Refinement of corticospinal neuron activity during skilled motor learning

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Abstract

The learning of motor skills relies on plasticity of the primary motor cortex as task acquisition drives the remodeling of cortical motor networks[1,2]. Large scale cortical remodeling of evoked motor outputs occurs in response to the learning of skilled, corticospinal-dependent behavior, but not simple, unskilled tasks[1]. Here we determine the response of corticospinal neurons to both skilled and unskilled motor training and assess the role of corticospinal neuron activity in the execution of the trained behaviors. Using *in vivo* calcium imaging, we found that refinement of corticospinal activity correlated with the development of skilled, but not unskilled, motor expertise. Animals that failed to learn our skilled task exhibited a limited repertoire of dynamic movements and a corresponding absence of network modulation. Transection of the corticospinal tract and aberrant activation of corticospinal neurons show the necessity for corticospinal network activity patterns in the execution of skilled, but not unskilled, movement. We reveal a critical role for corticospinal network modulation in the learning and execution of skilled motor movements. The integrity of the corticospinal tract is essential to the recovery of voluntary movement after central nervous system injuries and these findings should help to shape translational approaches to motor recovery.

The corticospinal circuit is the principal mediator of skilled, dexterous movements requiring precise execution and modulation of motor patterns. The learning of skilled movements depends on both the integrity of the corticospinal tract as well as the plasticity of cortical motor networks. Attenuation of cortical plasticity impairs the learning, but not the execution of previously learned complex motor behaviors[2]. The plasticity of these cortical motor networks has been known for as long as cortical organization has been studied, with stimulation of individual cortical points able to elicit distinct movements[3]. Electrical micro-stimulation in primate and rodent cortex has been used to demonstrate the labile nature of individual cortical sites[4] and how both learning and injury can reshape motor representations, or maps[5-7]. As rodents learn a skilled forelimb task, motor representations of the musculature involved in the trained movement increase, extending into neighboring map areas[1,2]. This large-scale physiological reorganization does not occur in animals trained on a simple, unskilled, lever-press task[1].

The distinction between skilled and unskilled behavior is a critical one, as both learning and execution of skilled, dexterous movements are dependent upon corticospinal output. Damage to the motor cortex or transection of the corticospinal tract impairs the execution of previously trained, skilled forelimb movements[8,9]. Furthermore, transient silencing of motor cortex shows disruption of fine motor control, but not the gross motor movements of a trained forelimb behavior[10]. In fact, once trained, the engrained movements on an unskilled lever press task can be faithfully executed in the absence of the entire motor cortex[11]. It is likely that complex, skilled behaviors require an active pattern of sensory feedback to fine-tune forelimb movements[12].

Dynamic movement adaptation during skilled learning

In order to determine the role of corticospinal neurons in the cortical reorganization that occurs during skilled, but not unskilled, motor learning, we modified the MotoTrak isometric pull task (Vulintus, Inc.) to require precise modulation of forelimb movements. We employed both skilled and unskilled versions of the task in combination with two-photon microscopy in head-fixed mice (Fig.1a-b). In order to train the skilled task, behavior was first shaped with water reward provided in response to mice exerting an isometric pull force on the manipulandum through a series of increasingly reduced reward windows, until mice reached the training window of 15-18 g (Ext. Data Table 1). Mice were trained to pull with a peak isometric force within this narrow window over a period of 22.8 ± 4.0 days, a period that was divided into three phases: early, middle, and late. Successful retrieval significantly increased over training with a rise in mean peak force (Fig. 1d,e). Moreover, the trained pull movement became significantly more consistent with learning as the correlation of the dynamics of each pull movement to that preceding it (pull-to-pull correlation) increased (Fig. 1f). Mice that did not improve by at least 25% total success rate over their baseline performance were categorized as non-learners (Fig. 1d). Non-learners trained for an average of 22.8 ± 4.4 days, similar to those that learned the task. Furthermore, in the non-learners neither force nor pull-to-pull correlation increased across training. Pull dynamics in non-learners were more consistent during the early phase than in mice that successfully learned the skilled task. This lack of variability in non-learner pulls may indicate that animals failed to explore varied strategies for task success.

For unskilled motor learning, we trained mice on the established, adaptive, isometric pull paradigm. This unskilled version of the task involves the same forelimb movements as the skilled task but does not require precise adjustments to restrict pull strength within a specific

range of force. Following behavioral shaping, mice were proficient on the unskilled, adaptive task during the first days of learning and maintained this success rate throughout a training period of 16.2 ± 1.4 days. As mice trained on the unskilled task, mean peak pull force increased over the course of training. These pulls became slightly, but not significantly, more consistent with training. While both the skilled and unskilled tasks produce the same gross motor movement, learning of the skilled pull movement requires active refinement and likely involves adaptive modulation of cortical output.

Corticospinal neuron recruitment during skilled learning

In order to observe corticospinal activity during the learning of skilled and unskilled isometric pull, we used retrograde transduction at spinal levels C7/8 (segments controlling distal forelimb musculature) to express the genetically encoded calcium indicator GCaMP7f specifically in corticospinal neurons. Following transduction, two-photon microscopy was used to record calcium transients from corticospinal somata in layer 5b. We classified activity patterns of individual corticospinal neurons based on calcium peaks during pull movements and inter-pull intervals (Extended Data Fig.1a, b). A third category of neurons were indiscriminately active across trials. Approximately one quarter of all cells were classified as pull-active in skilled and unskilled learners, while a smaller percentage were pull-active in those that failed to learn the skilled task (Fig. 1j). The proportion of cells per category did not change dramatically during learning; however, using CellReg[13] we found that individual neurons were not restricted by category across learning. Approximately 67% of neurons from both skilled and unskilled animals changed classification between the early and middle time points. In contrast, between the middle and late time points, 67% of neurons changed classification in the skilled group, but only 44% of

neurons changed categories in the unskilled group. All neurons that we recorded from projected to restricted spinal cord levels (C7/8), therefore a certain amount of functional redundancy in these projections would be expected to support the observed network-wide effects of motor learning, rather than cell-specific patterns during trained movement execution.

Across all trials we observed distinct temporal response profiles for each classification of corticospinal neurons. On both skilled and unskilled tasks, we found an initial suppression of activity in pull-active neurons that gave way to higher calcium responses during movements (Extended Data Fig.1b). Interpull interval-active neurons displayed a complementary response pattern, with the largest responses prior to reach onset, while indiscriminately active neuron responses were largely unperturbed during movement (Extended Data Fig.1b). Skilled training resulted in the greatest separation of temporal responses in corticospinal classifications, suggesting an increase in inhibition prior to movement onset. An initial suppression of activity would be in line with previous findings that selective inhibition of motor cortex networks prior to reach onset is essential for prepared movements[14].

As animals showed improved success on the skilled task, we observed a larger proportion of pull-active neurons associated with successful trials (Fig. 1k). Animals that failed to learn the skilled task showed no change in the proportion of corticospinal neurons that are associated with the few successful trials performed. Training on the unskilled, adaptive task, drove a transient increase in success-associated, pull-active neurons that diminished late in training. These changes in pull-active neurons during successful trials suggest an active modulation of

corticospinal neuron activity across learning as well as the specific refinement of corticospinal networks during learning of skilled movements.

In order to examine the relationship between corticospinal activity and forelimb movement, we compared changes in corticospinal neuron activity correlation across all pulls to learning mediated changes in pull-to-pull correlation. As pull-to-pull correlation increased in animals that learned the skilled task, we observed a commensurate increase in corticospinal neuron activity correlation across pull behavior (Fig. 1l). In contrast, mice that failed to learn the task exhibited high pull-to-pull correlations throughout training and no overall change in activity correlation. On the unskilled task, mice showed increased pull consistency with training; however, corticospinal activity correlation was largely unchanged. These data suggest that skilled learning shapes the activity of relevant corticospinal output networks and that the unskilled task is independent of refinement of these networks. Furthermore, the non-learners demonstrated a limited repertoire of dynamic movements and a corresponding absence of network modulation, which likely underlies their failure to develop expertise on the skilled task.

Corticospinal-dependence of skilled behavior

We used bilateral lesions of the medullary pyramids (pyramidotomy) to determine the role of corticospinal neurons in execution of the skilled isometric pull. Pyramidotomy permanently impairs dexterous motor control on the skilled forelimb reach task[8]. We first tested this in freely moving animals with similar training parameters as used in head-fixed mice. In freely moving mice, the differences in the duration of training required for skilled versus unskilled behavior were exacerbated, with training in freely moving mice lasting 11.4 ± 0.2 days and 34.7 ± 2.0

days on the unskilled and skilled tasks, respectively. Performance of mice in the skilled group steadily increased throughout training with a rise in mean peak force (Fig. 2d). Unlike in head-fixed mice, pull-to-pull movement correlation in freely moving mice showed only a slight increase with learning (Fig. 2e).

Freely moving mice in the unskilled, adaptive group showed no effect of training on successful performance and plateaued early (Fig. 2c). These results are in line with what we observed in head-fixed mice on the unskilled task. No significant change was found in mean pull force over training; however, pull-to-pull consistency improved significantly over time. The difference between freely moving and head-fixed isometric pull behavior is not unprecedented, as similar discrepancies have been demonstrated when comparing freely moving and head-fixed single pellet retrieval behavior[15].

We selected the skilled range of 15-18 g based upon pilot studies during which all animals could pull well above the target range; however, we sought to ensure that the effects of training were independent of animal strength. In order to do so, we trained a separate group of freely moving mice to pull the isometric handle above a 15 g static threshold. In this static threshold group, mice showed an increased performance across 7.0 ± 1.1 training days (Fig. 2c). Mean peak forces stayed consistent across training and pull-to-pull correlation remained largely unchanged (Fig. 2e). These results suggest that the lower success on the skilled version of the task was not due to an inability to pull above the lower bound of the skilled pull range, but rather the ability to properly modulate the force exerted. This is evident in the standard deviation from the mean as mice trained to pull in the narrow window of the skilled task exhibited a standard deviation of

1.20 g, while those trained on the unskilled adaptive or static threshold isometric pull had a standard deviation of 4.00 g and 2.77 g, respectively. The unskilled tasks allowed for greater flexibility in strategies for mediating task success.

In order to determine the dependence of each task on the corticospinal tract, we transected the corticospinal tract bilaterally at the level of the medullary pyramids (pyramidotomy) (Fig. 2a,b). This restricted transection of the corticospinal tract significantly impaired performance only on execution of the skilled isometric pull (Fig. 2i). Pull force was not significantly reduced in the absence of the corticospinal tract; however, animals performing each of the tasks showed reduced pull-to-pull consistency following pyramidotomy (Fig. 2k). These results demonstrate that skilled isometric pull is corticospinal tract-dependent. In contrast, success on the unskilled task was unaffected by bilateral pyramidotomy. In fact, after injury, both force and success rate improved in mice performing the unskilled, adaptive isometric pull (Fig. 2i,j). This improvement may be a continuation of learning as mice were tested on the unskilled task for an average of 10.0 ± 0 days following injury and 11.4 ± 0.2 days prior to injury. Despite the increased performance after injury, animals exhibited reduced consistency in pull-to-pull movements (Fig. 2k). On mice trained with the static 15 g threshold there was no significant effect of pyramidotomy on pull success or force (Fig. 2i,j). These results indicate that the impairment observed in the skilled group is not due to weakness caused by corticospinal injury, but rather by the impairment of movement modulation and lack of viable compensatory movement driven by spared descending supraspinal circuits. As with the animals in the adaptive group, pull-to-pull movement correlation was dramatically decreased on the static threshold task (Fig. 2k). All three groups showed a severe reduction in pull consistency after corticospinal tract injury, suggesting

that the gross movements of the unskilled versions of the task are not corticospinal dependent but rather rely on other motor structures.

Injury decouples activity from behavior

We next used bilateral pyramidotomy in combination with *in vivo* imaging to determine the effects of injury on corticospinal activity during head-fixed isometric pull. As in freely moving animals, bilateral pyramidotomy severely impaired skilled isometric pull performance in head-fixed mice (Fig. 3a). In addition, pull-to-pull movement correlation decreased during execution of the skilled movement over the two weeks assessed post injury (Fig. 3c). Unlike in freely moving mice, pull force was significantly attenuated in head-fixed animals (Fig. 3b). The poor performance of the non-learning mice prior to injury was not significantly impacted by pyramidotomy. Non-learning mice showed no significant decrease in pull force or pull-to-pull correlation after injury. In contrast to skilled behavior, the execution of the unskilled behavior was largely unaffected by pyramidotomy. Neither success rate nor pull-to-pull correlation were significantly affected by injury (Fig. 3a,c). Unlike in freely moving mice, pull force showed a mild decrease following pyramidotomy. The differences observed between freely moving and head-fixed mice on the skilled task is likely due to the more restricted movement of head-fixed animals that could prevent compensatory adaptation of movements. Indeed, freely moving mice demonstrated a greater proportional decrease in pull-to-pull correlation but less severe drop in success rate after injury compared to head-fixed animals.

Calcium transients recorded from C7/8 projecting corticospinal showed increased volatility in cell classification after injury. By the second week imaged post-injury, the proportion of

corticospinal neurons classified as pull-active decreased to 20 % in the skilled learners, and only 11 % in the non-learners (Fig. 3g). Coincident with the decrease in pull-active neurons, there was an increase in inter-pull active neurons in the learners and indiscriminately active neurons in the non-learning animals. Mice executing the unskilled task showed a roughly even distribution of corticospinal neuron classifications in the second week. Between the late learning phase and the second week tested post-injury, nearly two-thirds of corticospinal neurons changed classification in mice performing the skilled isometric pull: 63.4 % of those that learned the task and 61.9 % of those that did not. In the unskilled group, only 34.8 % of corticospinal neurons changed categories. These percentages are similar to the changes observed during mid-to-late learning. Just as before injury, overall population activity showed greater separation between pull-active and inter-pull active neurons on the skilled task than on the unskilled task (Ext. Fig. 2b). This indicates a likely role for corticospinal collateral projections to other motor regions in execution of the task after injury. Indeed, corticospinal collaterals to red nucleus have been reported to support limited skilled motor recovery after bilateral pyramidotomy[16].

Following injury, we found a decoupling of corticospinal activity from pull movements. As success rates dropped on the skilled task, there was a commensurate reduction in success-associated pull-active neurons (Fig. 3h). No such change was noted in the non-learners or in mice trained on the unskilled task, as there were few pull-active neurons associated with success prior to injury. In skilled mice, as pyramidotomy resulted in a severe decrease in pull-to-pull correlation there was only a slight decrease in corticospinal neuron activity correlation across pull behavior (Fig. 3i), consistent with a decoupling from corticospinal activity and a role for intact collaterals in behavioral output after injury. Mice that failed to learn the task exhibited

little change in pull-to-pull correlation or corticospinal activity correlation during pulls following injury. On the unskilled task, mice showed no relationship between activity and behavioral correlation either before or after injury.

Corticospinal activity regulates skilled movement

As pyramidotomy permanently interrupts corticospinal projections, leaving brainstem collaterals intact, we sought to determine the effects of transient disruption of corticospinal neuron activity on skilled movement. In order to do so, we selectively expressed Channelrhodopsin-2 (ChR2) in corticospinal neurons projecting to C7/8 spinal cord via retrograde transduction (Fig. 4a). A fiber optic cable was attached to a cannula affixed over primary motor cortex and mice were trained to proficiency on the unskilled or skilled forelimb reach over 6.5 ± 0.61 or 18 ± 1.2 days, respectively. Following training, a 473 nm laser triggered by IR sensor was used to activate ChR2 and disrupt corticospinal networks (Fig. 4b). Optogenetic stimulation of C7/8 corticospinal neurons significantly impaired skilled isometric pull performance and reduced pull force (Fig. 4c,d). Pull-to-pull movement correlation was not altered in any consistent way by aberrant corticospinal activation (Fig. 4e). In contrast to the effects of corticospinal activation on execution of the skilled behavior, mice trained to pull on the unskilled, adaptive isometric pull were largely unaffected by laser-mediated activation of corticospinal neurons. Mice exhibited no significant reduction in success rate or pull-to-pull correlation when performing the adaptive isometric pull during IR-triggered laser ON sessions (Fig. 4c,e). There was a significant decrease in pull force during adaptive isometric pull, consistent with a role for the corticospinal neurons modulating force (Fig. 4d). These results confirm that the patterned corticospinal activation observed via *in vivo* imaging is critical for execution of skilled, but not unskilled movements.

Discussion

Motor cortex plays multiple roles in skilled motor control. While early instructional outputs are critical for task acquisition of both skilled and unskilled behavior, primary motor cortex eventually disengages from task execution of unskilled movements as they can be performed during cortical silencing or even in the complete absence of motor cortex[11,17]. Unskilled tasks do shape layer 2/3 motor network activity and structure[18]; however, execution of unskilled tasks does not drive the large scale alterations in evoked motor outputs characteristic of skilled learning[1]. Skilled behavior acquisition results in both reorganization of motor maps[1] as well as structural remodeling of the corticospinal neurons that are engaged in the behavior[19,20]. We show here that corticospinal neurons are actively engaged in the execution of skilled forelimb movements during motor learning and that activity of these networks is refined in concert with the development of consistent movement execution. Motor training drives declines in movement variability when the reward rate is high as animals attempt to replicate successful trials[21]. Indeed, we found that increased success rate occurred in parallel with increased consistency of trained movements on the skilled task. In contrast, mice that exhibited more limited movement variability throughout training failed to learn.

The execution of our trained, skilled forelimb task was impaired by both transection of the corticospinal tract and by disruption of corticospinal neuron activity through optogenetic activation. The unskilled version of the same task failed to recruit corticospinal neurons and execution was neither impaired by pyramidotomy nor was it significantly perturbed by aberrant corticospinal activation. We did not observe a disengagement of corticospinal neurons from skilled forelimb movements over the course of training; however, it is possible that longer term

training could drive skilled movement execution to be corticospinal and motor cortex independent. In our study, skilled mice were trained an average of 34.7 ± 2.0 days (freely moving) and 22.8 ± 4.0 days (head-fixed), which would be in line with what others have described as mid-range training[17]; however, this training followed 16.9 ± 1.2 days (freely moving) and 16.4 ± 1.7 days (head-fixed) of behavioral shaping on the tasks. This behavioral shaping surely drove refinement of corticospinal networks prior to our imaging sessions.

Both skilled and unskilled isometric pull tasks utilize the same muscle movements and are therefore likely to engage similar motor circuits; however, only execution of the skilled task was found to depend on the integrity of the corticospinal tract. The development of expertise occurred rapidly on unskilled tasks and showed no significant correlation between corticospinal activity and movement kinetics; whereas skilled expertise required a training-mediated refinement of movement kinetics and corresponding increase in task-associated corticospinal neuron activity. These findings illustrate a key role for modulation of corticospinal networks in the learning and maintenance of skilled movements and provide a powerful toolset for assessing how compensatory mechanisms and corticospinal circuit plasticity can shape recovery neurological injury.

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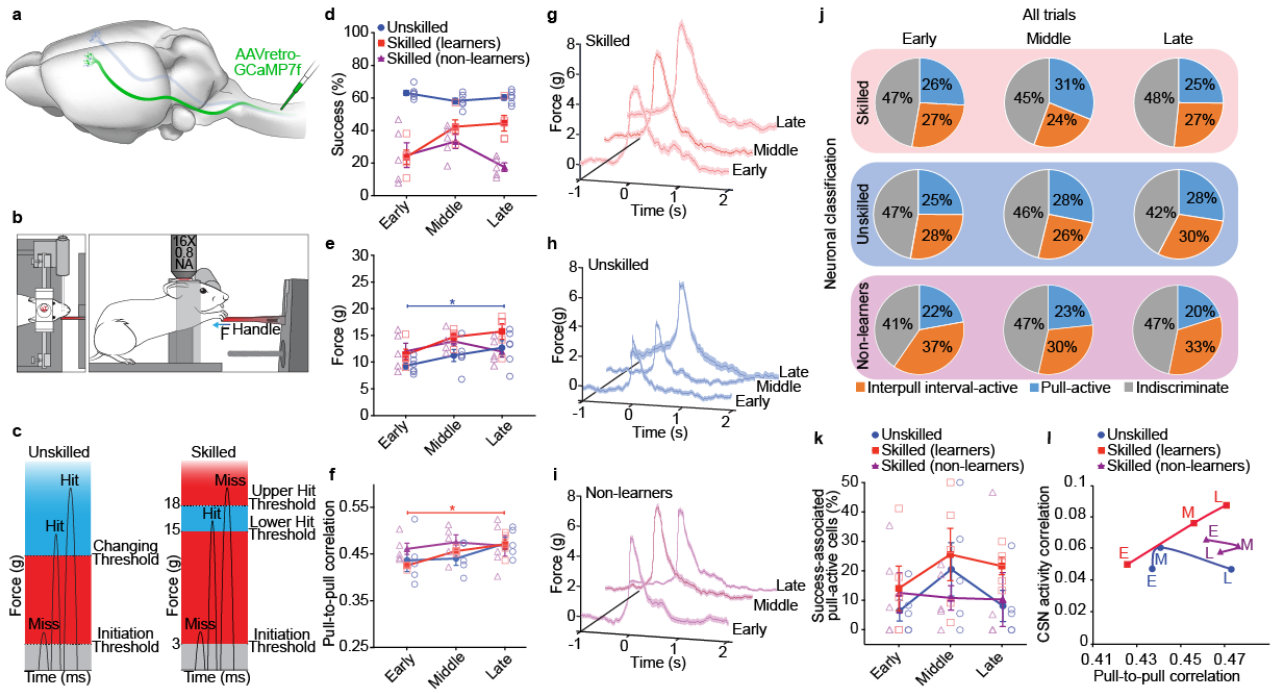


Figure 1. Corticospinal motor networks are refined during skilled task learning

a. AAVretro-GCaMP7f transduction at C7/8 labels corticospinal neurons in M1. **b.** Head-fixed MotoTrak isometric pull task under two-photon microscope. **c.** Behavioral paradigms in skilled and unskilled versions of the task. **d**. Performance on the isometric pull tasks at three distinct learning phases. Mice showed no learning curve on the unskilled task, whereas half the mice were able to learn the skilled task (n=5, repeated measures ANOVA P=0.0198, $F_{2,8}$=6.73) while others were classified as non-learners. **e.** Changes in pull force across training (n=6, repeated measures ANOVA P=0.0481, $F_{2,10}$=4.98, *Bonferroni post-hoc P<0.05). **f.** Average pull-to-pull correlation coefficients are significantly higher after learning in skilled mice (n=5, repeated measures ANOVA, P=0.007, $F_{2,8}$=22.13, *Bonferroni post-hoc P <0.05). **g-i.** Kinetics of average pull force over time, across training. Two second reward window starts at time 0. **j.** Classification of corticospinal neurons active during all trials, plotted across training: pull-active (active during movement), inter-pull interval-active (active before and after movement), or

indiscriminately active (active independent of pull movement). **k.** Percentage of pull-active corticospinal neurons with at least 40 % of spikes occurring during successful pulls. Skilled learning drives a moderate increase in success-associated neurons. **l.** Pairwise correlation analysis of activity across movements shows a linear increase with pull-to-pull correlation values across training in mice that learned the skilled task, suggesting a strong interaction between corticospinal activity and movement. Data presented as mean ± s.e.m.

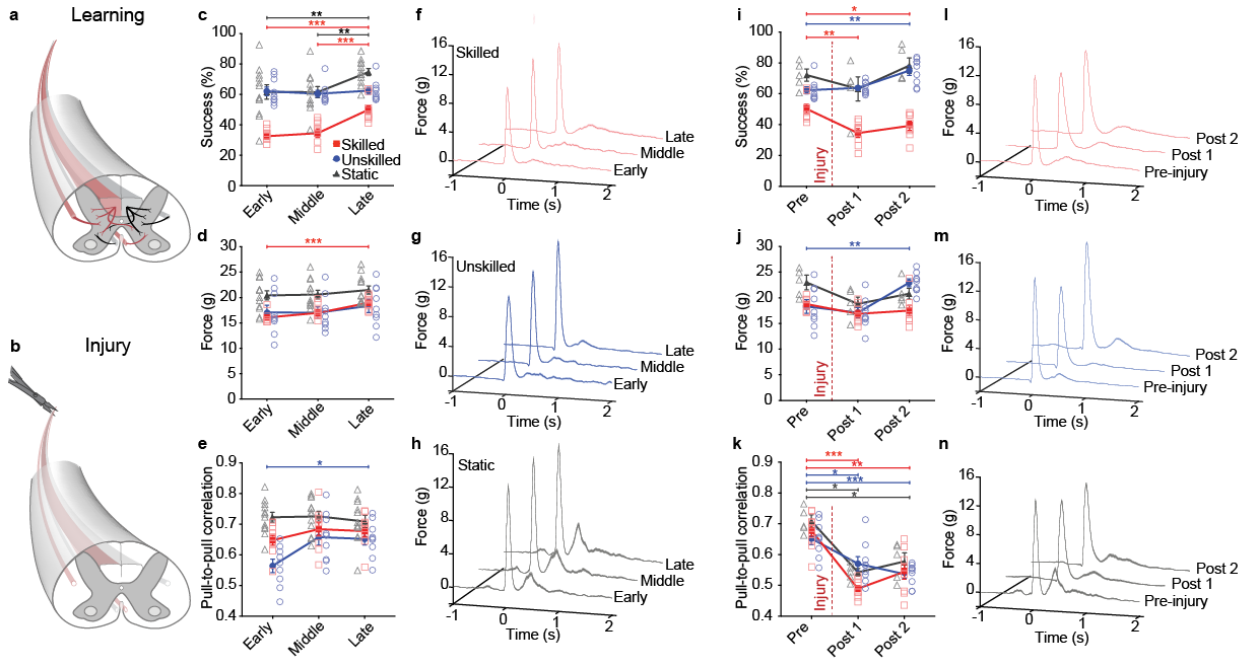


Figure 2. Skilled isometric pull depends on the corticospinal tract. a,b. Schematic showing intact corticospinal tract and effects of bilateral pyramidotomy. **c.** Success rate of freely moving mice on the isometric pull task with three paradigms: adaptive threshold, 15g static threshold, and 15-18g skilled threshold. Performance improves with training on the skilled and static conditions (repeated measures ANOVA, skilled: n=8, P<0.0001, $F_{2,14}$=41.2; static: n=12, P=0.002, $F_{2,22}$=9.2). **d.** Average pull force increases during skilled training, with limited variability (repeated measures ANOVA, n=8, P=0.004, $F_{2,14}$=12.1). **e.** Pull-to-pull correlation across training. **f-h.** Kinetics of average pull force over time, across training. Two second reward window starts at time 0. **i.** Success on skilled isometric pull is impaired for two weeks after bilateral pyramidotomy (post 1, post 2, repeated measures ANOVA, n=8, P=0.0004, $F_{2,14}$=17.7). Mice trained on the unskilled, adaptive task continue to improve after injury; preinjury (repeated measures ANOVA, n=9, P=0.0011, $F_{2,16}$=11.0). **j.** Average pull force is not significantly affected by pyramidotomy in the skilled or static paradigms. Mice on unskilled adaptive task exhibit a significant increase in pull force after injury (repeated measures ANOVA, n=9, P=0.0022,

$F_{2,16}$=13.4). **k.** Average pull-to-pull correlation coefficients are significantly reduced following injury in all conditions. **l-n.** Average isometric pull kinetics following bilateral pyramidotomy. Bonferroni post-hoc values *P <0.05, **P <0.01, ***P <0.001. Data presented as mean ± s.e.m.

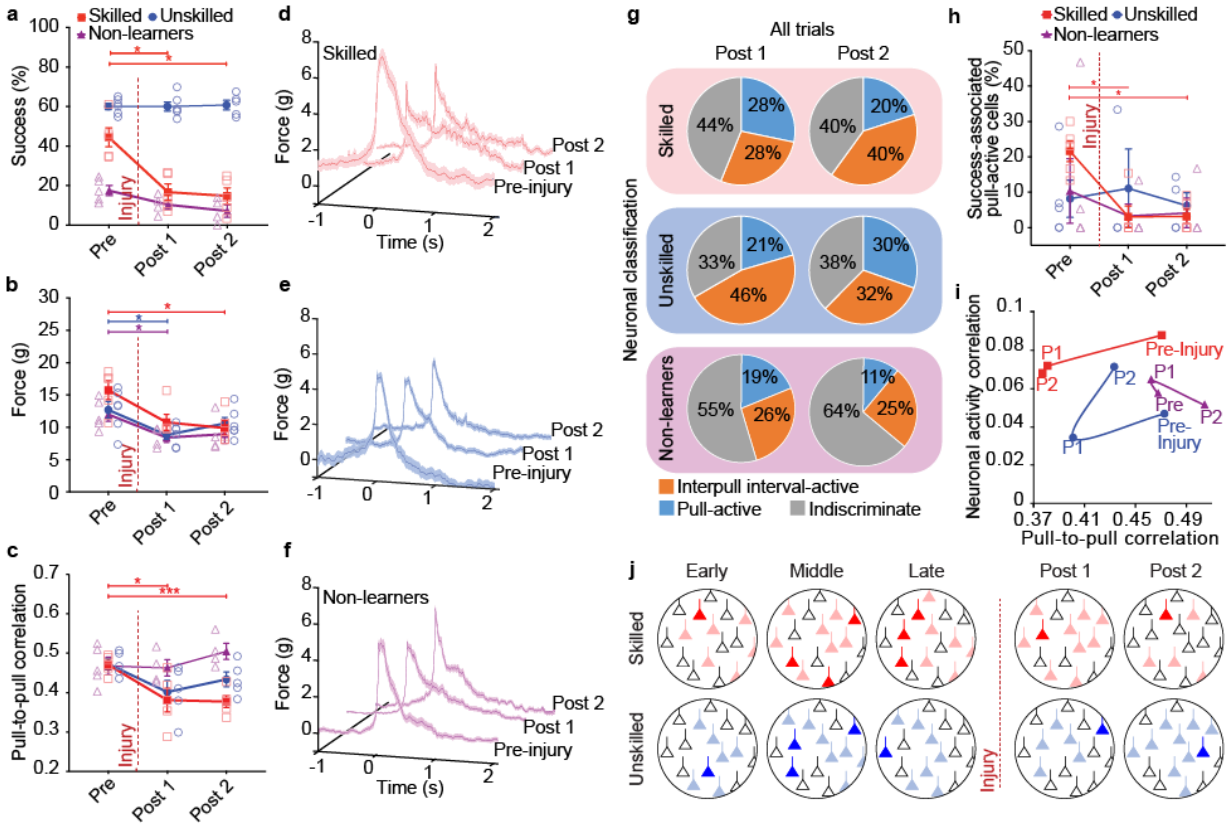


Figure 3. Pyramidotomy decouples corticospinal activity from skilled task performance a. In head-fixed mice, success rate is impaired on skilled, but not unskilled isometric pull over two weeks following bilateral pyramidotomy (post 1, post 2) (repeated measures ANOVA, n=5, P=0.0032, $F_{2,8}$=14.6). **b.** Average pull force is impaired in head-fixed mice. **c.** Average pull correlation coefficients are significantly impaired in skilled mice (repeated measures ANOVA, n=5, P=0.0046, $F_{2,8}$= 25.1). **d-f.** Average isometric pull kinetics show the effects of pyramidotomy on pull profiles. **g.** Pyramidotomy resulted in changes in corticospinal neuron classifications at 2 weeks post-injury in all groups. **h.** Skilled mice show a significant drop in the percentage of success-associated pull-active corticospinal neurons (greater than 40 % spikes during successful pulls) following injury (repeated measures ANOVA, n=5, P=0.0038, $F_{2,8}$=19.6). **i.** Pairwise correlation analysis of activity across movements. In skilled mice, the precipitous drop in pull-to-pull movement correlation is met with only a moderate decrease in

corticospinal activity correlation after injury. **j.** Model of corticospinal network modulation across motor learning and injury. A similar proportion of corticospinal neurons in M1 are active during pull movements in both skilled and unskilled movements across training (shaded neurons); however, categorization of individual neurons changes across learning. Skilled task acquisition correlates with an increase in the proportion of pull-active corticospinal neurons associated with successful trials (saturated neurons). Pyramidotomy disrupts success-associated circuits. Data presented as mean ± s.e.m.

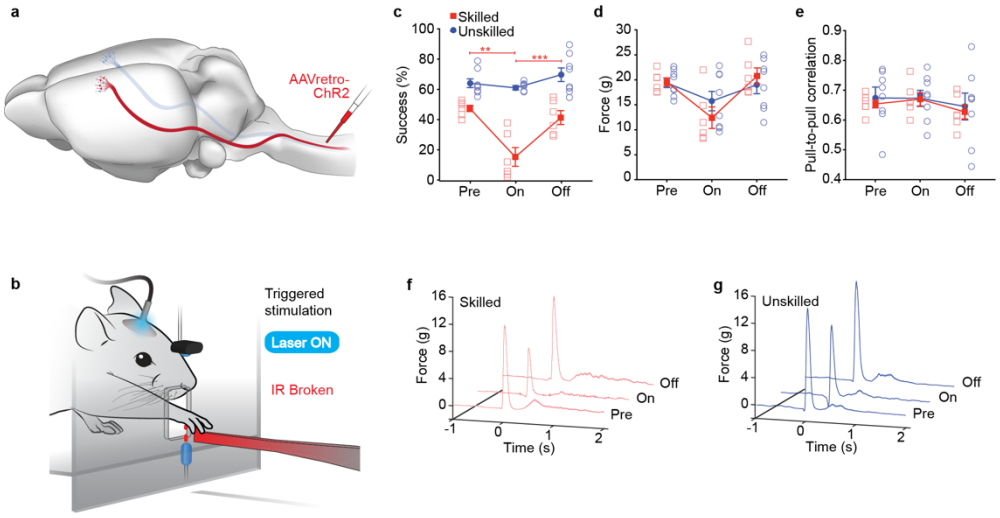


Figure 4. Disruption of corticospinal activity impairs skilled motor output

a. Illustration of corticospinal transduction strategy with AAVretro-ChR2-tdTomato transduction at C7/8. **b.** Illustration of IR-sensor- triggered optogenetic stimulation of M1 with 473 nm fiber coupled laser. **c.** Skilled and unskilled performance assessment on the isometric pull task averaged across the last 3 training days (Pre), during laser on stimulation of ChR2-expressing corticospinal neurons (On), and one day after laser on session (Off). Skilled mice showed a significant decrease in skilled performance during laser stimulation (repeated measures ANOVA, n=6, P=0.003, $F_{2,10}$=22.3). **d.** Pull force is moderately attenuated during laser on sessions. **e.** Average pull-to-pull correlation coefficients are not consistently disrupted during laser on sessions. **f-g.** Kinetics of average pull force during testing sessions. Data presented as mean ± s.e.m.

Methods

Animals

All animal work was approved by the Weill Cornell Medicine Institutional Animal Care and Use Committee. C57BL6/J *Rosa26-LSL-tdTomato* and C57BL/6J mice (Jackson Laboratory) were housed in disposable plastic cages on a reverse light cycle. Experiments were conducted on adult male (19.4 ± 0.56 g) and female (16.5 ± 0.42 g) mice during the dark period.

Surgery

Spinal cord transduction

Mice (n = 29, 5.83 ± 0.35 weeks) were deeply anesthetized with isoflurane (5% for induction, 1-2% for surgery) heart rate was monitored, and body temperature controlled using SomnoSuite small animal anesthesia system (Kent Scientific). Subcutaneous injection of the analgesic buprenorphine (0.1 mg / kg) and meloxicam (2 mg / kg) was given immediately after the animal was anesthetized. The fur covering the skin on the dorsum over the cervical spinal cord was shaved and the mouse secured in a stereotaxic (Stoelting Co.). Sterile eye lubricant was applied to both eyes to prevent corneal drying during surgery and, 0.1 ml of local anesthetic bupivacaine (0.5% solution) was infiltrated into the intended incision site. The skin was cleaned with three sets of alternating Betadine scrub and 70% alcohol then painted with Betadine solution. A midline incision over the vertebra undergoing laminectomy was made, the skin and dorsal musculature incised and retracted to expose the lamina, and partial laminectomy was performed on vertebral body C6 exposing spinal cord segment C7/C8. To minimize spinal motion due to the breathing, we secured the vertebral column at the spinous process of the second thoracic vertebra (T2). The T2 process was slightly elevated and held with spinal cord clamps (Narishige International) to allow exposure of the vertebral interspace and facilitate intraspinal injections.

For in vivo imaging: Two 300 nl injections of AAV2-retro-pkg-Cre (Addgene 24593, 1.4 x 10^{13} GC/ml) and AAV2 retro-syn-jGCaMP7f-WPRE (Addgene 104488, 1 x 10^{13} GC/ml) [1:1 mixture], for optogenetic stimulation: Two 400 nl injection of AAV2retro-GAG-ChR2 (Addgene 28017, 7 x 10^{12} VG/ml) were made unilaterally into right spinal cord gray matter at C7/C8 (0.5 mm lateral, 0.5-0.8 mm ventral) were made using a pulled glass pipette with a tip diameter of 35 µm and a Nanoject III programmable injector (Drummond) at a speed of 1 nl/s. The pipet was kept in place for additional 4 min to avoid leakage. The double layer of dorsal musculature was closed with 7-0 vicryl absorbable surgical suture (Ethicon) and skin closed with 7 mm surgical wound clips (Fine Science Tools). Mice were given 1 ml saline (0.9% solution) subcutaneously after surgery and buprenorphine twice daily for 3 days post-operatively.

Cranial window implantation

Two weeks after spinal cord transduction, the mice were anesthetized and prepared for surgery, utilizing the technique detailed in the spinal transduction section. Additionally, mice received subcutaneously a single dose of dexamethasone (0.1mg/kg) to prevent swelling of the brain. The head was placed in a stereotaxic device for stabilization and secured with ear bars and nose restrainer. To remove the skin over the skull, an incision was made at the base of the skull followed by two oblique cuts that converge to the midline. A craniotomy of ~ 3-4 mm was performed over the motor cortex by thinning the skull with a dental drill. Saline was used while drilling to keep the skull moist and help removal of the bone with minimizing bleeding of the dura. A sterile 5mm glass coverslip was permanently affixed on top of the dura by applying a cyanoacrylate-based adhesive gel (Loctite 454) around the glass. After the glass was secured, a

rectangular aluminum head bar (Narishige International) was affixed to the skull with C&B-Metabond dental adhesive cement.

Cannula implantation

During the spinal cord transduction surgery, the mice additionally received subcutaneously a single dose of dexamethasone (0.1 mg/kg) to prevent swelling of the brain. The head was placed in a stereotaxic device for stabilization and secured with ear bars and nose restrainer. To remove the skin over the skull, an incision was made at the base of the skull followed by two oblique cuts that converge to the midline. A craniotomy of 3 mm was performed over the forelimb motor cortex area (AP = 0.5, ML = 1.7 mm) by thinning the skull with a dental drill. Saline was used while drilling to keep the skull moist and help removal of the bone with minimizing bleeding of the dura. A 2.5 mm or 1.25 stainless steel cannula with guide (Thorlabs, CFM22L02) as implanted using Vetbond cyanoacrylate glue (3M) and C&B-Metabond dental adhesive cement (Parkell).

Water restriction

C57BL/6J and Ai14 *LSL-tdTomato* (C57BL/6J background) mice (n = 63) were induced to water restriction over a course of 10 days. Briefly, mice were taken off *ad lib* water and given 2 ml water per day for the first 3 days, 1.5 ml water for the subsequent 3 days, and finally 1 ml water for the final 4 days. Mice were maintained at or above 80% pre-restricted weight and held at 1 ml water per day for the duration of the experiment.

Isometric pull task

The MotoTrak behavioral system (Vulintus, Inc.) was used to assess only the right forepaw function. Mice were habituated for 3 days in either the MotoTrak conditions: acrylic chamber for freely moving or head-fixed on the restraint device (MAG-1, Narishige). In both conditions, the pull behavior was first shaped assisting mice to reach the isometric pull with water reward association. Head-fixed mice were divided into two groups: skilled and unskilled. In *skilled isometric pull* behavior training, the water reward was first given for a peak isometric pull force between 5 and 20 g (≥75 trials with success of 50% for three days within one week) then, the range was gradually reduced to pull with a force between 13-19 g (≥ 50 trials) and then to 15-18 g (≥ 50 trials). Graduation for each training phase required an improvement by at least 25% from their baseline, otherwise the mice were considered as non-learners. Motor learning in the narrow range 15-18 g was divided in three phases: early (first 3 days), middle (15.0 ± 4.3 days later) and late (11.6 ± 3.2 days after middle). In *unskilled isometric pull* behavior training, the adaptive threshold was set to the 50th percentile of the prior 10 trials, starting at 3 g. Additionally, the head-fixed mice underwent imaging of neuron activity in 2 to 4 fields of view over the forelimb motor cortex during a recording session of 6min. The freely-moving mice were divided in three groups: static, skilled and unskilled. The skilled and unskilled training were similar as described in the head-fixed mice. In *static isometric pull* behavior training, water reward was first given for an isometric pull force above 5 g. The threshold was increased stepwise to 8 g, 13 g, and finally 15 g.

Corticospinal tract lesion - *bilateral pyramidotomy*

Mice were deeply anaesthetized with isoflurane; an incision of 1 cm was made over the ventral midline. Gently we pushed aside the adipose tissue, we used the manubrium of sternum as a

reliable landmark to locate the pyramids ventrally. A small incision was made through the dura with an insulin syringe 28 gauge to facilitate the lesion of the pyramidal tract rostral to atlas, bilaterally at a depth of 0.5 mm with a 15° ophthalmic microscalpel. The skin was closed with 5-0 nylon surgical suture.

In vivo imaging

A Thorlabs Bergamo 2-photon microscope with SpectraPhysics InsightX3 dual beam laser was used to record calcium dynamics. GCaMP7f signals in corticospinal neurons were recorded at 940 nm using a 16X water-immersion objective (Olympus, WD 3.0 NA 0.8, D32 diameter) in a single imaging plane at depth 650 to 750 mm. All images were acquired at a rate of approximately 30 Hz per frame of 515 x 512 pixels using ThorImageLS 3.2 software with an 8 kHz galvo/resonant scanner speed. ThorSync 3.2 software was used in synchrony to store the frame times, triggers and signals coming into and out of the microscope for an off-line data analysis.

Optogenetic Stimulation

For optogenetic stimulation of corticospinal neurons, mice (n = 18, 6.67 ± 0.86 weeks) were trained on the skilled and unskilled freely-moving, isometric pull tasks with the fiber optic cable attached during all training. After reaching success rate improvements of at least 25% over baseline for three consecutive days, mice were tested with IR-triggered optogenetic stimulation. As mice reached through the MotoTrak slot to the manipulandum, interruption of an IR beam (Fig. 4b) triggered an Arduino-controlled 473 nm fiber-coupled laser (20 mW). ChR2 activation of corticospinal neurons was performed over at least 50 trials within a 15 minute session. Mice

were tested again with the laser off the following day. We analyzed success rate, force, pull-to-pull correlation, and pull kinematics. ChR2 expression in corticospinal neurons was visually confirmed through contraction of the right forepaw following optogenetic stimulation in freely moving mice. Laser intensity was increased from 0 mW to 50 mW until movement was evoked. In total,13 of 15 mice showed a response by 20 mW. The remaining 2 mice showed no response, even up to 50 mW stimulation intensity, and were excluded from the study.

Movement Analysis

Movement period was identified using peak voltage and width extracted from MotoTrak and ThorSync Software. Starting time was marked when the force exceeded a 3 g threshold and end time was marked as the start time plus the width of the pull. For the skilled group, peaks between 15-18g were labelled as successful. Successful trials in the adaptive group were labelled based on the changing threshold recorded in MotoTrak's acquisition software. Peak voltage was extracted in all trials per animal for group analysis over learning and injury. Pull correlation was calculated as the average correlation coefficient between consecutive trials within each session. Kinetics plots were created as an average of continuous force output during all trials in all sessions for each time point. The integral of these plots was calculated to quantify observable changes in force and width. Correlation of patterned activity, and inter-trial variability, were compared to success rate and variability in pull dynamics (force/time).

Imaging Analysis

Images were initially pre-processed in FIJI (ImageJ, NIH) with a Gaussian noise filter (Sigma radius: 2.00). Data analysis was performed in MATLAB (MathWorks) using Constrained

Nonnegative Matrix Factorization (CNMF) [22]. First, non-rigid motion correction removed motion artifacts. Then the motion-corrected file was split into patches and run through CNMF, de-noising and de-mixing spatially overlapping signals. Neurons were selected by radius and the merging threshold (how close components had to be considered the same). Fluorescent traces and $\Delta F/F_0$ were calculated and deconvolved for each identified component. Traces containing less than 5 distinct calcium events were excluded from all further analysis. $\Delta F/F_0$ data was min-max normalized and synchronized with output from the MotoTrak acquisition software through ThorSync Software.

Cell Classification

Cells were classified based on the mean value of activity during movement epochs [23]. Frames of forelimb movement were identified as described above, and movement traces were shuffled 10000 times, so each movement's position in time was randomized. The average activity of individual neurons was calculated for each shuffle and was compared to activity during recorded movements. Recorded values that were above the 85 percentile of the shuffled data were classified as movement active while the remaining cells were classified as interpull interval-active. Neurons that passed a fifth percentile threshold for average activity and number of distinct events were classified as indiscriminately active.

Fluorescence Analysis

Activity of classified cells was averaged across all trials per session then averaged across animals to observe each group during movement epochs. CellReg [13] was used with the spatial footprints calculated through CNMF to detect neurons active across imaging sessions. From these cells

identified as consistent across sessions, the percentage of cells that maintained the same classification and the percentage that switched classification were calculated. Movement active cells were separated and analyzed based on spike timing estimated through CNMF. Spikes occurring during successful or failed pulls were quantified for each neuron in the session. Cells that contained greater than 40% of spikes during successful trials were classified as success-active. The percentage of these cells over the total number of active cells was compared across time points in each animal.

Activity Correlation

Activity correlation during movement was calculated using pairwise correlation analysis. Activity during all distinct movements was concatenated to create a matrix of temporal activity that is movement specific. Pearson's coefficients were calculated between pairs of concatenated activity and were averaged across pairs and animals. This activity correlation was plotted against pull-to-pull correlation detailed above.

Statistical analysis

All statistical analysis was performed with GraphPad Prism software version 9.0.0. Parametric tests were used. Inter-group comparisons were tested with two-way ANOVA, with group and learning phase (time) as the independent factors, and post-hoc comparisons were carried out using Bonferroni tests. Learning phase (time)-related changes within a group were tested with repeated measures ANOVA. Data presented as the mean ± SEM. $P < 0.05$ was considered statistically significant.

Data availability Datasets generated during this study will be available in the public repository (odc-sic.org).

Code availability MATLAB scripts developed for the imaging data analysis will be made available upon reasonable request to the corresponding author.

Acknowledgements We thank C. Schaffer (Cornell University) for training in two-photon microscopy methods. This work was supported by Burke Foundation, the Craig H. Neilsen Foundation, and the National Institutes of Health Common Fund DP2 NS106663 to EH.

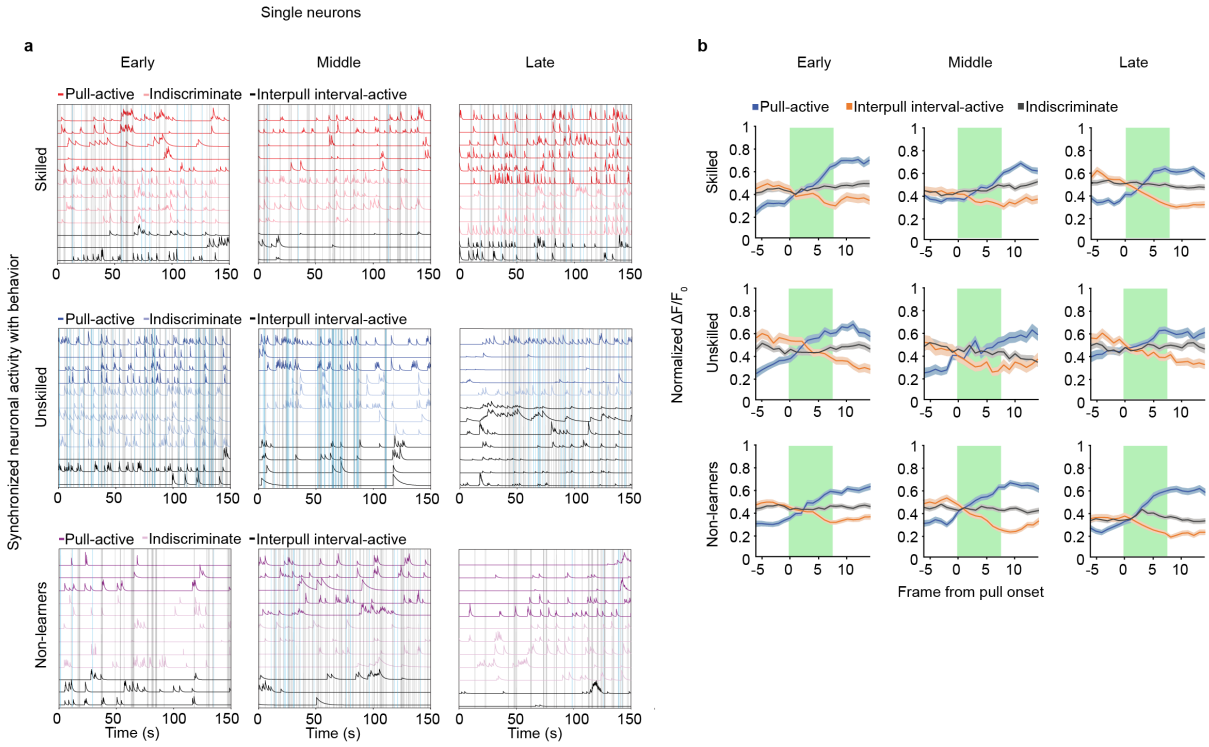
Author contribution NS and EH designed the study; NS, FM, YML performed experiments; AMS designed and developed the behavioral task; SFA generated all illustrations; FM analysed all imaging data; NS, FM, YML, and EH wrote the manuscript; NS and FM made equal contributions to the study.

Competing interests AMS is a co-owner and employee of Vulintus, Inc., which sells products used in data collection for this research. AMS and Vulintus had no editorial authority over the decision to publish.

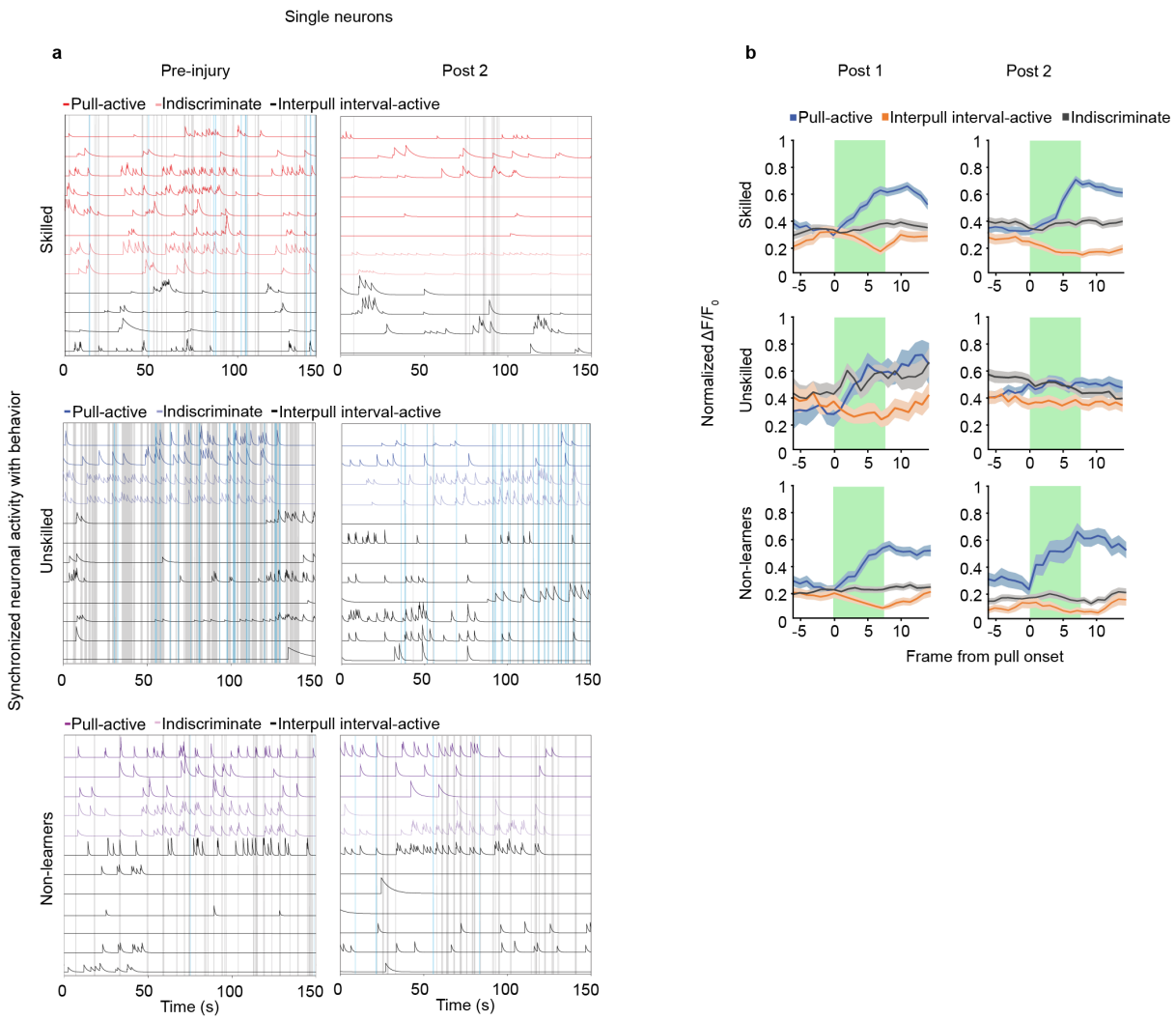
Additional information

Extended data is available for this manuscript

Correspondence and requests for materials should be addressed to EH



Extended Data Figure 1. Corticospinal neuron activity during isometric pull. a. Examples of corticospinal neuron activity from representative mice. Traces show fluorescence changes ($\Delta F/F_0$) from individual corticospinal neurons in skilled (top), unskilled (middle), and non-learners (bottom). Blue bars indicate successful pull trials, and gray bars represent failed trials. **b.** Activity of corticospinal neurons classified into pull-active, inter-pull interval active, and indiscriminately active across pull movement onset and offset. Green represents frames during pull behavior. Data presented as mean $\Delta F/F_0 \pm$ s.e.m.



Extended Data Figure 2. a. Example of corticospinal neurons activity from representative mice. Traces show fluorescent changes ($\Delta F/F_0$) of individual corticospinal neurons from skilled (top), unskilled (middle), and non-learners (bottom) mice. Blue bars represent successful pulls while gray bars represent failed attempts. **b.** Activity of cells around pull movement onset and offset after bilateral pyramidotomy. Green represents frames during pull behavior. Data presented as mean $\Delta F/F_0 \pm$ s.e.m.

Extended Data Table 1. Number of days for each stage of behavior

		Freely moving			Head-fixed			Cannulated	
	Threshold	Skilled	Unskilled	Static	Skilled (learners)	Unskilled	Skilled (non-learners)	Skilled	Unskilled
Preshaping days (mean ± sem)		3.7±0.2	3.4±0.2	3.8±0.2	3.4±0.7	4.7±0.4	3.6±0.7	4.5±1.4	N/A
Training days (mean ± sem)	Adaptive	N/A	11.4±0.2	N/A	N/A	16.2±1.5	N/A	N/A	6.5±0.61
	Phase 1	7.4±0.6 (5-20g)	N/A	5.0±0.4 (5g)	5.0±0.5 (5-20g)	N/A	4.6±0.2 (5-20g)	3.1±0.6 (5-20g)	N/A
	Phase 2	0.9±0.2 (8-20g)	N/A	1.5±0.2 (8g)	2.6±0.5 (8-20g)	N/A	2.6±0.4 (8-20g)	1±0 (8-20g)	N/A
	Phase 3	8.6±1.2 (13-19g)	N/A	4.0±0.5 (13g)	8.8±1.7 (13-19g)	N/A	10.6±2.8 (13-19g)	4.8±0.7 (13-19g)	N/A
	Phase 4	34.7±2.0 (15-18g)	N/A	7.0±1.1 (15g)	22.8±4.0 (15-18g)	N/A	22.8±4.4 (15-18g)	18±1.2 (15-18g)	N/A
Postinjury days (mean ± sem)		14.1±0.1	10.0±0.0	12.4±0.9	10.4±0.7	12.2±1.3	9.3±0.8	N/A	N/A

Extended Data Table 2. Number of corticospinal neurons recorded during imaging sessions

	Early	Middle	Late	Post 1	Post 2
Skilled	267	369	366	276	345
Unskilled	231	160	215	39	181
Non-learners	492	450	442	230	192

Extended Data Table 3. Statistics reporting, by figure

Figure number	Statistical test	*n*	Descriptive stats (AVG, variance)	*P* value	Degrees of freedom
1d	Repeated measures (RM) one-way ANOVA	5 (skilled) 6 (unskilled) 5 (non-learners)	mean±sem	0.0198 (skilled) 0.0970 (unskilled) 0.1715 (non-learners)	F(2, 8) = 6.731 (skilled) F(2, 10) = 3.016 (unskilled) F(2, 8) = 2.263 (non-learners)
1e	Repeated measures (RM) one-way ANOVA	5 (skilled) 6 (unskilled) 5 (non-learners)	mean±sem	0.0662 (skilled) 0.0481(unskilled) 0.4296 (non-learners)	F(2, 8) = 4.037 (skilled) F(2, 10) = 4.977 (unskilled) F(2, 8) = 0.9257 (non-learners)
1f	Repeated measures (RM) one-way ANOVA	5 (skilled) 5 (unskilled) 5 (non-learners)	mean±sem	0.0065 (skilled) 0.2040 (unskilled) 0.6400 (non-learners)	F(2, 8) = 22.13 (skilled) F(2, 8) = 2.188 (unskilled) F(2, 8) = 0.2687 (non-learners)
1k	Repeated measures (RM) one-way ANOVA	5 (skilled) 5 (unskilled) 5 (non-learners)	mean±sem	0.5629 (skilled) 0.2930 (unskilled) 0.9422 (non-learners)	F(2, 8) = 0.6077(skilled) F(2, 8) = 1.464 (unskilled) F(2, 8) = 0.0227 (non-learners)
2c	Repeated measures (RM) one-way ANOVA	8 (skilled) 9 (unskilled) 12 (static)	mean±sem	<0.0001 (skilled) 0.6302 (unskilled) 0.0019 (static)	F(2, 14) = 41.18 (skilled) F(2, 16) = 0.3817 (unskilled) F(2, 22) = 9.190 (static)
2d	Repeated measures (RM) one-way ANOVA	8 (skilled) 9 (unskilled) 12 (static)	mean±sem	0.0043 (skilled) 0.4516 (unskilled) 0.2690 (static)	F(2, 14) = 12.09 (skilled) F(2, 16) = 0.7961 (unskilled) F(2, 22) = 1.388 (static)
2e	Repeated measures (RM) one-way ANOVA	8 (skilled) 9 (unskilled) 12 (static)	mean±sem	0.3149 (skilled) 0.0064 (unskilled) 0.4966 (static)	F(2, 14) = 1.242 (skilled) F(2, 16) = 7.589 (unskilled) F(2, 22) = 0.6483 (static)
2i	Repeated measures (RM) one-way ANOVA	8 (skilled) 9 (unskilled) 5 (static)	mean±sem	0.0004 (skilled) 0.0011 (unskilled) 0.1357 (static)	F(2, 14) = 17.71 (skilled) F(2, 16) = 11.01 (unskilled) F(2, 8) = 3.311 (static)
2j	Repeated measures (RM) one-way ANOVA	8 (skilled) 9 (unskilled) 5 (static)	mean±sem	0.1210 (skilled) 0.0022 (unskilled) 0.1948 (static)	F(2, 14) = 2.525 (skilled) F(2, 16) = 13.36 (unskilled) F(2, 8) = 2.299 (static)
2k	Repeated measures (RM) one-way ANOVA	8 (skilled) 9 (unskilled) 5 (static)	mean±sem	<0.0001 (skilled) 0.0013 (unskilled) 0.0015 (static)	F(2, 14) = 23.02 (skilled) F(2, 16) = 12.67 (unskilled) F(2, 8) = 17.01 (static)
3a	Repeated measures (RM) one-way ANOVA	5 (skilled) 6 (unskilled) 5 (non-learners)	mean±sem	0.0032 (skilled) 0.9716 (unskilled) 0.0678 (non-learners)	F(2, 8) = 14.62 (skilled) F(2, 10) = 0.01879 (unskilled) F(2, 8) = 6.995 (non-learners)
3b	Repeated measures (RM) one-way ANOVA	5 (skilled) 6 (unskilled) 5 (non-learners)	mean±sem	0.0100 (skilled) 0.0180 (unskilled) 0.0631 (non-learners)	F(2, 8) = 16.38 (skilled) F(2, 10) = 8.286 (unskillled) F(2, 8) = 6.893 (non-learners)
3c	Repeated measures (RM) one-way ANOVA	5 (skilled) 5 (unskilled) 5 (non-learners)	mean±sem	0.0046 (skilled) 0.0703 (unskilled) 0.3689 (non-learners)	F(2, 8) = 25.05 (skillled) F(2, 8) = 4.187 (unskilled) F(2, 8) = 1.129 (non-learners)
3h	Repeated measures (RM) one-way ANOVA	5 (skilled) 5 (unskilled) 5 (non-learners)	mean±sem	0.0038 (skilled) 0.7858 (unskilled) 0.6501 (non-learners)	F(2, 8) = 19.59 (skilled) F(2, 8) = 0.1248 (unskilled) F(2, 8) = 0.3408 (non-learners)
4c	Repeated measures (RM) one-way ANOVA	6 (skilled) 8 (unskilled)	mean±sem	0.0028 (skilled) 0.1267 (unskilled)	F(2, 10) = 22.27 (skilled) F(2, 14) = 2.506 (unskilled)
4d	Repeated measures (RM) one-way ANOVA	6 (skilled) 8 (unskilled)	mean±sem	0.0491(skilled) 0.0459 (unskilled)	F(2, 10) = 6.165 (skilled) F(2, 8) = 12.17 (unskilled)
4e	Repeated measures (RM) one-way ANOVA	6 (skilled) 8 (unskilled)	mean±sem	0.3566 (skilled) 0.4789 (unskilled)	F(2, 10) = 1.133 (skilled) F(2, 14) = 0.7526 (unskilled)