PNAS RESEARCH ARTICLE | NEUROSCIENCE

Movement-dependent electrical stimulation for volitional strengthening of cortical connections in behaving monkeys

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Edited by Peter Strick, University of Pittsburgh Brain Institute, Pittsburgh, PA; received October 20, 2021; accepted April 29, 2022

Correlated activity of neurons can lead to long-term strengthening or weakening of the connections between them. In addition, the behavioral context, imparted by execution of physical movements or the presence of a reward, can modulate the plasticity induced by Hebbian mechanisms. In the present study, we have combined behavior and induced neuronal correlations to strengthen connections in the motor cortex of adult behaving monkeys. Correlated activity was induced using an electrical-conditioning protocol in which stimuli gated by voluntary movements were used to produce coactivation of neurons at motor-cortical sites involved in those movements. Delivery of movement-dependent stimulation resulted in small increases in the strength of associated cortical connections immediately after conditioning. Remarkably, when paired with further repetition of the movements that gated the conditioning stimuli, there were substantially larger gains in the strength of cortical connections, which occurred in a use-dependent manner, without delivery of additional conditioning stimulation. In the absence of such movements, little change was observed in the strength of motor-cortical connections. Performance of the motor behavior in the absence of conditioning also did not produce any changes in connectivity. Our results show that combining movement-gated stimulation with further natural use of the "conditioned" pathways after stimulation ends can produce use-dependent strengthening of connections in adult primates, highlighting an important role for behavior in cortical plasticity. Our data also provide strong support for combining movement-gated stimulation with use-dependent physical rehabilitation for strengthening connections weakened by a stroke or spinal cord injury.

electrical conditioning | movement | behavior | cortical plasticity | use-dependent plasticity

Significance

We describe an electrical-conditioning protocol in adult behaving monkeys in which stimuli gated by voluntary movements were used to strengthen connections between motor-cortical neurons involved in those movements. Movement-gated stimulation created a plastic landscape in which repetition of the movements that gated conditioning stimuli produced strengthening of cortical connections, in a use-dependent manner, long after stimulation had ended, a finding that is both unique and clinically relevant. In the absence of such behavior, little change was observed in the strength of connections. Similarly, movements alone did not produce changes in connectivity. Our data highlight a critical role for behavior in plasticity and provide strong support for combining movement-gated stimulation with use-dependent rehabilitation for strengthening connections weakened by injury or disease.

Spike-timing-dependent plasticity (STDP) refers to correlated activity of neurons that leads to long-term strengthening or weakening of the connections between them, depending on the order of firing of presynaptic and postsynaptic cells (1–7). Although much evidence supports the necessity of correlated neuronal activity for synaptic plasticity to occur, physical movements and behavioral factors, such as motivation, stress, attention, and reinforcement, which occur at very different timescales from STDP (8), are also known to play crucial roles in modulation of functional plasticity (9–17). This adds a layer of complexity to the neuronal computations underlying plasticity processes and the recovery, mediated by such mechanisms, from an injury to the central nervous system (18, 19). Thorndike (20, 21) argued that a connection is significantly modified only when associated with outcomes important to the animal's behavior, suggesting a volitional dimension to the control of plasticity, perhaps through the release of neuromodulators such as dopamine and acetylcholine that play important roles in reward (22, 23) and attention (24, 25) circuits, respectively. Correlated activity of neurons, while often necessary, is not always sufficient to induce plasticity. However, a relatively small number of studies have explored the role of behavioral context in the regulation of synaptic plasticity (9–14, 17, 19).

Motivated by the Hebb–Stent (or STDP) learning rule (1–5) and Thorndike's law of effect (20), we sought to determine the extent to which an activity-dependent stimulation protocol that produced coactivation of neurons at two motor-cortical sites could be exploited to modulate the connectivity between them, both in the presence and absence of a relevant behavioral context. While a number of laboratories (summarized in ref. 26), including ours, have developed conditioning paradigms for inducing STDP, the role of behavior in synaptic plasticity remains largely uninvestigated. To dissect this role, we implemented an electrical-conditioning paradigm in adult behaving monkeys in which stimuli were delivered during volitional movements that activated neurons at a presynaptic site. The gated stimuli were delivered to a postsynaptic site in an attempt to boost the firing of postsynaptic neurons while the presynaptic neurons were active. Delivery of movement-gated stimulation resulted in small increases in the strength of cortical connections immediately after conditioning. Remarkably, when

Author contributions: S.M. conceived the original idea; S.M., S.W., E.E.F., and S.I.P. designed research; S.M. and S.W. performed research; S.M. and S.W. analyzed data; and S.M., E.E.F., and S.I.P. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2116321119/-/DCSupplemental.

Published June 27, 2022.

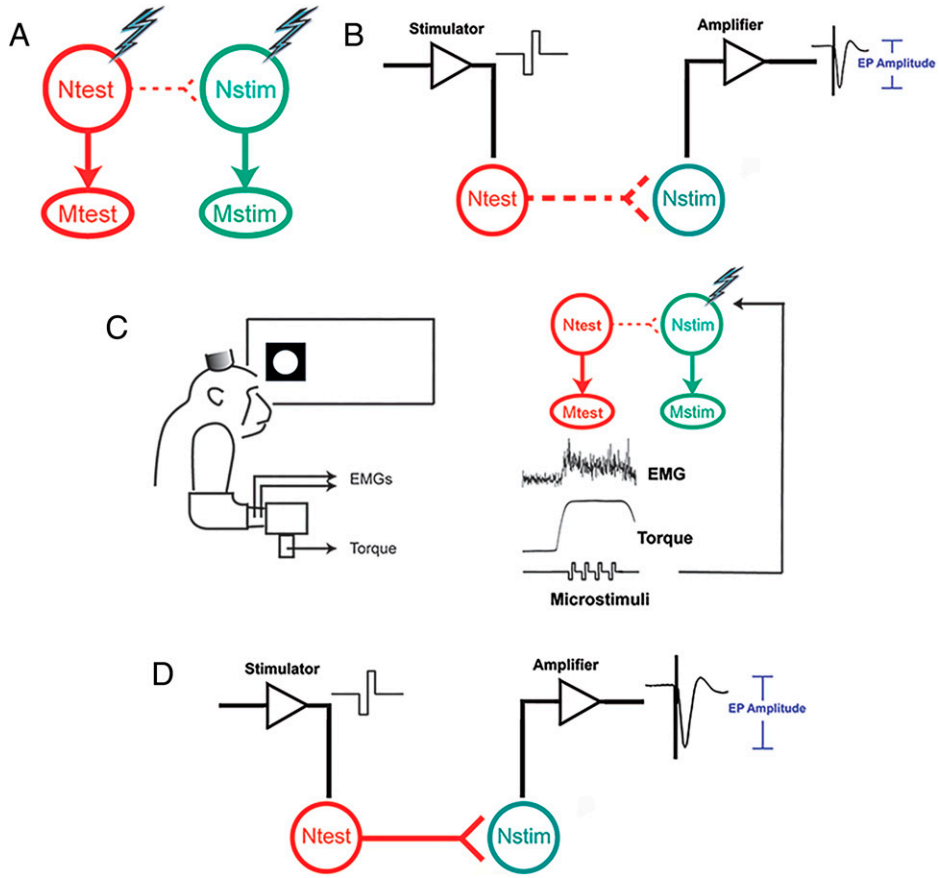

Fig. 1. Electrical-conditioning protocol and experimental design. (*A*) Trains of microstimuli were delivered at two sites in the motor cortex, Ntest and Nstim, to document their motor outputs (Mtest and Mstim, respectively). (*C*) During conditioning, cursor hold inside the Mtest target gated delivery of (single) stimuli to Nstim, while the monkey performed a wrist flexion–extension target-tracking task. (*B* and *D*) Setup for assessing conditioning changes. Cortical responses at Nstim evoked by delivery of single test stimuli at Ntest were used to document changes in the strength of the connection from Ntest to Nstim before (*B*) and after (*D*) delivery of movement-gated stimulation.

paired with further repetition of the movements that gated the conditioning stimuli, there were substantially larger increases in the strength of connections, without additional delivery of conditioning stimulation. Importantly, neither behavior alone nor conditioning alone produced similar effects. Note that the term "behavior," as used here, involves both voluntary movements and accompanying behavioral modulators, such as stress, motivation, attention, and reinforcement. Each of these variables may play distinct roles in modulation of plasticity (9–19), but they were not controlled or differentiated in our study.

Our results suggest that movement-gated stimulation creates a plastic landscape in which repetition of the behavioral context presented during conditioning drives cortical strengthening long after stimulation has ended. Taken together, our data highlight a crucial role for behavior in modulation of synaptic plasticity. They also provide support for combining movement-gated stimulation with use-dependent physical rehabilitation for strengthening motor pathways weakened by injury or disease.

Results

Movement-Dependent Stimulation Protocol and Experimental Design. Two adult monkeys (Y and U) were trained to perform a randomly alternating center-out wrist flexion–extension target-tracking task with their right hands. Monkeys were trained to hold the cursor inside presented targets for 1.6 s and were rewarded with fruit sauce at a variable reinforcement ratio after successful completion of trials. After learning the target-tracking task, monkeys received chronic bilateral implants, consisting of custom-made electrode arrays (whose design is described in ref. 27; also see *SI Appendix, SI Materials and Methods*), with platinum-iridium (Pt/Ir) microwires targeting sites in layer V of the sensorimotor cortex. All tested site pairs were in the left primary motor cortex in both animals, contralateral to their responding hands.

Motor outputs of two reciprocally connected neuronal sites, termed Ntest and Nstim, in the primary motor cortex were assessed using trains of intracortical microstimulation (ICMS; for details, see *SI Appendix, SI Materials and Methods* and Fig. 1*A*). Movement-related activity at Ntest and Nstim was inferred from their motor outputs (Mtest and Mstim, respectively). The strength of synaptic connections between cortical sites was documented by the size of evoked potentials [EPs (28, 29)] in local-field-potential (LFP) recordings, referred to as EP amplitude, recorded at one site after biphasic charge-balanced single test stimuli were delivered at a second site (Fig. 1 *B* and *D*). The perturbation of activity in one set of neurons followed by the quantification of its impact, or the evoked response, at other sites provides a directed approach to assess connectivity within and across brain regions, given a direct correlation between EP amplitudes and the strength of connections (28).

Movement-gated stimulation was delivered to Nstim during movements that were expected to activate neurons at Ntest. The conditioning stimulation likely boosted the firing of neurons at Nstim and produced coactivation of the two cortical sites. To achieve this, delivery of (single) conditioning stimuli

at Nstim was gated by cursor hold inside the Mtest target. For example, if the motor output of Ntest was flexion, the monkey received flexion-gated stimulation at Nstim while the cursor was held inside the flexion target, as shown in Fig. 1*C*. Hence, conditioning stimuli were delivered during the plateau phase of torque or the tonic phase of Mtest electromyographic (EMG) activity. Movement-gated stimulation was delivered at 10 Hz at an intensity that was subthreshold for movement, as long as the cursor remained in the Mtest target. Delivery of conditioning was preceded and followed by delivery of (single) test stimuli, which were also subthreshold for movement, at Ntest to assess the strength of its connection to Nstim (Fig. 1 *B* and *D*). A detailed description of how conditioning and test currents were chosen in our experiments is included in *SI Appendix*, *SI Materials and Methods*.

Thus, two types of electrical stimuli were delivered in our experiments: (single) movement-gated stimuli, which were used for conditioning, and test stimuli, which were used to document motor outputs and the strength of cortical connections. Conditioning stimuli were delivered during wrist flexion or extension; test stimuli were always delivered with the monkey's wrist at rest.

Immediate Effects of Movement-Gated Conditioning. Fig. 2 shows stimulus-triggered averages (StTAs) of cortical field potentials at Nstim evoked by delivery of single test stimuli at Ntest. The time course of the experimental session is shown in Fig. 2, *Inset*. Here, movement-gated stimulation was delivered for 90 min and was preceded and followed by delivery of test stimuli to document changes in connectivity from Ntest to Nstim. Single test stimuli were delivered at several time points after conditioning up to an hour. The monkey performed the flexion–extension target-tracking task throughout the session. Connectivity changes were quantified as percent changes in the average EP amplitude after conditioning relative to the preconditioning level, according to the following equation:

$$\text{Percent Change in EP Amplitude} = \frac{(\text{Average Postconditioning EP Amplitude} - \text{Average Preconditioning EP Amplitude})}{\text{Average Preconditioning EP Amplitude}} \times 100.$$

Delivery of movement-gated stimulation produced a 34% increase in the strength of the connection from Ntest to Nstim when assessed 5 min after the end of conditioning (cf blue and red traces in Fig. 2). Notably, the EP amplitude continued to grow in an incremental manner over time, while the monkey performed the wrist task, during the postconditioning period (instead of decaying back to the preconditioning level). At the 60-min time point (orange trace), the EP was 54% larger compared to the preconditioning level (blue trace), suggesting a role for postconditioning behavior in modulation of conditioning-induced strengthening of cortical connections.

Movement-Gated Conditioning Combined with Postconditioning Behavior Produces Further Strengthening of Cortical Connections. To assess the role of postconditioning behavior in modulation of conditioning-induced cortical plasticity and examine the time course of washout, we documented connectivity changes for 2 wk after conditioning. During the 2 wk, we conducted 2.5-h behavioral sessions every weekday. Single test stimuli were delivered at multiple time points during these sessions to assess the strength of the connection from Ntest to Nstim while monkeys performed the wrist target-tracking task. Note that no additional conditioning stimulation was delivered during these behavioral sessions.

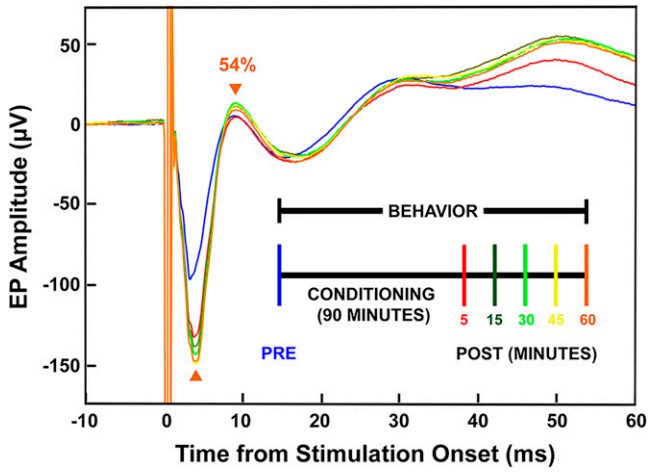

Fig. 2. Postconditioning behavior modulates conditioning-induced cortical plasticity. StTAs of cortical responses were used to document changes in connectivity from Ntest to Nstim. *Inset* shows the time course of the experimental session. Single test stimuli were delivered at several time points (5, 15, 30, 45, and 60 min) after conditioning. The EP amplitude was characterized as the size of the trough-to-peak (indicated by the orange arrowheads) deflection in the early component (≤15 ms) of the evoked response, which was likely the product of a monosynaptic connection from Ntest to Nstim (28). Color of the StTA curves in the plot corresponds to events in the *Inset* timeline.

Fig. 3 shows the experimental timeline (Fig. 3*A*) and connectivity changes (Fig. 3*C*) at a pair of sites in the left primary motor cortex of monkey Y, shown in Fig. 3*B* with Ntest in red and Nstim in green. Preconditioning motor outputs were assessed using trains of ICMS whose delivery at Ntest activated wrist extensors at a lower threshold intensity compared to flexor muscles, so extension-gated stimulation was delivered to Nstim during conditioning. Note that preconditioning delivery of ICMS trains at Nstim activated both wrist flexors and extensors.

Successive performance of the wrist task for days after conditioning produced dramatic strengthening of the connection from Ntest to Nstim, as seen by an increase in the size of cortical potentials evoked at Nstim (Fig. 3*C*). The postconditioning EP amplitudes in many cases were over 100% larger compared to the preconditioning level. Second, the larger postconditioning EPs coincided with higher frequency of wrist extensions, which, in this case, occurred on days 8, 9, and 10 after conditioning. During these 3 d, the size of the EP grew incrementally, both during each session and over the three sessions, from 90% (on day 8 at $t = 0$ time point; day 8/$t = 0$) to 261% (on day 10/$t = 135$; indicated by the orange asterisk), which was the maximum gain observed during the 15-d conditioning-and-behavior experiment. Substantial gains were also seen both before (on days 0, 1, 2, and 3) and after this 3-d period. Third, there were changes in the size of the EP between the last (i.e., $t = 135$) time point of a behavioral session and the first (i.e., $t = 0$) time point of the subsequent session, possibly due to bidirectional homeostatic adjustments of synaptic strength (30, 31) during sleep (32, 33). This change was more pronounced when the latter session occurred after a weekend and resulted in a decrease in the strength of the connection toward preconditioning levels (cf day 10/$t = 135$ and day 13/$t = 0$ in Fig. 3*C*). Finally, the resultant cortical plasticity persisted for over 2 wk with the size of the EP still 149% larger (or the connection 2.5-fold stronger) on day 14 (at $t = 135$ time point) after conditioning.

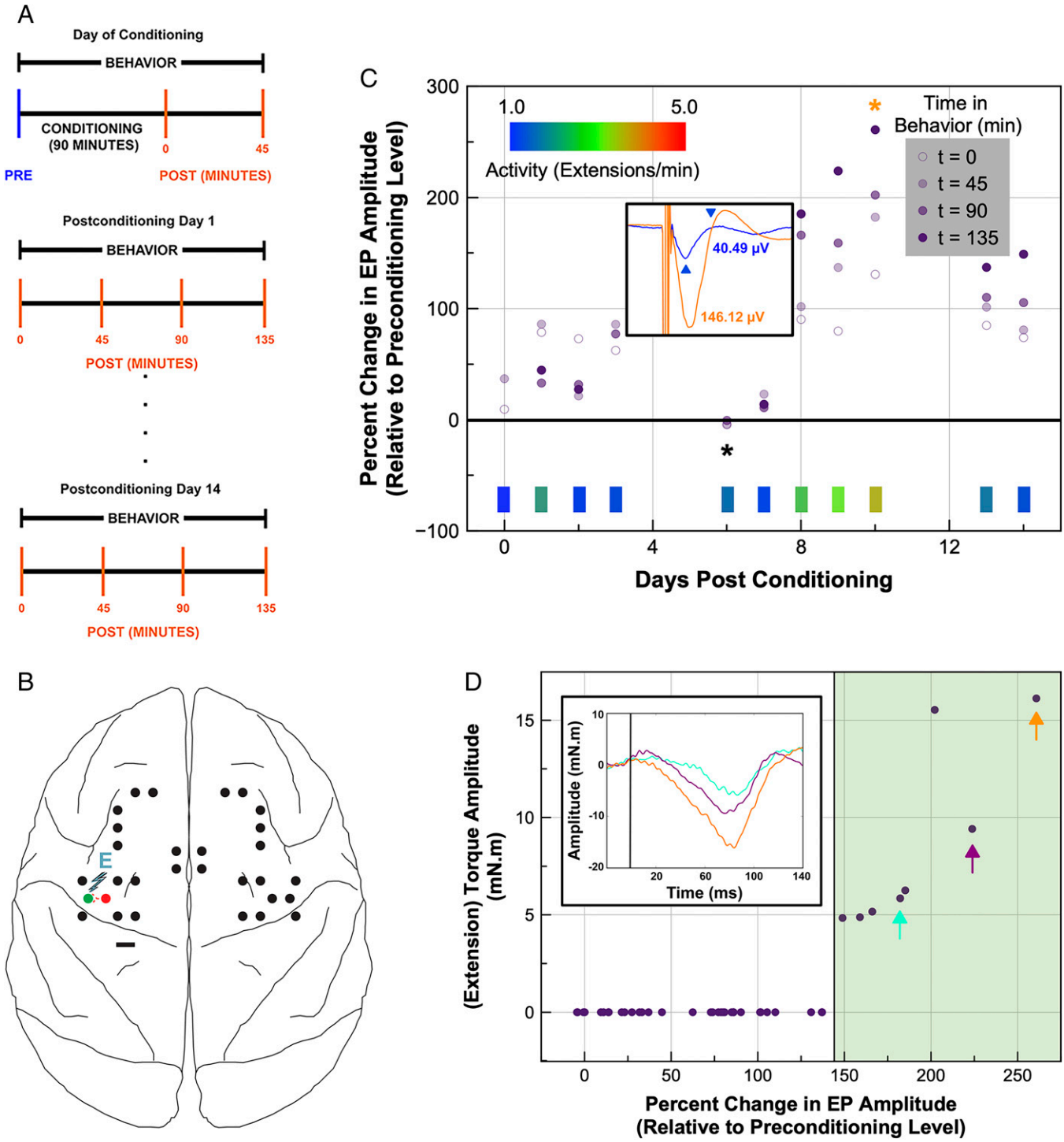

Fig. 3. Movement-gated stimulation combined with behavior produces volitional strengthening of cortical connections. (*A*) Movement-gated conditioning was delivered on day 0 (for 90 min) and was preceded and followed by delivery of single test stimuli on the same day (at two time points; 0 and 45 min) and during behavioral sessions that occurred every weekday over the subsequent 2 wk. Test stimuli were delivered at four time points (0, 45, 90, and 135 min) during these behavioral sessions. (*B*) Schematic of the implant in monkey Y with the conditioning site pair, whose data are shown in *C*, indicated in red and green. Extension-gated stimulation was delivered at Nstim (green site) during conditioning. E, extension. (Scale bar, 3 mm.) (*C*) The percent change in EP amplitude was used to document the effect of combining conditioning and behavior on cortical connectivity during the 15-d experiment. Connectivity was assessed at the orange time points shown in the timeline in *A*, represented here in different shades of violet. Color bars (at the bottom of the plot) overlaid on a graded three-color heatmap (shown in the top left corner) indicate the average frequency of wrist extensions during each behavioral session. The maximum and minimum values of the connectivity dataset are indicated by orange and black asterisks, respectively. *Inset* in the center of the plot shows StTAs of the preconditioning EP (blue) and the EP evoked at the maximum gain (orange), with color-matched labels indicating the respective EP amplitudes. The EP amplitude was characterized as the size of the trough-to-peak (indicated by blue arrowheads) deflection, shown here for the preconditioning EP. The same trough and peak were used to characterize postconditioning amplitudes. Note that behavioral sessions only occurred on weekdays; lack of data on days 4, 5, 11, and 12 is due to their occurrence over weekends. (*D*) Plot shows amplitude of stimulus-triggered extension torques as a function of cortical-connectivity changes. *Inset* shows StTAs of wrist torques (with flexion and extension responses represented by positive and negative deflections, respectively) at three selected cortical-gain values indicated in the main plot by color-matched arrows.

We also investigated the effect of combining conditioning and behavior on the late component of the Ntest-to-Nstim EPs and the motor output of Ntest. At the maximum gain of 261% in the magnitude of the early component (measured between 2.5 and 15.0 ms after stimulus delivery; orange asterisk in Fig. 3*C*), there was a concomitant increase of 315% in the amplitude of the late component of the EP (measured between 15 and 100 ms), relative to the preconditioning level. Output effects were quantified with StTAs of flexion–extension wrist torques (16) evoked by delivery of the same single test stimuli at Ntest that were used for documenting the strength of its connection to Nstim (in Fig. 3*C*). Fig. 3*D* shows the magnitude of torque responses evoked from Ntest as a function of postconditioning cortical-connectivity changes. Preconditioning test stimuli did not evoke any wrist torques (indicated by the origin of the plot), as expected, since currents that were subthreshold for movement were used for assessing connectivity, and test stimuli were always delivered with the monkey's wrist at rest. Delivery of extension-gated conditioning combined with behavior strengthened the horizontal connection from Ntest to Nstim (Fig. 3*C*). No postconditioning torques were evoked when cortical gains were less than 148%. Once this threshold was exceeded (marked by the green-shaded region in the plot), postconditioning single test stimuli, delivered to Ntest, now evoked wrist torques by means of both the horizontal projections to Nstim, which were strengthened by our intervention, and its direct projection to the muscles. The convergence of the two inputs produced greater descending input at the level of the spinal cord, which likely increased firing of motoneurons, such that the same subthreshold (single) test stimuli now evoked wrist torques. In concordance with delivery of extension-gated stimulation (Fig. 3*B*), postconditioning test stimuli evoked extension (as opposed to flexion) torques whose amplitudes were strongly correlated to gains in cortico-cortical connectivity (Pearson's correlation coefficient = 0.84 in the green-shaded region of the plot), demonstrating a reinforcement in the output of motor-cortical sites that was consistent with the potentiation of the synaptic connection between them (6).

Conditioning Alone or Behavior Alone Is Ineffective in Modulating Cortical Connectivity. To delineate the individual contributions of conditioning and behavior, we performed two controls at the same pair of left motor-cortical sites (in monkey Y) shown in Fig. 3*B*. The first control (performed 3 wk after the end of the conditioning-and-behavior experiment) assessed the effect of behavior alone by documenting changes in Ntest-to-Nstim EPs for 15 d without delivery of any conditioning stimulation on day 0 (Fig. 4*A*). The second control assessed the contribution of conditioning through delivery of extension-gated stimulation, followed by documentation of connectivity changes without the monkey performing the wrist task after conditioning on day 0 and during subsequent days. Due to practical challenges associated with monkeys sitting in the experimental recording booth without doing a task or receiving any reinforcements, (single) test stimuli were delivered only at the start of each postconditioning session (i.e., at $t = 0$; Fig. 4*C*) for this control. As shown in Fig. 4 *B* and *D*, both behavior alone and conditioning alone produced small cortical-connectivity gains (≤35%). Importantly, extension-activity levels were comparable across the conditioning-and-behavior and behavior-alone experiments (1.81 ± 0.84 and 2.15 ± 0.82 extensions/min, respectively, mean ± SD; Figs. 3*C* and 4*B*). Additionally, the same amount of test stimulation (at the same current amplitude and frequency and at identical time points; Figs. 3*A* and 4*A*) was delivered during the 15-d behavior-alone control conducted at the same site pair, which rules out any appreciable contribution of the repeated test stimulation (or its interaction with behavior) on the plasticity observed with conditioning-and-behavior (in Fig. 3*C*). Lastly, there was a trend toward depression of the connection over time, especially in the second week, associated with both behavior alone and conditioning alone. These control experiments suggest that conditioning or behavior, in isolation, is relatively ineffective in strengthening cortical connections.

Cortical Strengthening Produced by Combining Conditioning and Behavior Is Direction Specific. In a subset of experiments, we also explored connectivity changes in the reverse direction (i.e., from Nstim to Ntest). Here, single test stimuli were delivered at Nstim to document changes in the strength of its connection to Ntest. We found that modulation of cortical EPs obtained by combining conditioning and behavior was direction specific, producing little to no changes in the reverse direction. *SI Appendix*, Fig. S1 shows connectivity changes in both the forward and reverse directions at a second pair of left motor-cortical sites during another 15-d conditioning-and-behavior experiment. Combining conditioning and behavior at this pair of sites (shown in *SI Appendix*, Fig. S1*A*) produced a maximum gain of 166% in the forward direction (on day $9/t = 0$; indicated by the orange asterisk; *SI Appendix*, Fig. S1*B*), while the maximum gain in the reverse direction was 26% (which occurred on day $2/t = 135$; also indicated by the orange asterisk; *SI Appendix*, Fig. S1*C*).

Connectivity Changes Obtained with Conditioning and Behavior, Delivered Individually or Together, across Cortical Sites. We investigated the effect of conditioning and behavior, delivered individually or together, on the strength of motor-cortical connections in the forward and reverse directions across five to eight site pairs in the two monkeys. Conditioning-and-behavior experiments were conducted at four site pairs in monkey Y and at three site pairs in monkey U (although only six out of those seven site pairs were tested in the reverse direction). The effect of behavior alone was tested at four site pairs in each monkey, while conditioning alone was tested at two site pairs in monkey Y and at three pairs in monkey U. Fig. 5 *A* and *B* document the connectivity changes across site pairs, showing all individual data points, whose means are shown in Fig. 5 *C* and *D*, respectively. Substantial cortical-connectivity gains were obtained only when conditioning was combined with behavior, and these gains were restricted to the forward direction (Fig. 5 *A* and *B*, green data points).

Due to repeated assessment of EP amplitudes over time at individual connections and the presence of unbalanced data (arising from unequal sample sizes across groups), we implemented a linear mixed model to compare changes within and across intervention groups (34), details of which can be found in *SI Appendix*, *SI Materials and Methods*. The linear-mixed-model statistical analyses revealed a significant overall difference between the conditioning-and-behavior and behavior-alone groups ($F_{1,33.226} = 20.462$, $P < 0.0001$; effect size = 1.54, very large effect; Fig. 5*C*), as well as a differential interaction of group type over time ($F_{49,273.248} = 2.611$, $P < 0.0001$), which was also confirmed individually in the two animals (monkey Y: $F_{30,123.364} = 3.355$, $P < 0.0001$; effect size = 1.76, very large effect; and monkey U: $F_{30,115.119} = 1.872$, $P < 0.01$; effect size = 0.95, large effect). Changes obtained by combining conditioning and behavior were also significantly different from conditioning alone ($F_{1,17.220} = 8.143$, $P = 0.01$; effect size = 0.97, large effect; Fig. 5*C*), while there was no significant difference between the behavior-alone and conditioning-alone groups

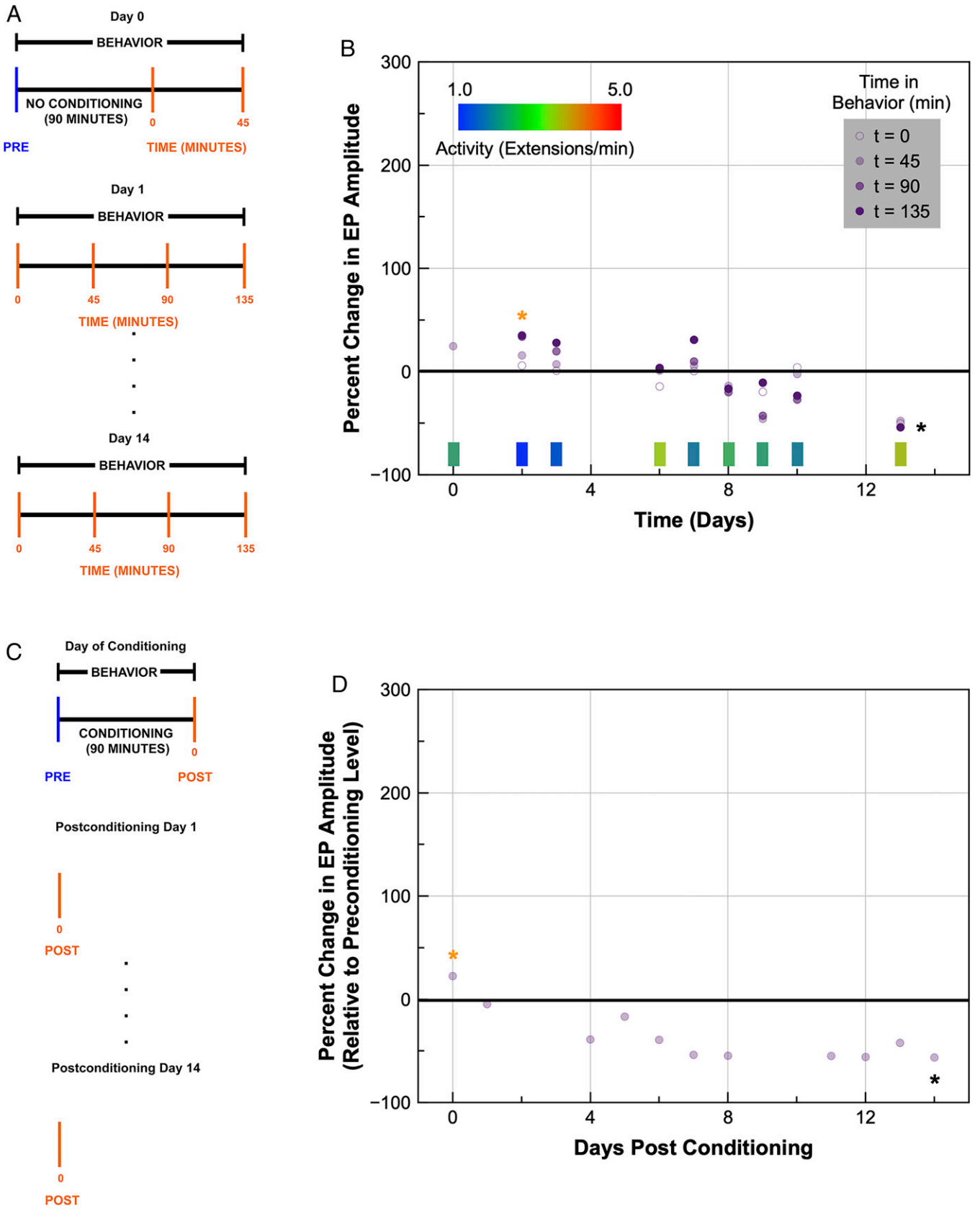

Fig. 4. Behavior alone or conditioning alone is relatively ineffective in modulating cortical connectivity. (*A*) Timeline of behavior-alone control. Single test stimuli were delivered on day 0 and during behavioral sessions (at four time points; 0, 45, 90, and 135 min) that occurred every weekday over the subsequent 2 wk. (*B*) The percent change in EP amplitude, relative to the initial assessment level denoted by "PRE" in *A*, was used to document connectivity changes with behavior alone. Activity *Inset* and color-bar descriptions are the same as in Fig. 3*C*. (*C* and *D*) Timeline and connectivity data for the conditioning-alone control. Data for both plots come from the same pair of left motor-cortical sites shown in Fig. 3*B*. The maxima and minima of the two datasets in *B* and *D* are indicated by orange and black asterisks, respectively.

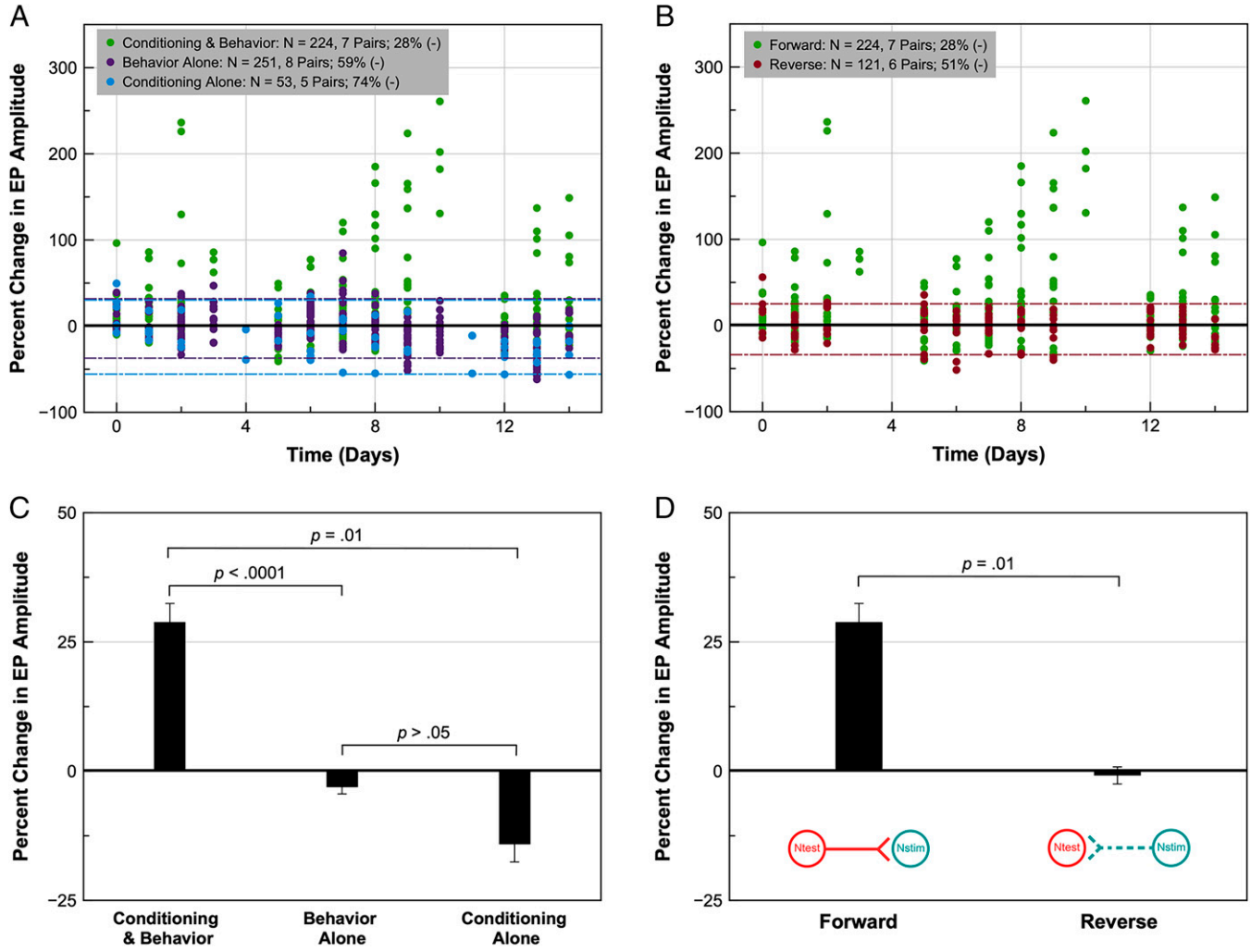

Fig. 5. Connectivity changes obtained with conditioning and/or behavior across cortical sites. (*A* and *C*) The percent changes in EP amplitude in the forward direction produced by conditioning and behavior, delivered individually or together, are shown for all tested site pairs. Individual data points are shown in *A*, with the corresponding means and significance levels shown in *C*. Similarly, *B* and *D* compare connectivity changes observed with conditioning and behavior, delivered together, in the forward and reverse directions. Color-coded dashed lines show 5th and 95th percentiles for the corresponding control distributions in *A* and *B*. Note that the 95th-percentile values for both controls are overlapping in *A*. Sample sizes for all conditions are listed in *A* and *B*, which include both the total number of data points (denoted by "N"), and the corresponding number of cortical site pairs tested under each condition. (−) indicates percentage of changes with negative polarity (i.e., post < pre). Error bars in *C* and *D* represent SEM. *P* values were obtained by using linear-mixed-model analyses.

($F_{1,20.334} = 4.141$, $P > 0.05$; Fig. 5*C*). Additionally, connectivity changes produced by conditioning and behavior, delivered together, in the forward direction were significantly larger than those in the reverse direction ($F_{1,27.984} = 7.544$, $P = 0.01$; effect size = 1.48, very large effect; Fig. 5*D*). Details of effect-size calculations and thresholds used for interpretation of the magnitude of observed effects can be found in *SI Appendix, SI Materials and Methods*.

We also found that connectivity gains in the forward direction obtained immediately after conditioning (22.10 ± 1.38, mean ± SE) were smaller than the maximal changes (123.71 ± 2.62, mean ± SE; e.g., see orange asterisks in Fig. 3*C* and *SI Appendix*, Figs. S1*B* and S2 *B* and *D*) produced by further repetition of wrist movements, without delivery of any additional conditioning stimulation. A paired-samples *t* test further revealed that postconditioning StTAs immediately after conditioning (i.e., on day 0/$t = 0$) were not significantly different from preconditioning StTAs [$t(6) = 1.826$, $P > 0.05$]. Conversely, postconditioning StTAs corresponding to the maximal changes, obtained through repetition of "conditioned" movements, were significantly larger than preconditioning StTAs [$t(6) = 2.713$, $P = 0.03$; effect size = 2.67, very large effect].

While conditioning and behavior, when combined, produced significant gains in the strength of forward connections, the magnitude and duration of cortical plasticity observed across site pairs were variable (cf Fig. 3*C* and *SI Appendix*, Figs. S1*B* and S2 *B* and *D*). Effect sizes, calculated at the maximal post-conditioning changes obtained during the 15-d conditioning-and-behavior experiments, ranged from small to very large across connections and animals (monkey Y: 0.39 to 5.09 and monkey U: 0.64 to 2.75). We also quantified the dependence of cortical modifications on the initial strength of the connection by calculating the Pearson's correlation coefficient between gains obtained during the 15-d experiments and the corresponding baseline EP amplitudes. This was done for both the maximum and average changes obtained with conditioning and/or behavior (in the forward direction). The analysis revealed that there was a weak dependence, at best, of the magnitude of gains on the initial strength of the connection, which ranged from −0.36 to 0.29 across interventions (*SI Appendix*, Table S2). Second, in some cases, gains were slowly accumulated over successive behavioral sessions (Fig. 3*C* and *SI Appendix*, Figs. S1*B* and S2*D*), while they were accrued over faster timescales at other cortical site pairs. In a striking example, shown in *SI Appendix*, Fig. S2*B*, the

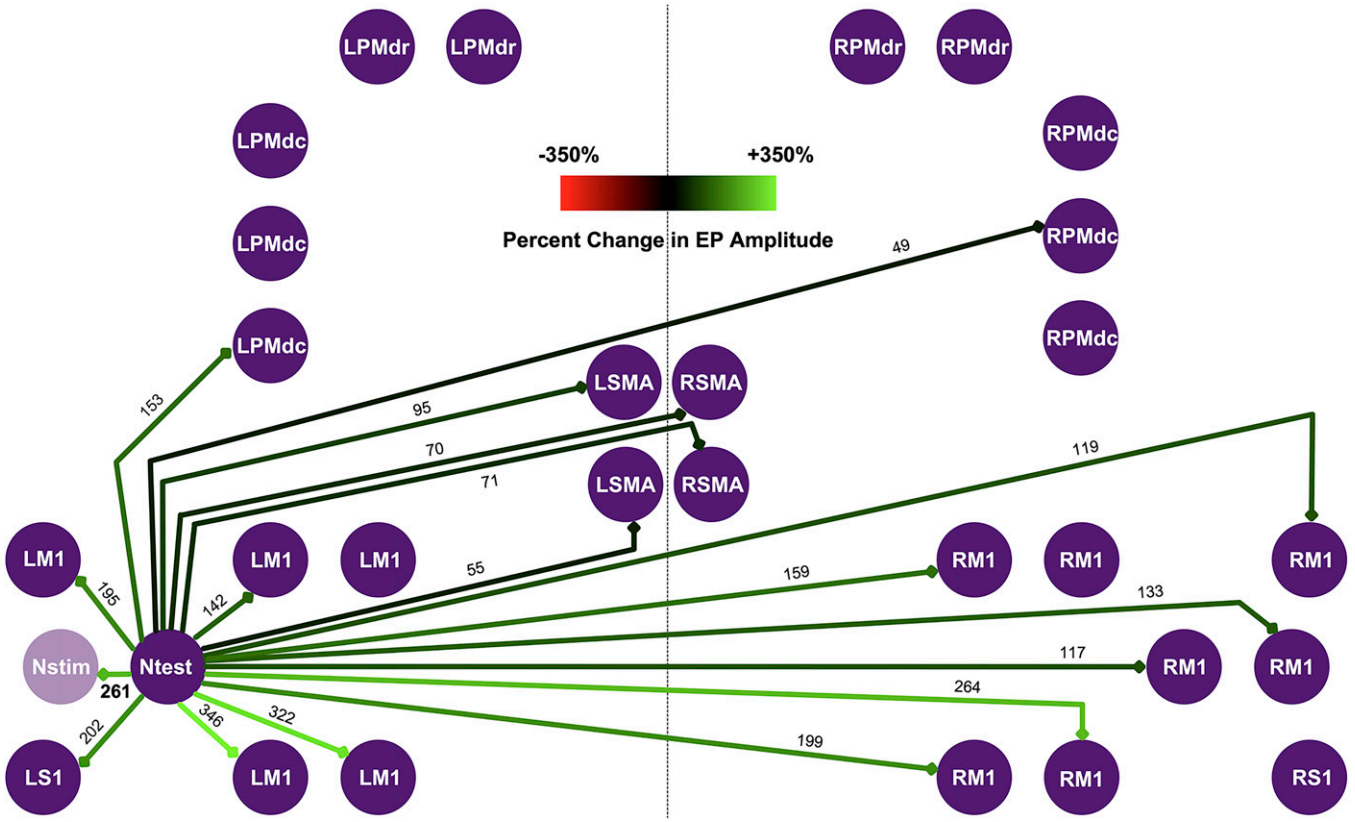

Fig. 6. Global effects of combining conditioning and behavior. The percent change in EP amplitude was used to document global connectivity changes produced by combining conditioning and behavior. Concurrent potentiation in the strength of all outgoing connections from the presynaptic site (Ntest) was observed when there was a 261% strengthening (indicated in bold; also see orange asterisk in Fig. 3*C*) of the connection from Ntest to Nstim. Colored arrows denote the magnitude of connectivity changes imposed on a graded three-color (red–black–green) scale (*Inset*); numbers indicate percent changes. The schematic represents the implant of monkey Y. Lack of arrows implies that there were no clear connections, as assessed through EPs in StTAs, between sites. M1, primary motor cortex; PMdc, caudal subdivision of the dorsal premotor cortex; PMdr, rostral subdivision of the dorsal premotor cortex; S1, primary somatosensory cortex; SMA, supplementary motor area. Prefixes L and R indicate the left and right hemispheres, respectively. Note that both Ntest and Nstim are sites in LM1.

strength of the forward connection increased, when conditioning was combined with behavior, from −5 to 237% within an individual (135-min) behavioral session on day 2 after conditioning. However, the resultant plasticity was short-lived and extinguished over the weekend that followed the session (in contrast to the more persistent effect shown in Fig. 3*C*).

Lastly, we assessed the directionality of all connectivity changes (relative to baseline levels) obtained with conditioning and/or behavior across the 15-d duration of our experiments (Fig. 5*A*). We found that 28% of the changes obtained by combining conditioning and behavior were negative. In stark contrast, 59% and 74% of the changes were negative with behavior alone and conditioning alone, respectively, indicating higher levels of depression. A paired-samples t test further revealed that postconditioning StTAs corresponding to the minimal changes (e.g., see black asterisks in Fig. 3*C* and *SI Appendix*, Figs. S1*B* and S2 *B* and *D*), obtained by combining conditioning and behavior, were not significantly different from preconditioning StTAs [$t(6) = -1.811$, $P > 0.05$]. On the other hand, postintervention StTAs corresponding to the minimal changes were significantly smaller than preintervention StTAs with both behavior alone ($Z = -2.521$, $P = 0.01$, Wilcoxon signed-rank test; effect size = 0.45, small effect) and conditioning alone [$t(4) = -3.427$, $P = 0.03$, paired-samples t test; effect size = 0.61, medium effect].

Global Effects of Combining Conditioning and Behavior. STDP-based stimulation protocols have often been found to produce global modifications of synaptic strength, affecting connections beyond the site of induction (27, 35–39). To test site specificity of the cortical potentiation induced by combining conditioning and behavior, we documented changes in the strength of outgoing connections from Ntest and Nstim to other implanted sites. We found that the cortical gains observed with conditioning and behavior, delivered together, were not restricted to Ntest-to-Nstim, but the potentiation propagated outward to affect all connections from the presynaptic site (Ntest; Fig. 6), while the strength of connections from Nstim to other implanted sites showed little change (*SI Appendix*, Fig. S3).

In the example shown in Fig. 6, a 261% potentiation in the Ntest-to-Nstim connection (orange asterisk in Fig. 3*C*), produced by combining conditioning and behavior, resulted in a concurrent increase in the size of all outgoing EPs from Ntest. The magnitude of potentiation was variable, ranging from 49 to 346%, across sites, which spanned different cortical areas (primary motor cortex, primary somatosensory cortex, premotor cortex, and supplementary motor areas) and both the left and right hemispheres. Importantly, gains were not correlated to the motor outputs of the cortical sites or their distance from Ntest or Nstim. Motor outputs across cortical sites ranged from flexion, extension, and both flexion and extension to neither flexion nor extension. In a second example shown in *SI Appendix*, Fig. S4, a 237% increase in the strength of the connection from Ntest to Nstim (orange asterisk in *SI Appendix*, Fig. S2*B*), similarly, produced concurrent potentiation in all outgoing connections from Ntest that ranged from 66 to

242%. Again, the sites spanned different cortical regions and both hemispheres, and potentiation was not related to their motor outputs or their distance from Ntest or Nstim. Second, potentiation of the Ntest-to-Nstim connection produced very small (bidirectional) changes in the strength of all outgoing connections from Nstim, including its connection to Ntest (*SI Appendix*, Fig. S3). Lastly, this pattern of global plasticity required induction of potentiation and was absent when there was no substantial change in the strength of the connection from Ntest to Nstim. As shown in *SI Appendix*, Fig. S5, a −1% change in the strength of Ntest-to-Nstim connection was accompanied by changes ranging from −38 to 41% across sites that received inputs from Ntest. Note that all sites that received inputs from Ntest or Nstim, assessed through presence of EPs in StTAs, were included in these analyses.

Discussion

This study describes an activity-dependent stimulation protocol in which electrical stimuli gated by voluntary movements were used to produce simultaneous activation of neurons at motor-cortical sites in adult behaving monkeys. Movements corresponding to the motor output of a presynaptic site, during which neurons at that site were likely active, gated delivery of (single) stimuli to a postsynaptic site in an effort to produce coactivation of cells at the two sites. Delivery of movement-gated stimulation resulted in small increases in the strength of cortical connections immediately after conditioning. Gains in cortical connectivity and motor output, induced by STDP-based conditioning approaches, have been reported before by our laboratory (6, 7, 16, 27) and other researchers (40–42). In these studies, conditioning-induced plasticity decayed over minutes to several days (depending on the duration and frequency of conditioning sessions). In contrast, when movement-gated stimulation was paired with further repetition of the movements that gated the conditioning stimuli, there were substantially larger increases in the strength of cortical connections, without any additional delivery of conditioning stimulation. Moreover, conditioning alone or behavior (used here to describe both voluntary movements and associated levels of behavioral modulators, such as attention and motivation) alone were relatively ineffective in modulating cortical connectivity. Second, the cortical plasticity produced by combining conditioning and behavior was directional, strengthening connections in the forward direction with little to no changes in the reverse direction. Lastly, there was a global, but selective, spread of plasticity from the conditioned sites that resembled a presynaptic pattern of propagation of potentiation, previously described in an in vitro study of STDP (35).

Comparison with Related Activity-Dependent Stimulation Protocols. An examination of the general effects of cortical microstimulation can be found in *SI Appendix*, *SI Discussion*. The current conditioning paradigm is related to protocols used in our previous studies, in which cortical or spinal stimulation was triggered by multiple motor-unit action potentials (MUAPs) in forelimb-EMG recordings (16, 43). Lucas and Fetz (16) showed that such MUAP-triggered cortical stimulation produced a shift in the ICMS-evoked movement representation of the presynaptic site (associated with the recorded triggering muscle) toward that of the postsynaptic stimulated site, presumably due to strengthening of the cortical connection from the presynaptic to the postsynaptic site. In neural-network simulations, the changes in intracortical connectivity were consistent with STDP mechanisms (44). In the second study, McPherson et al. (43) improved forelimb-motor recovery in rats with incomplete cervical spinal cord injury by delivering long-term MUAP-triggered spinal stimulation, which may have strengthened spared corticospinal connections to neurons below the lesion.

These two previous studies (16, 43) and the current investigation used signals related to muscle activity as a surrogate for movement-related firing of motor-cortical neurons. However, it is worth considering the differences between MUAP-triggered and movement-gated stimulation with regard to the underlying plasticity mechanisms. MUAP-triggered stimulation is designed to synchronize stimulus-driven action potentials of postsynaptic neurons with spikes of presynaptic cortical cells that are coactivated with EMG in the triggering muscle. This coupling is tightest between corticomotoneuronal (CM) cells and motoneurons of their target muscles in primates (45), due to their monosynaptic connections. CM-cell spikes are also synchronized with firing of other cortical neurons, as shown by peaks in their cross-correlograms (46). Spike-triggered averages of EMG demonstrate that CM cells fire 6 to 25 ms before MUAPs of their target muscles (45), indicating that MUAP-triggered cortical stimulation is within the window for STDP (1) to strengthen intracortical synapses of CM and synchronized cells.

Despite evidence for temporal coupling (45), the directional tuning of CM cells and their target motor units is not always the same (47, 48). Moreover, many populations of last-order premotor neurons contribute to motor-unit firing, in addition to CM cells (49), making the timing between CM-cell and motor-unit firing probabilistic. The relative timing of firing of non-CM motor-cortical cells, synchronized with CM cells, and motor units is expected to be broader, given the intervening synapses (making the coupling weaker). When using trains of ICMS to document cortical representation, as in the Lucas and Fetz study (16), it is also important to take into account that repetitive stimulation with trains activates additional pathways through temporal summation, making the contribution of CM-cell activation to the evoked output both unknown and quite possibly minor. Despite these complexities, results in the Lucas and Fetz study (16) are consistent with an STDP mechanism (44), suggesting that the population of cortical neurons that produced the shift in the direction of ICMS effects had a statistically higher probability of firing less than ∼30 ms before delivery of the MUAP-triggered stimulus pulses that activated their postsynaptic targets in the motor cortex.

In contrast, it is difficult to attribute the plasticity produced by movement-gated stimulation to a similar STDP mechanism. Although the firing rates of wrist-muscle MUAPs and wrist-related neurons in the primary motor cortex are simultaneously elevated during movement, the relative timing of conditioning-stimulus pulses and presynaptic cortical spikes would be essentially random because movement-gated stimuli were delivered at a constant frequency. Thus, the number of presynaptic cortical spikes occurring before and after individual conditioning-stimulus pulses in the current study were likely equal, leading to as many weakening as strengthening STDP events. It is possible that the effects of the conditioning stimulation itself during the 1.6-s duration of the cursor hold, interacting with the complex excitatory–inhibitory circuits in the motor cortex, establish spike-stimulus timings that promote STDP (50). However, since the population of cortical cells contributing to the measured variable (i.e., EP amplitude) is unknown (e.g., the proportion of cells with CM vs. only cortico-cortical projections), it is difficult to formulate a specific hypothesis about potential circuit mechanisms. Alternatively, primarily non-Hebbian forms of

plasticity may underlie the changes in connectivity reported in the current study. At the very least, increases in connection strength that occur long after delivery of conditioning stimulation must involve mechanisms other than STDP (which are discussed below). Neuromodulatory systems, for example, can influence STDP rules—by acting via acetylcholine, monoamines, and other signaling molecules—and bridge the gap between the timing of spikes and their behavioral outcome (51, 52).

Lastly, the output measures used to assess the effect of conditioning were different across the three protocols—namely, amplitude of cortico-cortical EPs (used here), ICMS-evoked torques (16), and motor performance (43)—making it difficult to infer the relationships between the underlying plasticity mechanisms. In addition, the frequency and duration of the conditioning sessions and the timeline for testing the effects of conditioning were variable across the three studies. The largest changes in connectivity in the present study occurred with further performance of a motor task after a brief period of conditioning. Similar (and longer) conditioning periods were used by Lucas and Fetz (16), but the effect of postconditioning behavior on ICMS-evoked torques was not evaluated. Rats in the McPherson et al. study (43) received many hours of daily MUAP-triggered stimulation for months; this led to sustained postconditioning changes, which could involve mechanisms similar to those in the current study.

Role of Behavior in Cortical Plasticity. Our results show that movement-dependent stimulation transformed the adult primate motor cortex into a plastic landscape, generally associated with motor learning, in which use-dependent plasticity was observed with further natural use of the conditioned pathways long after stimulation had ended. In contrast, conditioning alone was relatively ineffective in modulating the strength of cortical connections. Moreover, the size of cortical gains produced by combining conditioning and behavior was often positively correlated with the monkey's wrist-activity levels (e.g., Fig. 3*C*, days 8, 9, and 10), suggesting a role for motivated behavior in cortical plasticity. In a relevant study supporting this argument, Nishimura and coworkers (18) used Granger-causality analysis to demonstrate signal flow in the high-gamma frequencies from the nucleus accumbens to the primary motor cortex in the first 4 to 5 wk following a cervical spinal cord injury in macaque monkeys. This interaction was critical to rehabilitation-mediated recovery of forelimb motor function. Pharmacological inactivation of the nucleus accumbens, involved in the regulation of motivation-driven effort (53–55), during this early phase after injury abolished the high-gamma activity, leading to deficits in recovery of finger dexterity. This study provides strong support for the role of motivated behavior in cortical plasticity and functional motor recovery.

Two other studies provide critical support for the role of behavior in synaptic plasticity. Ahissar et al. (12) found that strengthening of cortical connections, produced by a conditioning protocol that coactivated neurons involved in those connections, was strongly dependent on the behavioral context of the stimuli that induced such modifications. Correlated activity of neurons, while necessary, was not sufficient for the induction of plasticity. Second, Perez and coworkers (19) found that volitional activity during a paired stimulation protocol enhanced corticospinal transmission in humans with spinal cord injury. Transmission was significantly improved when the paired stimulation was combined with relevant isometric movements, compared to delivery of stimulation at rest, underscoring the role of volitional activity, which occurs at a very different timescale from STDP (8), in boosting synaptic plasticity.

Synaptic tagging (56–58) and changes in neuronal excitability (59) are two mechanisms by which synapses and neurons "primed" during the plasticity-induction state can be marked for further modifications. Such non-Hebbian mechanisms may be involved in the effects observed with conditioning and behavior, delivered together, in the present investigation. In this scenario, synapses between neurons at Ntest and Nstim that are active during wrist flexion or extension may have been tagged by movement-gated stimulation and further strengthened by repetition of those movements in a use-dependent manner. In the absence of conditioning stimulation, no such priming occurs, and performance of an already-learned motor task does not produce an appreciable increase in the strength of associated cortical connections, as has been observed previously by Kilgard and coworkers (60), indicating that cortical plasticity, while functionally relevant, is unnecessary for continued task performance. Similarly, tagging synapses, through movement-gated stimulation, in the conditioning-alone control primes synapses whose strength does not increase due to nonuse in the absence of subsequent motor behavior. Lastly, we can only speculate that the effects of the conditioning stimulation itself establish spike-stimulus timings that promote strengthening of the connections from Ntest to Nstim. Hebbian mechanisms would then preclude concurrent strengthening of connections in the reverse direction (i.e., from Nstim to Ntest).

Interplay between Various Forms of Plasticity. Our study shares features of STDP, such as directional modulation of synaptic strength (1–5) and a presynaptic spread of potentiation (35). However, connectivity changes observed in our experiments were likely also affected by non-Hebbian forms of plasticity, such as homeostatic plasticity [driven by cell-wide—and not synapse-specific—mechanisms such as global synaptic scaling (61, 62)] and slow-onset synaptic potentiation (56, 63), and the interactions between Hebbian and non-Hebbian forms of plasticity (30, 31). For example, there were bidirectional changes in the strength of cortical connections, produced by combining conditioning and behavior, between the last time point of a behavioral session and the first time point of the subsequent session (e.g., Fig. 3*C*), possibly due to homeostatic adjustments of synaptic strength that occurred during sleep (32, 33). The changes were more pronounced when the latter session occurred after a weekend, and it resulted in a general decrease in connection strength toward preconditioning levels. Sleep is known to produce bidirectional changes in connection strength (32, 33). Induction of plasticity during awake states is often accompanied by secondary modifications during sleep through various forms of metaplasticity (64, 65), which refers to neuronal changes that influence the capacity for subsequent synaptic plasticity. Synaptic tagging (56–58) and changes in neuronal excitability (59) are, once again, implicated as mechanisms by which neurons primed during awake states can be marked for further modifications during sleep, thus providing a bridge between plastic changes across brain states. Second, this interaction between different forms of plasticity may also be reflected in the bidirectionality of connectivity changes (relative to baseline levels) produced by conditioning and behavior, delivered individually or together, over the 15-d course of the experiments (Fig. 5*A*). The gains produced by combining conditioning and behavior likely offset the percentage of negative changes that were observed in the behavior-alone and conditioning-alone controls. Third, this interplay between gains

produced by conditioning-and-behavior and homeostatic mechanisms, seeking to compensate for increases induced by activity-dependent stimulation in uninjured animals, may have also resulted in the lack of plateau in cortical-connectivity gains that was observed in our study. If synapses are potentiated and the resultant increases in synaptic strength are very long-lasting, more resources (neurotransmitters, receptors, vesicles, and second messengers) would be needed to maintain such high levels of potentiation, which could potentially overwhelm the metabolic capacity of the system to stably sustain itself (61). These gains, however, may be more persistent in an injured environment, where neuronal pathways are weakened, and interventions seek to drive connection strength back to baseline levels.

Propagation of Cortical Plasticity. The global pattern of plasticity seen in our study has been observed before by Poo and coworkers (35), who found presynaptic propagation of long-term potentiation (LTP), produced by correlated presynaptic and postsynaptic activation, from the synapse of induction. In their study, which was conducted in sparse hippocampal cultures consisting of three or four neurons, LTP propagated retrogradely to glutamatergic synapses on the dendrites of the presynaptic neuron and laterally to those made by its axonal collaterals onto other glutamatergic cells. No lateral or forward propagation of LTP to or from the postsynaptic neuron or secondary propagation to synapses not directly associated with the presynaptic or postsynaptic neurons was observed. Also, as observed in our study (*SI Appendix*, Fig. S5), propagated potentiation required the induction of LTP. However, we have only investigated plasticity propagation in outgoing connections from the presynaptic and postsynaptic sites (Fig. 6 and *SI Appendix*, Figs. S3 and S4). Experimental time constraints associated with delivering sequential (test) stimulation at multiple sites precluded measurement of changes in incoming connections to the presynaptic and postsynaptic sites or secondary connections. Nonetheless, results from our experiments, conducted in behaving monkeys, bear remarkable similarities to some of the features of the plasticity-propagation pattern observed in the in vitro STDP study (35). Such selective propagation was found to be mediated by a retrograde messenger that rapidly crosses the synapse where potentiation was induced, signaling presynaptic modifications that produce a global, yet specific, pattern of connectivity changes at a timescale similar to plasticity induction (66, 67).

Conclusions. Our results demonstrate that movement-dependent conditioning, combined with repetition of the gating movements, can produce significant strengthening of connections in the motor cortex of adult behaving monkeys. They indicate a crucial role for behavior—specifically, voluntary movements and motivation—in modulating Hebbian-like plasticity. Physical rehabilitation and use-dependent movement interventions, such as body-weight-supported treadmill training (68) and constraint-induced therapy (69), have been widely explored as treatment options after neurological injuries, but they typically—and at best—lead to partial recovery of motor function. Our data provide strong support for combining movement-gated stimulation with such use-dependent physical therapies for enhancing motor recovery after a stroke or spinal cord injury. Since our stimulation protocol uses a noninvasive gating signal (i.e., movement), subsequent studies will assess its clinical applicability by using less-invasive surface electrodes or superficial scalp electrodes for delivery of conditioning stimulation. Augmenting motivation through electrical stimulation of dopaminergic neurons in the nucleus accumbens or the ventral midbrain may further enhance motor recovery promoted by movement-gated stimulation and physical rehabilitation (70), and exploration of such combinatorial interventions may represent important future directions. They will also help dissect the role of behavior—and behavioral modulators—in synaptic plasticity.

Materials and Methods

All animal-handling, training, and surgical procedures were approved by the University of Washington's Institutional Animal Care and Use Committee, and they conformed to the NIH's *Guide for the Care and Use of Laboratory Animals* (71). Experiments were conducted in two adult male *Macaca nemestrina* monkeys that were trained to perform a randomly alternating center-out wrist flexion–extension target-tracking task with their right hands. After learning the motor task, monkeys received chronic bilateral implants, consisting of custom-made electrode arrays, with Pt/Ir microwires targeting sites in layer V of the sensorimotor cortex. Disposable surface patches were used to record EMG activity from wrist flexors and extensors. Conditioning and/or behavior experiments were conducted in animals who had already learned the wrist-motor task. All tested site pairs were in the left primary motor cortex of the animals. Cortical LFPs, EMGs, and wrist torques were recorded during experimental sessions. Bipolar cortical stimuli were also delivered during these sessions. Stimulus-evoked potentials (28, 29) were used for documenting changes in cortical connectivity induced by our interventions. Stimulus-evoked responses in the flexor and extensor muscles, so-called motor-evoked potentials, and evoked wrist torques (16) were used to characterize changes in the motor output of cortical sites. Lastly, linear mixed-effects models (34) and paired-samples tests were used for assessing statistical significance of observed changes. We also quantified the magnitude of the changes using the effect-size metric (72, 73).

For detailed descriptions of (1) animals, (2) behavioral training, (3) fabrication of cortical implants and surgeries, (4) EMG electrodes, (5) recordings and stimulation, (6) documentation of cortico-cortical connectivity and motor outputs, (7) delivery of conditioning and/or behavior, (8) connectivity analyses, and (9) statistics, refer to *SI Appendix, SI Materials and Methods*.

Data Availability. All data used in this study are publicly available. Neurophysiological recordings and analyses code have been deposited in the Harvard Dataverse and can be accessed at https://dataverse.harvard.edu/dataverse/Movement-DependentStimulation (74).

ACKNOWLEDGMENTS. We thank Larry Shupe and Jatin Sonavane for providing programming, hardware, and software assistance. We also thank Rebekah Schaefer, Andrew Bogaard, and Robert Robinson for assistance with animal care, handling, training, and surgeries. This research used statistical consulting resources provided by the Center for Statistics and the Social Sciences at the University ofWashington. We especially thank Sara LaPlante for assistance with linear-mixed-model analyses. This work was supported by NIH Grants RR00166 and NS12542 and NSF Center for Neurotechnology Grant EEC-1028725.

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