not experience a significant change ($P > 0.05$) in preferred direction between MC_1 and MC_2 (**Fig. 3a**). All three neural populations were combined, as no significant differences were evident for each separate comparison. In general, we found that the vast majority of neurons reverted back to their task-related firing patterns during MC_2 in comparison with MC_1 (89 ± 5% and 83 ± 4.5%, mean ± s.d., without significant changes, $P > 0.05$, in preferred direction and modulation depth, respectively, $n = 6$ experiments). We also did not find evidence of significant differences for manual control sessions associated with 'early brain control' (87 ± 8% and 80 ± 3%, mean ± s.d., $P > 0.05$, without changes in preferred direction and modulation depth, $n = 6$ experiments). Moreover, the presence of a unimodal distribution of changes (**Fig. 3b**,**c**) suggests a small degree of instability of the neuron-behavior relationship during the two sessions[7,28,35,36]. Alternatively, these changes could reflect subtle changes in task performance.

What are the neural dynamics of switching (that is, MC_1 → brain control → MC_2)? We measured the directional modulation relationship across sessions. For each transition, relatively rapid changes in preferred direction and modulation depth were evident for direct units (**Fig. 4a**,**b**). Similar dynamics were evident for indirect neurons, albeit with a reduction of modulation (**Fig. 4c**,**d**). Moreover, the properties of both direct and indirect neurons remained relatively stable during each state.

Stability of indirect neural properties across days

To further test the link between indirect units and brain control, we examined their properties across consecutive days of proficient brain control. For instance, if the properties of the indirect population remain constant across days of proficient brain control, then this would suggest that they have an active role. We selected a population of stable indirect neurons, all of which had stable waveform shapes, ISI distribution and preferred direction during manual control (**Fig. 5a**). The activities of these neurons were compared across two consecutive days of brain control (task performance: day 3, 97%; day 4, 98%). There was no significant difference in either the preferred direction or modulation depth for these examples ($P > 0.05$, bootstrap analysis). There were also neurons that were robustly modulated during manual control, but were consistently not modulated during each daily brain control session (**Fig. 5b**). In general, individual indirect neurons maintained a relatively fixed neuron-behavior relationship for consecutive days of brain control (comparison of $n = 3$ experiments; percentage of neurons with stable parameters: preferred direction, 87 ± 4%; modulation depth, 81 ± 2%; $n = 16$–20 indirect neurons). Notably, this was not significantly different from the neuron-behavior relationship for manual control described above ($P > 0.05$, bootstrap analysis).

We also compared the distribution of changes across days for both direct and indirect neurons at the population level (**Fig. 5c**). Notably, the indirect neuron distribution was also not significantly different from that for direct neurons (indirect, −19 ± 12°; direct, −15 ± 9°; mean ± s.d., $P > 0.05$). Across multiple experiments, we also found that the population dynamics were very similar across consecutive days (indirect, 0.6 ± 11°; direct, −4 ± 10°; mean ± s.d., $n = 3$ experiments). This was also evident for the modulation depth ratio distributions (indirect, 1.0 ± 0.14; direct, 1.07 ± 0.15; mean ± s.d., $n = 3$ experiments). Together, these data indicate that indirect neurons maintained a relatively fixed neuron-behavior relationship during brain control. The similarity with the direct neurons further suggests that the indirect population may have an active role during brain control.

DISCUSSION

Our results show that large-scale modifications of the motor cortex network are associated with learning neuroprosthetic control. We consistently observed that learning brain control was linked to modifications of both direct and indirect neurons. Although a similar fraction of both neural populations experienced a change in preferred direction, there were clear differences in their relative modulation. Thus, the process of learning neuroprosthetic control differentially modifies groups of neurons on the basis of their causal relation to movements. Notably, these large-scale changes were markedly stable over time and readily coexisted with the cortical activity patterns associated with actual upper limb movements.

Large-scale modifications associated with learning

One goal of the field of BMI is to allow skilled control of an external artificial actuator while minimizing the learning required[12,23,37]. A related hypothesis is that by tapping the existing cortical network

for manual control, brain control can be achieved in a rapid and intuitive manner. In support of this possibility are studies demonstrating that motor cortex spiking can be dissociated from movements[21,38], imagined movements result in patterns of activity in the absence of movement[18] and an arbitrary activity pattern may be achieved through learning[21,23]. Past research has evaluated brain control with biomimetic decoders that capture the relationship between neural activity and a movement parameter[11–13,18]. Multiple studies, however, have reported that learning is required to achieve skilled control[12,13,18,23,26].

We also observed a requirement for learning when a new set of neurons and a decoder were introduced[23,27]. Moreover, we noted shifts in the preferred direction of both direct and indirect neurons. Our results also show for the first time, to the best of our knowledge, that the surrounding indirect neurons are differentially modified. This was evident only after stabilization of performance and not during the initial learning process. Stability of recordings and the decoder are likely to be important for skill acquisition and the observed neural modifications[23,27]. It is important to note, however, that the majority of BMI studies have not used such conditions, instead relying on decoders that are retrained daily[12,13,27,39]. It remains unclear how the indirect neurons are modified under those conditions.

What is the role of indirect neurons? The stability of indirect neural properties suggests that indirect neurons have an active supportive role in neuroprosthetic control. Our analysis of individual neurons indicates that such stability is present over long daily sessions as well for sessions on subsequent days. Notably, the stability of both direct and indirect neural properties is quite similar. This implies that, although the decoders cannot 'translate' indirect neural activity, this activity may shape the direct activity. However, it also remains possible that indirect activity may have a negative role. In this viewpoint, our observed reduction in modulation depth may allow more efficient brain control by avoiding interference with the direct neurons[22,24]. This may be related to the 'reweighting' phenomenon after perturbations to the decoder[22].

Changes in directional tuning with learning

Both indirect and direct populations experienced similar changes in preferred direction. Moreover, at the population level, there was no significant rotation. Notably, these widespread changes were closely linked to the process of learning. For example, both groups experienced an overall stabilization of tuning properties when task performance plateaued. This was also evident for neurons across days of brain control. This association suggests a link between learning neuroprosthetic control and the observed changes in preferred direction. After the initial switch to brain control during early sessions, performance was typically poor. This implies that the ensemble tuning properties (that is, those present during manual control) are not sufficient. It is reasonable to assume that error-correction mechanisms are recruited over this period. It is thus possible that the observed shifts in preferred direction are the result of cortical mechanisms to minimize task-related errors. This notion is supported by our recent finding of a strong correlation between the extent of instability of direct neural tuning properties and task performance during long-term brain control[23].

We also designed a task to closely approximate the initial conditions for manual control and brain control (that is, task 1). This task minimized the possibility that changes in limb position and posture[32,33] could by themselves result in widespread changes in preferred direction. With this task, we still noted large-scale changes. Notably, even when initial conditions were such that the animal could not predict an upcoming trial (that is, single trial switching between manual and brain control; **Supplementary Fig. 3**), changes in preferred direction were still evident. These results suggest that differences in initial conditions cannot completely account for the observed change in neural properties. It remains possible that differences emerging after the onset of movements (for example, absence of limb dynamics and proprioception) are triggers for the changes in neural properties. However, past studies have also found that brain control in the presence of limb movements (that is, where proprioception and limb dynamics are likely to be present in some form) have also resulted in modifications[13,40]. Moreover, changes in sensory responses should affect both the indirect and direct neurons equally (that is, a global change). Our observed differential modulation is not consistent with this possibility.

Differential modulation of direct and indirect populations

The distinction of direct versus indirect is an externally imposed causal link to cursor movements via the decoder. Although these populations were similarly modulated before learning brain control, stable skill acquisition was associated with differential modulation. Thus, learning proficient control through error-correction processes and visual feedback appears to be capable of differentially modifying populations of units with a causal link to movements.

Our finding is closely related to previous results, albeit at the level of neural ensembles, on modifications of single neurons and pairs of neurons through operant conditioning[21,24,41]. Studies of operant conditioning of single neurons found that nonconditioned adjacent neurons were largely correlated with the conditioned neurons[24,41]. Recent theoretical work suggests that spike timing–based plasticity could underlie changes in neural activity through operant conditioning[42]. Apparent differences in comparison to our findings may be the result of two factors. First, differential modulation was only evident after several days of practice. Our analysis of early sessions could be consistent with the correlated changes seen with daily conditioning of individual neurons. Second, learning neuroprosthetic control with larger ensembles may not be compatible with strategies that trigger correlated increases in neural activity.

Moreover, recent work on changes in neural activity in response to decoder perturbations suggest that error-correcting mechanisms can partially establish a link between neurons and their specific contributions to errors during brain control[22]. This finding may be related to our observation of differential modulation. Neural mechanisms of error correction are almost certainly recruited by this process. It seems reasonable to hypothesize that a common mechanism underlies both the initial establishment of proficient control as well as adjustments after a perturbation. Especially given the casual link between direct activity and cursor movements, direct neurons are more likely to contribute to errors than indirect neurons.

Reversibility of the modifications

The observed large-scale modifications were reversible in a state-dependent manner. Although several studies have documented changes in neural properties during brain control[12–14,21–23], the time course and reversibility of such changes remained unclear. We found that modifications to both direct and indirect neurons were rapidly reversible. This indicates that proficient neuroprosthetic control is associated with the formation of a cortical state that readily coexists with the long-standing network for natural motor control. When switching between control states, the cortical network appears to rapidly switch, without interference, based on task requirements.

Such rapid reversibility may contrast with the network changes associated with adaptation to novel force fields[4,5]. In a previous study, after adaptation to a new dynamical environment, motor cortex appeared to retain a 'memory trace' evident at the level of single neurons[5]. Although this may suggest a difference between motor adaptation versus neuroprosthetic learning, there are several factors that could account for the differences. Two such factors are the amount of time spent learning the task and the electrophysiological recording technique (for example, acute single neuron recordings versus chronic recordings could target different neuronal populations). In general, the exact mechanisms that allow for apparently rapid changes to cortical properties when switching control states remain unclear. They may be related to existing cortical mechanisms for switching among states during natural motor control[43]. It also points to the general ability to maintain multiple neuron-behavior relationships without interference[23,28,36].

In summary, our results indicate that learning neuroprosthetic control is associated with differential modulation of neuronal populations based on its causal link to movement control. Moreover, proficient control is linked to the formation of a stable large-scale set of neural activations.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureneuroscience/.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

K.G. and J.M.C. designed the experiments. K.G. and J.M.C. performed behavioral training. K.G. performed the experiments and analyzed the data. K.G. and J.M.C. wrote the paper. D.F.D., J.D.W., J.M.C. and K.G. performed surgical procedures. K.G., J.D.W. and J.M.C. revised the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Surgery. Two adult male rhesus monkeys (*Macaca mulatta*) were chronically implanted bilaterally in primary motor and premotor cortex with 3–4 arrays of 64 teflon-coated tungsten microelectrodes (8 × 8 array separated by ~500 μm, Innovative Neurophysiology; **Supplementary Fig. 4**). The specific location of implants in these two monkeys (monkeys P and R) was described previously[23]. All procedures were conducted in compliance with the US National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the University of California at Berkeley Institutional Animal Care and Use Committee.

Extracellular unit recordings. Unit activity was recorded using the MAP system (Plexon). Activity was sorted on-line before recording sessions (Sort-client, Plexon). Our experiments exclusively used recordings from contralateral M1. Near, far and direct units were from the contralateral M1. Two previously published datasets[20] were also included in the analysis. One dataset was from the ipsilateral M1 and the other was from the contralateral M1.

Consistent with previous reports[12,23,28–30], several months post-surgery, we found a subset of units whose waveform shape, amplitude and relationship to other units on a channel varied little from day to day (that is, sorting template in Sort-client required no modifications; **Fig. 5a**,**b**). The stationarity of such properties was the first criterion for a putative stable unit. Offline, we confirmed stationarity using principal component analysis (Wavetracker, Plexon). We also examined the ISI distribution. A Kolmogorov-Smirnov test was used to compare ISI distributions. After sorting, units with a clear refractory period (1.5–2 ms) were designated as putative single units. We also estimated the preferred direction of stable units as an additional measure of recording stability (see below).

Electromyography. Surface gold disc electrodes (Grass Technologies) were mounted on medical adhesive tape and placed on the skin overlying muscle groups. Muscle groups tested included pectoralis major, biceps, deltoid, triceps, trapezius, and forearm extensors and flexor muscles. EMG signals were amplified 10,000-fold (Grass Technologies). Offline, signals were high-pass filtered, rectified and smoothed by convolution with a 25-ms triangular kernel (Matlab, R2009b). Directional activation was estimated using the activity in a 300-ms window after movement onset.

Experimental setup and behavioral training. Monkeys were trained to perform a center-out delayed reaching task using the Kinarm exoskeleton (BKIN Technologies). The behavioral task consisted of hand movements from a center target to one of eight targets distributed over a circle 14 cm in diameter (that is, manual control). Target radius was 0.75 cm. Trials were initiated by entering the center and holding for a variable period (500–1,000 ms). The GO cue (center changed color) was provided after the hold period. A liquid reward was provided after a successful reach. Visual feedback of hand position was provided by a cursor precisely co-located with the center of the hand (radius of 0.5 cm).

Decoding parameters from neural ensembles. We used linear regression to map neural activity to kinematic parameters[23,26]. This was performed using functions available in Matlab (R2009b). For a subset of experiments (brain control task 2), the neural activity predicted joint position. These values were then converted into Cartesian coordinates. The cursor position was updated on the Kinarm projection screen at 10 Hz. We also tested direct prediction of hand velocity in Cartesian coordinates (brain control task 1; **Fig. 1a**). Neural activity was streamed over a local intranet via the PLEXNET client-server application (Plexon) and converted into 100-ms bins of spiking activity.

The number of neurons incorporated ranged from 10 to 45. This variability was a function of several factors. Primarily, we were limited by the availability of well-isolated neurons. The yield slowly decreased over time after implantation. The second limitation was the stability of an ensemble. Prior to each experiment, we monitored the activity in one array over days to identify possible stable units. Although such monitoring increased the probability of recording a stable ensemble, it did not guarantee stability. Thus, there were numerous failed experiments resulting from an inability to record the ensemble over the desired time period. The other factor was the need to monitor both direct and indirect neurons during the course of learning brain control.

We ensured that well-isolated units were part of both the direct and indirect populations. **Supplementary Figure 8** compares the properties of the two groups from one experiment (same experiment as shown in **Fig. 2a**). Using the linear decoder described above, we also compared the ability of randomly selected neurons from either the direct or indirect population to predict limb movement parameters. After running 100 such comparisons, we found that the mean correlation between the actual and predicted limb positions was very similar (direct population, $R = 0.77 \pm 0.06$; indirect population, $R = 0.74 \pm 0.05$; mean ± 2 s.d.).

Online brain control. As noted above, these animals had been previously trained to perform brain control task 2 (ref. 23). The task structure for brain control task 1 was new to them. New brain control experiments were performed over short periods of time (typically 3–6 d). There was consistent evidence of improvements in performance with practice (also see **Supplementary Fig. 2**). Animals were permitted to use a fixed decoder (that is, held constant after initial training on day 1) and stable recordings from a neural ensemble[23,27]. Each experiment consisted of a new set of stable neurons and a decoder that was fixed after training on day 1. Each experiment consisted of multiple daily sessions.

During brain control task 1, the animal kept his right upper limb in the exoskeleton. The animals were required to move their hand to the center target to start a trial and to keep it in the center target at all times (**Fig. 1**). A new cursor (under brain control) appeared at the start of the trial. The animals were required to move the cursor to the target by modulation of motor cortex activity (under velocity control). Hand position was continuously monitored during brain control. The trial was aborted with any change in hand position. To start a new trial, the animal had to move out of the center target and reposition his hand. Thus, the brain-controlled cursor was reset to the center target for each trial. During selected sessions, we concurrently performed video and surface EMG recordings from proximal and distal muscle groups (**Supplementary Fig. 1**). Monkey R was trained to perform this task in a single trial randomized fashion (switching between manual control and brain control trials; **Supplementary Fig. 3**).

For brain control task 2, the cursor was continuously under brain control. The task-related hand was removed from the exoskeleton and restrained on the side during brain control. The cursor was under continuous volitional control. Subjects were required to self-initiate each trial by moving the brain controlled cursor to the center. A trial was considered incorrect if the cursor failed to reach the target by 10 s after a GO cue. To start a trial, the cursor had to be held over the center target for 250–300 ms. The chance level of self-initiation was ~0.5 per minute. This value was determined through experiments where the task was performed by spontaneous neural activity (that is, the computer monitor was turned off while the cursor was controlled by spontaneous activity). In contrast, while engaged in the task, each subject self-initiated trials at a rate of 3–10 per min.

Data analysis. The majority of analysis was performed on late sessions (defined as sessions ≥ day 3 in an experiment). Early sessions occurred on days 1 and 2.

Preferred direction. Directional tuning was estimated by comparing the mean firing rate as a function of target angle during movement execution. In manual control, the time to target was relatively constant (~700 ms). In brain control, this period was variable and decreased with learning. For the analysis, a 500-ms window was used (starting 200 ms before movement). Our results did not depend on the specific time window (**Supplementary Figs. 6** and **7**).

The tuning curve was estimated by fitting the firing rate with a sine and a cosine as

$$f = [B_1, B_2, B_3] \times \begin{bmatrix} \text{const} \\ \sin\theta \\ \cos\theta \end{bmatrix} \quad (1)$$

where θ corresponds to reach angle and f corresponds to the firing rate across the different angles. Linear regression was used to estimate the B coefficients. The preferred direction was calculated using the following: preferred direction = $\tan^{-1}(B_2/B_3)$, resolved to the correct quadrant[44]. For units with changes in preferred direction, we ensured that regression captured ≥50% of the variance. Thus, the unit shown in **Figure 5b** was not included in the analysis of preferred direction changes.

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Modulation depth. Modulation depth was calculated as the peak-to-peak amplitude of the tuning curve. For **Figure 2c**, we ensured that the tuning fit was appropriate for the manual control trial. The modulation depth of the brain control was computed regardless of the fit. This ensured that units no longer modulated in brain control were included (for example, **Fig. 5b**). The modulation depth ratio was calculated by dividing the modulation depth under two conditions (for example, comparison of brain control/manual control). To compare multiple experiments and experimental conditions, we normalized each experiment to the mean modulation depth ratio for direct neurons. We also tested a nonparametric metric (difference between highest and lowest firing rate). We reached the same conclusion of a relative decrease for the indirect population.

Changes in directional tuning. A bootstrap resampling procedure was used to test significance of modulation depth and preferred direction changes[23,28]. By repeating this 2,000 times, we created a distribution corresponding to the null hypothesis (that is, no change in preferred direction). The confidence intervals were based on the specified *P* value using a percentile bootstrap. Comparison of modulation depth and firing rates were performed in an analogous manner.

Changes in mean preferred direction and mean modulation depth. A bootstrap statistic was also used to compare differences between populations (for example, **Fig. 2b**). The experimental values for each population were sampled with replacement 2,000 times. By taking the mean of each resample, we created a distribution of values. For comparison between conditions, we sampled one value from the respective zero mean distributions to create a distribution of absolute differences. We did not note any bias and the corresponding distributions were symmetric (**Fig. 2b**).

We also tested an alternative tuning measure[23]. Specifically, we reexamined the tuning analysis based on the actual path of the cursor (as opposed to the intended direction). A similar percentage of units experienced a change in preferred direction (path taken: 69 ± 8% versus intended direction: 67 ± 12% mean ± s.d., $n = 10$ sessions, $P > 0.05$). Moreover, a related hypothesis is that changes in individual preferred directions could also manifest as changes in ensemble firing patterns. A preliminary analysis of the reversible changes in firing patterns when switching from manual control to brain control are shown in **Supplementary Fig. 9** (refs. 45,46).

Modifications during a session. Neurons with a significant change were selected. To estimate values over the course of MC_1/BC/MC_2 trials, a moving window of trials (two sets of trials to each of eight targets) was used. Each individual parameter was then plotted over time (**Fig. 4a**,**c**). To calculate the baseline preferred direction change, we first determined the mean preferred direction during MC_1 for each neuron. This value was subtracted from all values during MC_1/BC/MC_2. Thus, the baseline was 'zeroed' for ease of comparison. As we were interested in examining the rapidity and stability of shifts, we took the absolute value of this. The traces in **Figure 4b**,**d** were the overall average.

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