

Motor Cortex Is Required for Learning but Not for Executing a Motor Skill

Highlights

- We train rats to execute spatiotemporally precise task-specific motor sequences
- We show that motor cortex is not required for executing the learned skills
- Motor cortex, however, is essential for acquiring the subcortically generated skills
- This suggests that motor cortex “tutors” subcortical motor circuits during skill learning

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In Brief

Motor cortex is widely credited with expanding the behavioral repertoire of mammals by enabling the acquisition and execution of new motor skills, but its specific contributions have been difficult to pin down. Using a novel motor skill learning paradigm, Kawai et al. show that motor cortex contributes to learning skills even when it is not required to execute them. This demonstrates a previously unappreciated role for motor cortex in “tutoring” subcortical circuits during skill learning.

Motor Cortex Is Required for Learning but Not for Executing a Motor Skill

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SUMMARY

Motor cortex is widely believed to underlie the acquisition and execution of motor skills, but its contributions to these processes are not fully understood. One reason is that studies on motor skills often conflate motor cortex's established role in dexterous control with roles in learning and producing task-specific motor sequences. To dissociate these aspects, we developed a motor task for rats that trains spatiotemporally precise movement patterns without requirements for dexterity. Remarkably, motor cortex lesions had no discernible effect on the acquired skills, which were expressed in their distinct pre-lesion forms on the very first day of post-lesion training. Motor cortex lesions prior to training, however, rendered rats unable to acquire the stereotyped motor sequences required for the task. These results suggest a remarkable capacity of subcortical motor circuits to execute learned skills and a previously unappreciated role for motor cortex in “tutoring” these circuits during learning.

INTRODUCTION

Motor skills underlie much of what we do, be it playing instruments or sports, signing our names, or tying our shoelaces. Learning a motor skill involves organizing actions into novel sequences that can be executed efficiently and reproducibly to solve a given task (Hikosaka et al., 2002; Luft and Buitrago, 2005; Shmuelof et al., 2012; Willingham, 1998). When learned, skills are retained long-term (Hikosaka et al., 2002; Park et al., 2013; Romano et al., 2010), suggesting that they are stored as lasting changes in motor control circuits (Dayan and Cohen, 2011; Ungerleider et al., 2002). However, how the mammalian brain divides the task of learning and executing motor skills across its distributed motor control network is not well understood (Figure 1).

Motor cortex is widely believed to play central roles in both motor skill learning and execution. Its projections to the spinal

cord are essential for generating independent joint and digit movements, or “dexterity” broadly defined (Alaverdashvili and Whishaw, 2008; Lawrence and Hopkins, 1976; Lawrence and Kuypers, 1968a; Lemon, 1993; Passingham et al., 1983; Whishaw, 2000; Figure 1A). Practice-induced changes in motor cortical control networks (Riout-Pedotti et al., 1998; Wang et al., 2011; Xu et al., 2009) can improve the quality and range of movements they can produce (Adkins et al., 2006; Classen et al., 1998; Kleim et al., 1998), a capacity that helps animals adapt their motor output to difficult control scenarios—an important aspect of many skills.

However, motor cortex is not the only “controller” capable of commanding spinal circuits for the purpose of generating movements. The phylogenetically older subcortical motor infrastructure is also quite sophisticated in this regard (Azim et al., 2014; Barnes, 2012; Esposito et al., 2014; Kuypers and Martin, 2011), as is evident from studies in rodents and primates with motor cortex or corticospinal tract lesions (Castro, 1972; Darling et al., 2011; Lawrence and Kuypers, 1968a; Passingham et al., 1983). Although dexterity is permanently compromised in these animals, other aspects of control survive remarkably intact, consistent with an ability of subcortical controllers to generate a variety of species-typical or “innate” movements and actions (Baker, 2011; Grillner and Wallén, 2004; Hikosaka, 1994; Honeycutt et al., 2013; Lawrence and Kuypers, 1968a; Philipp and Hoffmann, 2014; Stein et al., 1999; Figure 1A). Disrupting descending brainstem pathways can have far more devastating consequences for baseline motor control than lesions to the corticospinal tract (Lawrence and Kuypers, 1968b), highlighting the importance of these lower-level controllers.

If subcortical controllers are indeed essential for generating many types of basic movements and action patterns (Figure 1A), how are they engaged during skill learning and execution? Studies on motor skills that focus on facets explicitly requiring cortical control, i.e., dexterity (Karni et al., 1995; Kleim et al., 1998; Pol-drack et al., 2005) (Figure 1B), fail to address this question. However, the capacity to sequence and coordinate movements that—in theory at least—can be generated from a pre-established subcortical motor repertoire (Shmuelof and Krakauer, 2011; Yin et al., 2009) is a fundamental aspect of many motor skills.

Several studies have suggested that innate movement patterns can be quite flexible (Berkinblit et al., 1986; Grillner and

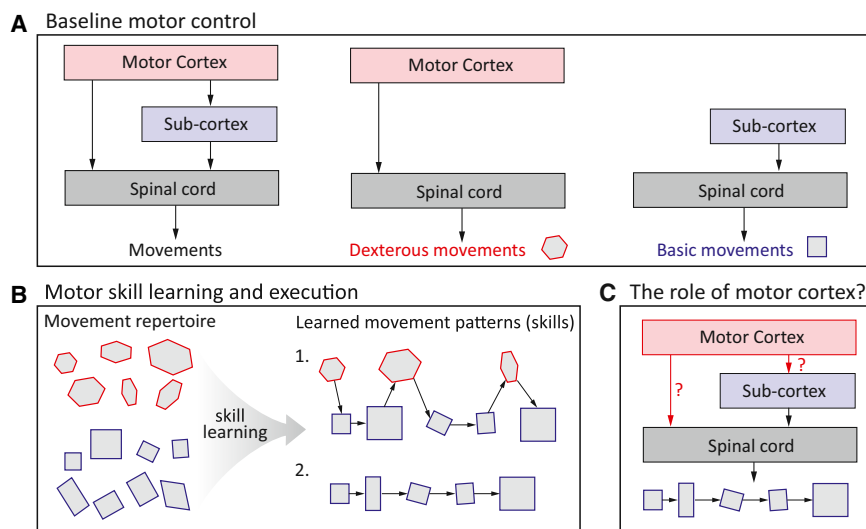


Figure 1. Addressing the Role of Motor Cortex in the Acquisition and Execution of Learned Motor Skills Independently of Motor Cortex’s Established Role in Dexterous Control

(A) A simplified schematic of the mammalian motor control system (left), distinguishing cortical (center), and subcortical (right) controllers. Direct cortical control of the spinal cord is necessary for generating dexterous movements. Subcortical controllers are capable of generating a range of species-specific action patterns and movements (Basic movements).

(B) We define motor skills as task-specific learned motor sequences. The component actions can, in principle, be generated by either cortical (red octagons) or sub-cortical (blue rectangles) controllers. Some motor skills require dexterity (1), whereas others can be built by sequencing and coordinating subcortically generated actions in novel ways (2).

(C) Using motor skills that do not require dexterity allowed us to investigate the role of motor cortex in the acquisition and execution of learned motor sequences separate from its role in dexterous control.

Wallén, 2004), leaving open the possibility that new motor behaviors can be formed by adapting subcortically generated motor programs to novel contingencies and demands (Berntson and Micco, 1976; Grillner and Wallén, 2004). But, if so, what are the limits of such plasticity, and what degree of autonomy do lower-level motor circuits have when it comes to executing learned motor skills? Is motor cortex an essential contributor regardless of the control challenges involved, or can subcortical circuits store and produce task-specific motor sequences as long as the components themselves can be generated subcortically (Figures 1B and 1C)?

A related question that cannot be cleanly addressed in tasks requiring dexterity is whether motor cortex has a role in learning that is distinct from its role in control. Motor cortex projects heavily to subcortical circuits (Akintunde and Buxton, 1992) and is known to modulate the expression of subcortically generated actions in context-specific ways (Drew et al., 1996, 2008; Ioffe, 1973; Stoltz et al., 1999). One hypothesis still to be tested is that such modulation, when repeated and consistent, fulfills a “tutoring” function that allows subcortical control networks to acquire, consolidate, and execute new motor sequences (Andalman and Fee, 2009; Shmuelof and Krakauer, 2011).

Addressing the above questions and, more generally, the interplay between cortical and subcortical motor circuits during skill learning would benefit from an experimental paradigm that dissociates motor cortex’s role in learning and generating task-specific motor sequences from its role in dexterous control. The rat presents a tractable and well suited model for such a paradigm. It is a good learner, amenable to being trained in complex behavioral tasks (Erlich et al., 2011; Poddar et al., 2013; Tervo et al., 2014), and capable of generating much of its action repertoire without motor cortex, i.e., subcortically (Castro, 1972). The latter is important because the ideal task for our inquiry is one that, in principle at least, can be solved by sequencing and/or adapting subcortically generated move-

ments and actions (Figure 1C). However, most established motor tasks, even in rats, are designed to interrogate the function and plasticity of cortical controllers (Óveczky, 2011), making them ill suited for the questions we ask here.

This motivated us to develop a new motor learning paradigm that trains rats to produce complex, spatiotemporally precise, and robustly maintained task-specific motor sequences without explicit requirements for dexterity. By means of motor cortical lesions and high-resolution behavioral analysis, we show that motor cortex is not required for executing the learned skills we train, implying that they can be stored and generated subcortically. Intriguingly, however, we find that motor cortex is essential for learning the very same skills, suggesting that it plays an essential role in guiding plasticity in downstream control circuits during skill learning.

RESULTS

A New Motor Skill Learning Paradigm for Rats

To dissociate learning and execution of complex task-specific motor sequences from the cortex-dependent challenge of generating dexterous movements, we developed a motor skill learning paradigm that rewards rats for pressing a lever with their forepaws in a temporally precise sequence—a difficult to learn but not necessarily a dexterous task (Figure 2A; Experimental Procedures). Although the relative timing of the lever-presses was prescribed, the movements that could be used were not, meaning that there were no explicit requirements for dexterity. Force requirements were also modest (<0.1 N to press the lever; Experimental Procedures), making the central challenge of the task to execute temporally precise and reproducible movement sequences.

After learning to press the lever for water, water-restricted animals were rewarded for increasingly precise approximations to the target sequence, which was a sequence of two lever-presses separated by a predefined inter-press interval (IPI). Because rats

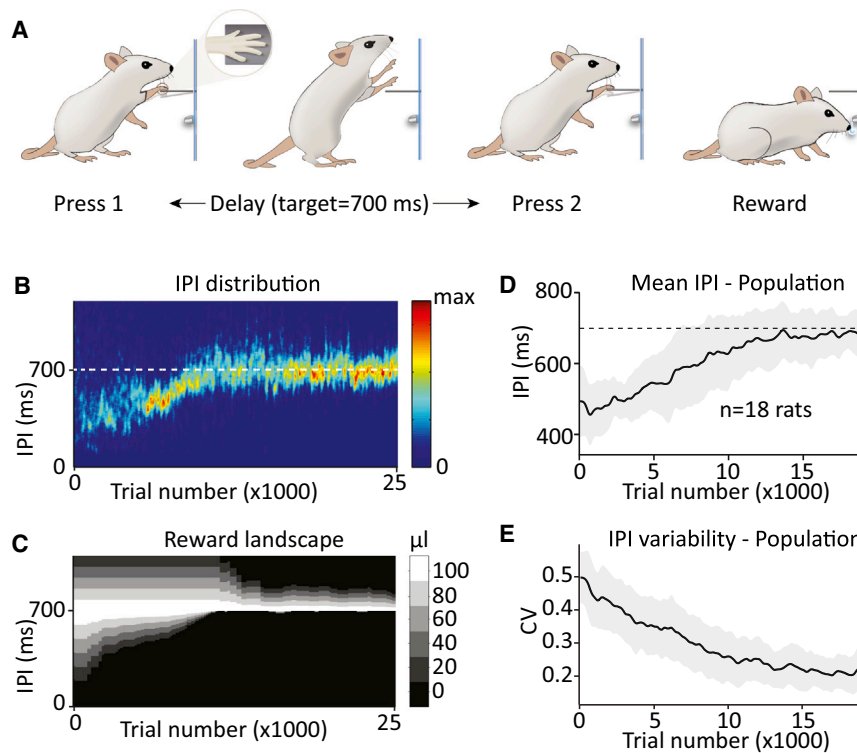


Figure 2. A Novel Paradigm for Training Temporally Precise Motor Sequences in Rats without Explicit Requirements for Dexterity

(A) Schematic of the task. Water-restricted rats are rewarded with water for pressing a lever twice in a prescribed temporal sequence.

(B) Density plot of the IPI distribution for a rat learning a 700-ms target IPI.

(C) The graded reward landscape for the rat in (B). The amount of water dispensed at a given IPI is automatically updated based on performance to ensure an average reward rate around 35% (Experimental Procedures).

(D and E) Learning curves showing the mean (D) and CV (E) of the IPI distribution as a function of training across all rats with a 700-ms IPI target ($n = 18$). The shaded region denotes SD across rats.

Rats Learn Complex and Stereotyped Movement Patterns

The movement patterns that emerged after weeks of training were highly complex and stereotyped (Movie S1), involving predominantly the forelimbs, although other movements, including hindlimb and whole-body movements, were often incorporated. Many of the motor elements

show a natural proclivity for pressing the lever in fast succession, i.e., within ~ 300 ms (Figure S1), we challenged them to overcome this natural tendency and learn new task-specific motor sequences by setting the target to longer IPIs (700 ms, $n = 18$; Figure 2B). During training, the range of rewarded IPIs was adjusted based on performance to ensure average reward rates around $\sim 35\%$ (Figure 2C; Experimental Procedures; Supplemental Experimental Procedures). If an IPI was not in the rewarded range, a new trial could only be initiated after 1.2 s without a lever-press. The end goal of training was asymptotically precise performance at the targeted IPI (Figures 2B and 2D; Experimental Procedures; Supplemental Experimental Procedures).

Converging on a behavior that reliably produced a 700-ms IPI took many weeks of training. Rats, on average, required $13,689 \pm 5,470$ (mean \pm SD, $n = 18$) trials or 35 ± 9 training days to reach “criterion” performance, defined as the mean IPI being within 10% of the target and the coefficient of variation (CV) of the IPI distribution being less than 0.25. The rewarded range was narrowed further until temporal precision reached asymptote (Supplemental Experimental Procedures), which occurred after $17,130 \pm 5,228$ total trials or 43 ± 10 training days (CV at asymptote, 0.20 ± 0.03 ; mean IPI, 701 ± 35 ms; Figures 2D and 2E).

Although not essential for reaching criterion performance on the task (Figure S2), all rats also learned to withhold lever-pressing for the prescribed 1.2 s following unrewarded IPIs, making task engagement more efficient. The median time to the next lever-press after unrewarded IPIs exceeded 1.2 s after, on average, $15,526 \pm 10,077$ trials ($n = 18$; Experimental Procedures).

making up the consolidated skills were not directly related to the act of pressing the lever and were perhaps incorporated through trial and error learning as a mechanism for producing the target IPI (700 ms) (Killen and Fetterman, 1988), which was more than double what rats prefer naturally (Figure S1). Thus, rats “solve” what is ostensibly an interval timing task in the motor domain by acquiring spatiotemporally precise movement sequences that produce the prescribed lever-press timing.

To better quantify changes to task-related movement kinematics, we also tracked the forepaws in a subset of rats throughout learning ($n = 4$) by tattooing them with colored ink and extracting color markers from high-speed (200 frames/s [fps]) movies (Figure 3A; Experimental Procedures; Movie S2). Analysis of the paw trajectories revealed gradual learning-related changes to forelimb movement patterns (Figures 3B and 3C) that were accompanied by reduced trial-to-trial variability (Figure 3D), culminating in precise and individually distinct forepaw trajectories (Figures 3E and 3F). When performance at the target IPI reached asymptote, the underlying kinematics largely stabilized (Figures 3D, 3F, and 3G). Moreover, as with human motor skills (Park et al., 2013; Romano et al., 2010), the learned behaviors were maintained even after many days without practice (Figure 3G), suggesting that they had been consolidated into motor control circuitry.

Motor Cortex Is Not Required for Performing the Learned Skills

To probe whether execution of the complex learned motor sequences requires motor cortex (Figure 4A), we lesioned it

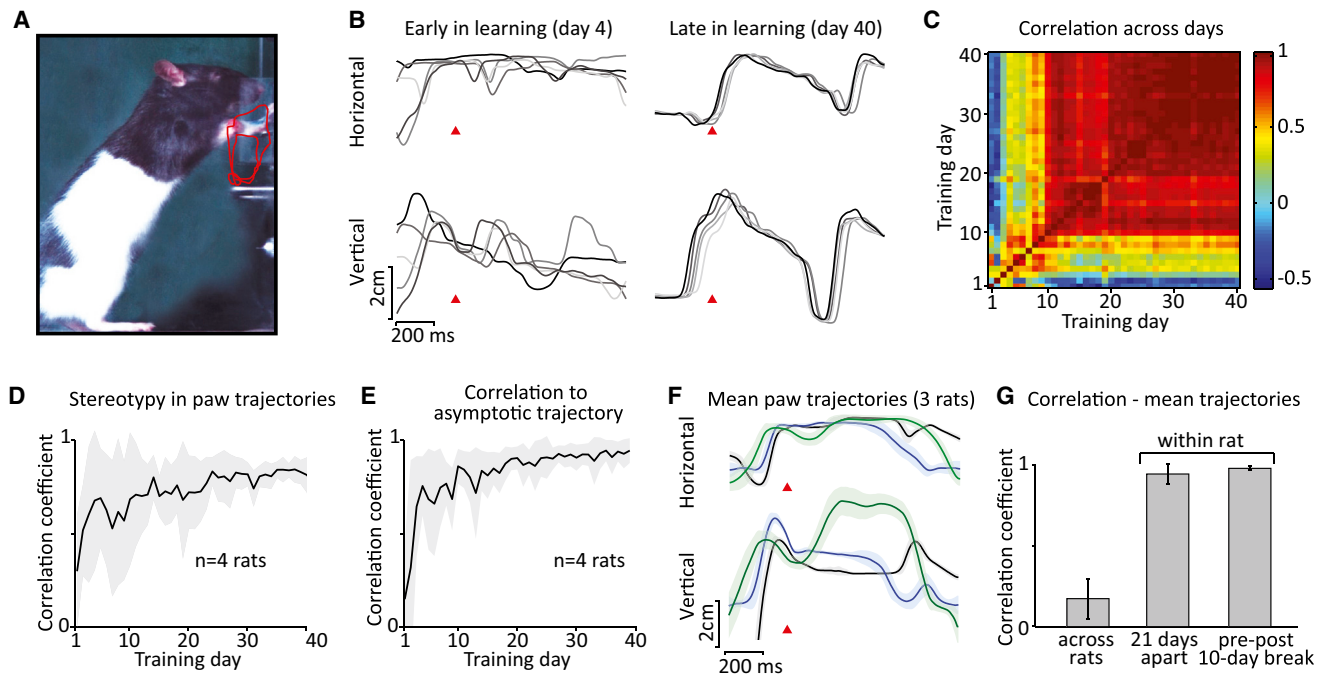


Figure 3. Rats Trained in Our Motor Skill Paradigm Learn to Produce Stereotyped, Idiosyncratic, and Stably Expressed Motor Sequences (A) Kinematic tracking of a rat's forepaw during a trial (red trace). Paws were tattooed with colored ink, and the color markers were extracted from high-speed movies (Experimental Procedures; Movie S2). (B) Forepaw trajectories for five consecutive trials early and late in learning for the rat in (A), aligned to the first lever-press (red arrows). (C) Correlation matrix for the mean paw trajectories associated with a narrow band of IPIs around the target (680–735 ms) over the course of learning for the rat in (A and B). (D) Stereotypy in paw kinematics estimated from the average pairwise correlation of trajectories around the target (680–735 ms) in daily training sessions, averaged across four rats. The shaded region denotes SD across rats. (E) Correlation between daily average paw trajectories and the trajectory at asymptotic performance averaged across four rats (Experimental Procedures). The shaded region denotes SD across rats. (F) The learned motor behaviors are precise but individually distinct. Mean trajectories (within the IPI range of 680–735 ms), aligned to the first tap (red arrow), for three rats at asymptotic performance. Data from single training sessions are shown. The shaded regions denote SD across trials. (G) Average correlation in mean trajectories (within the IPI range of 680–735 ms) across rats ($n = 8$ rats; 7 pairwise correlations for each), within rats 21 days apart during training ($n = 4$ rats), and before and after a 10-day break ($n = 8$ rats). In all cases, rats had reached asymptotic performance on the task. All rats for which the requisite kinematic tracking data was available were included. Comparison across 21 days was only possible for four of the rats (Experimental Procedures). We noted that restricting the analysis to the four rats that were reliably traced across all conditions yielded similar results for the different conditions. Error bars denote SD across animals.

after asymptotic performance was reached ($n = 11$ rats). We initially lesioned motor cortex in the hemisphere contralateral to the paw used for lever-pressing, or, when both paws were used, the paw that pressed the lever first. Induced lesions ($>23 \text{ mm}^3$ of cortical volume) were similar to those in previous studies, demonstrating motor cortical involvement in skilled reaching tasks requiring dexterity (Whishaw, 2000; Whishaw et al., 1986), and encompassed both primary and secondary forelimb representations; i.e., the area of cortex that controls the forelimb (Bonazzi et al., 2013; Neafsey et al., 1986; Figure 4B; Figure S3; Experimental Procedures).

As judged by their ability to move around the cage and manipulate food pellets, all lesioned animals were initially impaired on the contralateral side, consistent with prior reports (Castro, 1972). This initially depressed motor activity suggested that subcortical motor circuits were transiently affected by the sudden loss of cortical inputs (Seitz et al., 1999). By the end of the 10-day recovery period, however, animals were moving around

normally, implying that subcortical circuits had recovered to the point of supporting basic motor functions.

If motor cortex is required for storing and executing the learned motor sequences we train, then rats reintroduced to the task after the lesions would be expected to have significant performance deficits and altered movement kinematics. If, on the other hand, the acquired skills are stored subcortically and motor cortical input to the essential control circuits is not essential for generating them, then post-lesion task performance should be much less affected.

Remarkably, when reintroduced into the behavioral apparatus on the 11th day after the lesions, animals performed the task much as they had previously (Figures 4C–4E). Trial-to-trial variability, measured as the CV of the IPI distribution, was increased on the first days of post-lesion training (Figure 4E), consistent with the trend seen after 10-day breaks in unlesioned animals. The mean IPI also showed a trend comparable with what is seen after control breaks. On the first day of post-lesion training,

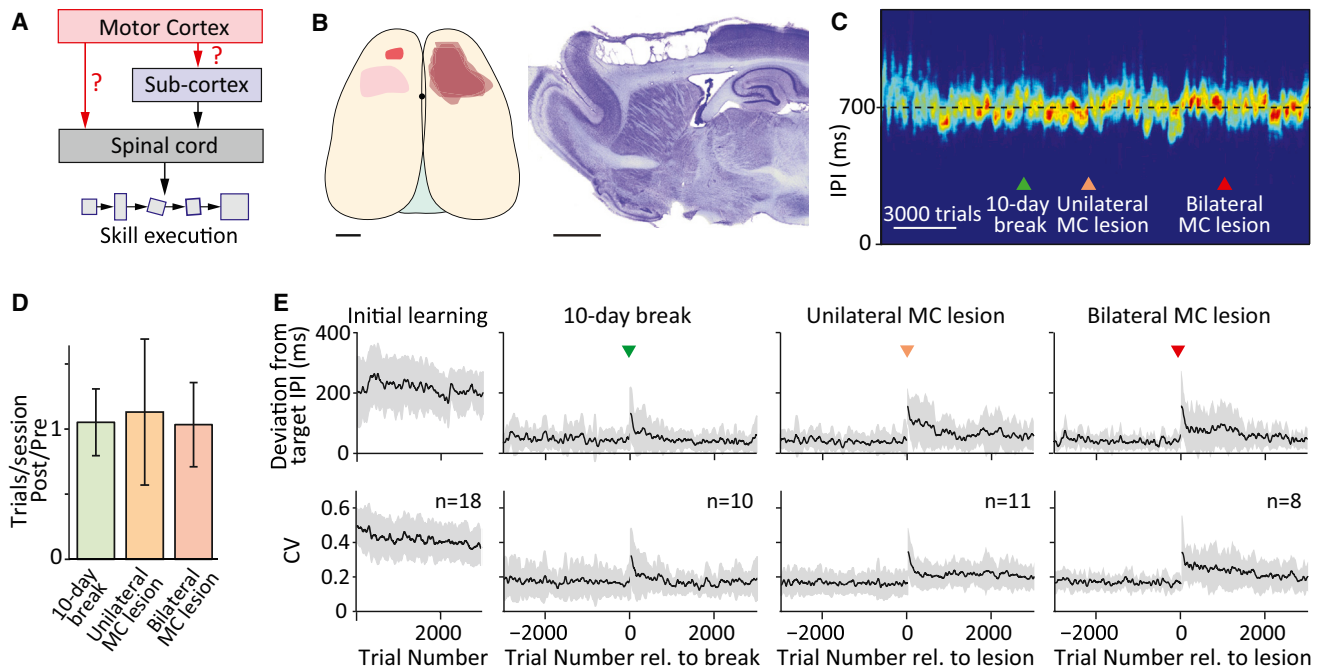


Figure 4. Probing the Necessity of Motor Cortex for Executing the Acquired Skills by Lesioning It after Learning

(A) Schematic showing the pathways through which motor cortex could drive skill execution (red arrows). (B) Motor cortex lesions. Left: schematic showing the outlines of the primary (pink) and secondary (red) forelimb motor cortex on the left hemisphere (Bonazzi et al., 2013; Neafsey et al., 1986) and the lesions from four rats overlaid on the right (Experimental Procedures). The black dot represents the approximate location of the bregma. Right: a Cresyl Violet-stained sagittal section (2.0 mm lateral from the midline) showing the induced lesion. Scale bars, 2 mm. (C) IPI density plot for a rat at asymptotic performance levels receiving a 10-day break from training and then a two-stage bilateral motor cortex (MC) lesion. (D) The number of trials performed in a session before and after a 10-day control break (green) or motor cortex lesions (orange and red) does not change significantly ($p > 0.1$ for all comparisons, Student's *t* test). Error bars denote SD across animals (the number of animals was as in D). (E) Mean (absolute deviation from target) and CV of IPI distributions during initial learning and relative to the different interventions across all rats in our experiments. The different numbers of animals for each condition reflect that not all rats were subjected to all interventions (Experimental Procedures). All manipulations were done after the animals had reached asymptotic performance on the task. The shaded regions denote SD across animals.

only 1 of 11 rats showed an effect on performance ($p < 0.05$) above and beyond what could be expected from a 10-day break in intact animals (Experimental Procedures). The affected individual recovered to normal performance levels by the 9th day of training.

The highly stereotyped and individually distinct movement patterns were also largely unaffected by the motor cortex lesions. This becomes strikingly evident when comparing high-speed movies of task performance before and after the lesions (Movie S3). Forepaw trajectories were also similar to pre-lesion already on the very first day of post-lesion training ($n = 7$; Figures 5A–5C), the only “outlier” being the rat that also showed a significant performance deficit (orange arrow in Figure 5C).

Interestingly, this was the only rat in our cohort to converge on a strategy contingent on precise positioning and movement of the distal digits (Movie S4). That performance and learned kinematics were compromised in this rat following the lesion is consistent with motor cortex being essential for certain types of digit movements (Alaverdashvili and Whishaw, 2008; Whishaw et al., 1991). This rat largely recovered its pre-lesion form after a few days of training, seemingly compensating for the loss of precise digit control (Movie S4). The forepaw trajectories also

became more similar to pre-lesion (correlation coefficient going from 0.51 on the first day after lesion to 0.79 on the third day), consistent with the gross structure of the learned motor sequence being stored downstream of motor cortex also in this rat.

To test whether motor cortex in the contralesional hemisphere was integral to the spontaneous recoveries we observed, we lesioned it in a subset of rats ($n = 8$) after a few additional weeks of training (range, 26–60 days). Following a 10-day recovery period, the now bilaterally motor cortex-lesioned animals still executed the skill similar to pre-lesion (Figures 4C–4E, 5B, and 5C; Movie S5). CV and deviation from target IPI were consistent with having a 10-day break for all but one animal, which transiently sped up the execution of its acquired movement sequence. However, the mean trajectories around the target IPI for this and all other lesioned rats remained similar to pre-lesion (Figures 5B and 5C).

These results show that motor cortex is not obligatory for storing or executing complex learned motor sequences. They further suggest that the function of subcortical motor circuits can be remarkably resilient to the loss of motor cortical input and can even support the execution of certain consolidated motor skills without it.

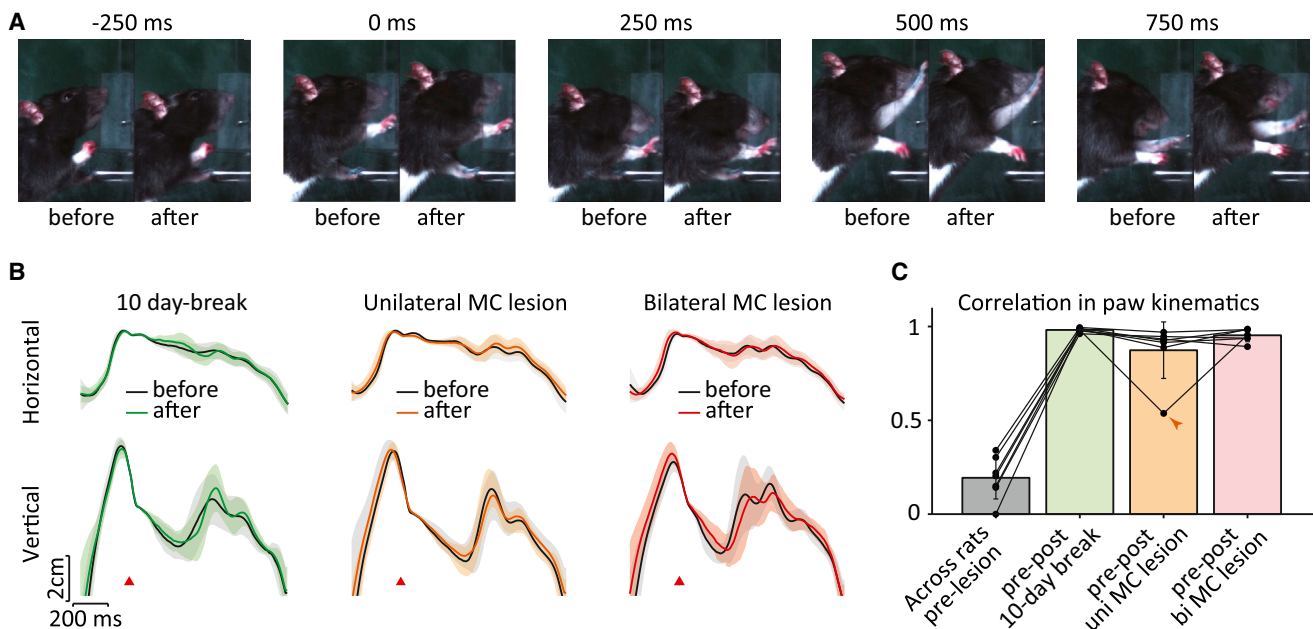


Figure 5. Learned Movement Kinematics Recover Spontaneously after Motor Cortex Lesions

(A) Snapshots from high-speed movies showing the rat's movement pattern on the last day before and on the first training day after lesion to motor cortex in the left hemisphere (see also [Movies S3](#) and [S5](#)). The times of the frames are relative to the first lever-press.

(B) Mean forepaw trajectories for the rat in (A) corresponding to a range of IPIs around the target (680–735 ms) on the last training day before and first training day after (left) a 10-day control break, (center) lesion to motor cortex contralateral to the paw first pressing the lever, and (right) lesion to motor cortex in the previously unlesioned hemisphere (right). The shaded regions represent SD across trials. Red arrows denote the time of the first tap.

(C) Correlation in average paw trajectories corresponding to IPIs around the target (680–735 ms) across different rats ($n = 7$ rats, six pairwise correlations each) and within rats ($n = 7$) for the conditions in (B). Shown are data from all rats for which kinematic tracking data were available for all conditions ($n = 7$). Lines connect the data points for individual rats. Correlations pre- and post-unilateral and -bilateral lesion were not significantly different from pre- and post-10-day control breaks ($p = 0.13$ and $p = 0.1$, respectively, paired t test). The orange arrowhead denotes the outlier rat (see text).

Lesioning Motor Cortex prior to Learning the Task

Next we asked whether motor cortex is essential for learning the skills for which it is not, when acquired, a required controller. This gets at whether motor cortex has a role in learning that is distinct from its role in control ([Willingham, 1998](#)).

A priori, there are two main possibilities. One is that motor learning is an extension of control ([Willingham, 1998](#)) and that circuits sufficient for generating a consolidated skill are also sufficient for learning it. Another possibility is that motor cortical projections to downstream motor circuits “tutor” these phylogenetically older control circuits during learning, allowing them to acquire and produce complex task-specific movement sequences ([Figure 6A](#)).

To arbitrate between these possibilities, we lesioned motor cortex bilaterally in untrained rats ($n = 11$) using the same protocol as for trained animals ([Figure 4B](#); [Experimental Procedures](#)). After recovering for 12 ± 4 days (range, 6–21 days), rats were introduced to the task for the first time.

Early in learning, there was little to distinguish lesioned and unlesioned animals in terms of task performance or relevant metrics of motor output ([Figures 6B–6H](#)). Across the first 1,000 trials of timed lever-press training ([Experimental Procedures](#)), the mean and CV of the IPI distributions were not significantly different for the two cohorts ([Figures 6B](#) and [6C](#)). The fraction of IPIs within 10% of the 700-ms target ([Figure 6D](#)) and average

reward rates ([Figure 6E](#)) were also similar, meaning that, during this initial exploratory phase of learning, lesioned rats generated the prescribed lever-press sequence at rates comparable to controls. Motivation for the task, measured as the number of lever-presses in a training session, was similarly unaffected by the lesions ([Figure 6F](#)). Furthermore, lesioned rats did not have any deficits in learning the association between the reward tone and the availability of water ([Figure 6G](#)). The number of taps outside of the trial context was also similar ([Figure 6H](#)). Therefore, exploratory lever-pressing behavior and the capacity to respond to reward—two essential ingredients for the trial and error learning process by which the skills are acquired—were, by our measures, unaffected by motor cortical lesions.

Skill Learning Is Severely Affected by Motor Cortex Lesions

Even though we found no major deficits in motivation or baseline control to prevent lesioned animals from engaging with the lever and learning from their interactions with it, none of the rats learned the task to criterion ([Figures 7A–7D](#)). This was despite being kept in training three times longer than it takes normal animals to master the task (97 ± 23 training days corresponding to $60,029 \pm 20,390$ trials, $n = 11$). In terms of precision of motor output (CV of the IPIs), lesioned animals reached asymptotic performance after $27,014 \pm 12,178$ trials or 53 ± 19 training days

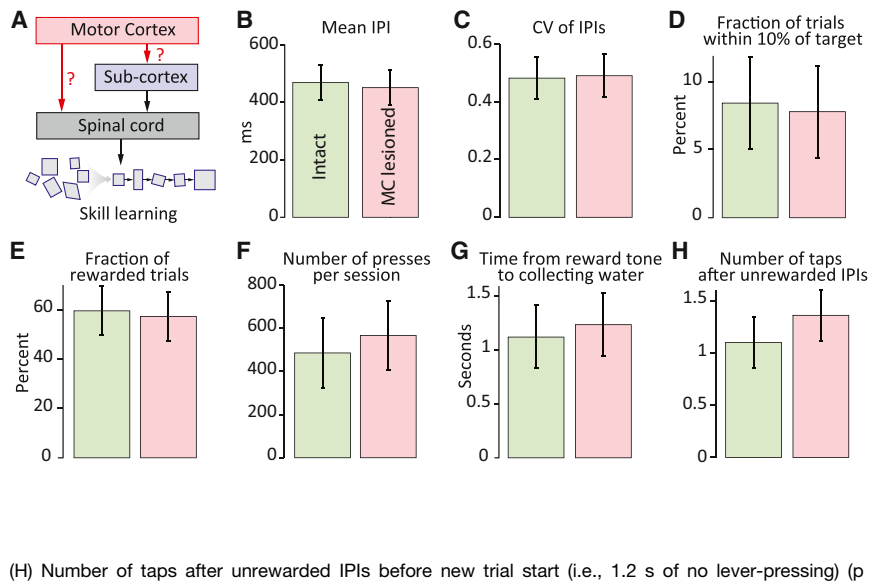


Figure 6. Probing the Necessity of Motor Cortex for Learning Subcortically Generated Skills

(A) Schematic showing the pathways through which motor cortex could influence subcortical activity (red arrows) and, therefore, support the acquisition of subcortically generated motor skills. (B–H) Motor cortex-lesioned rats do not show any apparent motivational or task-relevant motor control deficits early in learning. Shown are performance metrics for intact (gray bars, $n = 18$) and lesioned (pink bars, $n = 11$) animals in the first 1,000 trials of training.

(B) Mean of the IPI distribution ($p = 0.45$).

(C) CV of the IPI distribution ($p = 0.73$).

(D) Fraction of trials within 10% of the target IPI (700 ms) ($p = 0.29$).

(E) Fraction of rewarded trials ($p = 0.92$).

(F) Number of lever-presses per training session ($p = 0.71$).

(G) Time from reward tone to collecting water ($p = 0.19$).

(H) Number of taps after unrewarded IPIs before new trial start (i.e., 1.2 s of no lever-pressing) ($p = 0.36$).

Statistical significance was estimated using Student's *t* test. Error bars denote SD across animals.

(Experimental Procedures), at which point their timing variability (CV of the IPI distribution) was nearly double that of intact animals (0.38 ± 0.06) and only 27% lower than at the start of training (Figure 4C), with none of the animals below 0.25. The mean IPI across the population was also significantly lower than the 700-ms target at this stage of training (601 ± 67 ms), with only 3 of the 11 rats being within 10% of the target IPI (Figure 7B).

Furthermore, although all unlesioned animals learned to withhold lever-pressing for the prescribed 1.2 s after unrewarded IPIs (Figures 7E–7H; Experimental Procedures), only 1 of 11 lesioned animals mastered this aspect of the task within the time frame of training. Lesioned rats instead continued to press the lever prematurely after unrewarded IPIs, showing little affinity for learning the metastructure of the task (Figure 7E). Intriguingly, when normal animals learn the 1.2-s inter-trial delay, this aspect too is robust to motor cortical lesions (Figure S4), providing yet another example of a task-specific behavior that requires motor cortex for learning but not—when acquired—for retention and execution.

To rule out the possibility that the learning deficits we saw in motor cortex-lesioned animals were due to non-specific cortical tissue damage, we induced the same-size lesions 5 mm posterior to motor cortex, targeting parts of sensory and parietal cortices ($n = 3$ rats). Rats with these extra-motor cortical lesions learned the task and inter-trial delay as well as normal rats (Figure S5), indicating that the severe learning deficits we observed in motor cortex-lesioned animals (Figure 7) were due to the specific loss of motor cortical function.

DISCUSSION

To probe the role of motor cortex in the acquisition and execution of task-specific motor sequences independently of its established role in dexterous control (Figure 1), we developed a novel motor learning paradigm that trains spatiotemporally precise movement patterns in rats (Figures 2 and 3). Surprisingly, we

found that motor cortex is dispensable for executing such non-dexterous skills (Figures 4 and 5), implying that they can be stored and generated in subcortical motor circuits (Lashley, 1924, 1950). In contrast, when we lesioned motor cortex prior to training, animals failed to learn the task (Figures 6 and 7), suggesting a role for motor cortex in enabling downstream circuits to learn and execute certain types of motor skills.

Contrasts with Prior Studies on Motor Skill Execution

At first glance, our results may seem at odds with prior lesion studies implicating motor cortex in skill execution (Darling et al., 2011; Whishaw et al., 1986, 1991). Importantly, however, many of these studies trained animals to adapt species-typical reaching movements to novel and challenging food retrieving scenarios. Solutions to such tasks typically involve new and dexterous movements that are known to require corticospinal control (Bortoff and Strick, 1993; Darling et al., 2011; Lemon, 2008).

In contrast, our task was designed to probe the role of motor cortex in learning and executing task-specific motor sequences that, in principle at least, can be built from subcortically generated actions—a complementary dimension of motor skill learning. The central challenge of our task was not to refine the control of specific movements but, rather, to assemble pre-existing movements into novel, stereotyped, and reliably expressed sequences equal to a prescribed task.

That our conclusions relating to motor cortex's role in skill execution differ from previous studies is not a contradiction but, rather, a reflection of the heterogeneous nature of “motor skill learning” and the fact that different learning and control challenges can rely on different neural circuits and mechanisms (Ali et al., 2013; Thorn et al., 2010).

Contribution of Subcortical Circuits to Skill Execution

That the learned skills we train are preserved following large bilateral motor cortex lesions suggests a role for subcortical

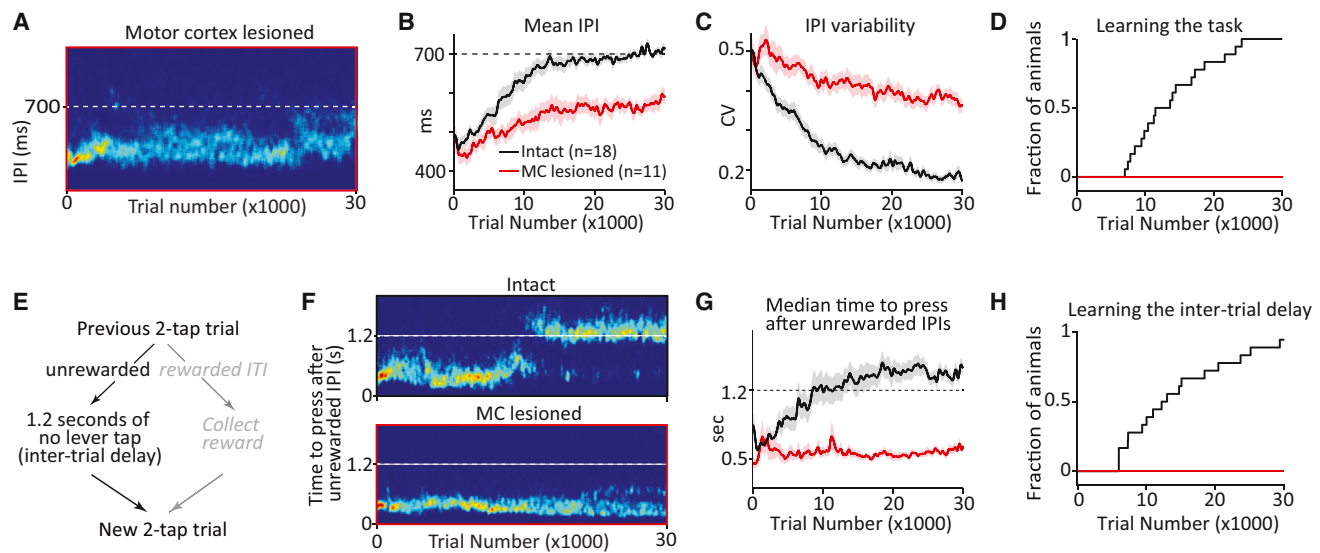


Figure 7. Motor Cortex Is Required for Learning a Skill that, when Acquired, Is Motor Cortex Independent

(A) Density plot of the IPI distribution for a bilaterally motor cortex-lesioned rat learning a 700-ms target IPI (compare this with the unlesioned example in [Figure 2B](#)).

(B and C) Learning curves showing the mean (B) and CV (C) of the IPI distribution in intact (black lines, $n = 18$) and lesioned (red lines, $n = 11$) rats learning a 700-ms IPI. The shaded area denotes SEM across animals.

(D) Cumulative histogram showing the fraction of animals that learned the task to criterion performance (see text) as a function of training.

(E) Rats were required to wait 1.2 s after unrewarded IPIs for a new trial to be initiated.

(F) Density plots showing the distribution of times between an unsuccessful second lever-press and the subsequent press for representative intact and motor cortex-lesioned animals.

(G) Median delay after an unsuccessful second lever-press in intact (black line) and motor cortex-lesioned (red line) animals. The shaded area denotes SEM across animals.

(H) The same as (D) but for learning the inter-trial delay aspect of the task to criterion ([Experimental Procedures](#)).

circuits in their storage and execution. Although the cerebellum ([Ito, 1984](#)) and basal ganglia ([Graybiel et al., 1994](#)) are known to support many aspects of motor control, their contributions to acquired skills are often presumed to be mediated through interactions with motor cortex ([Doyon et al., 2009](#); [Hikosaka et al., 2002](#)). Other subcortical motor structures, such as the reticular formation ([Baker, 2011](#); [Esposito et al., 2014](#)), red nucleus ([Massion, 1988](#)), and superior colliculus ([Philipp and Hoffmann, 2014](#)), are thought to encode species-specific movements and action patterns or serve as intermediaries for cortical commands ([Esposito et al., 2014](#)). Our results expand this view by showing that movements generated by subcortical controllers can be shaped, sequenced, and coordinated during skill learning to yield complex task-specific motor patterns that can be stored and produced independently of motor cortex. The limits of subcortical plasticity and the extent to which lower-level motor circuits can support other forms of complex learned motor behaviors remains to be understood.

Implications for Understanding Recovery after Motor Cortex Lesions

Functional recovery after motor cortical injury is often attributed to experience-dependent cortical reorganization and plasticity ([Nudo et al., 2001](#); [Sanes and Donoghue, 2000](#)), a process that hinges on the capacity of unlesioned motor circuits to acquire, through experience, new strategies to compensate for lost func-

tionality ([Krakauer, 2006](#)). Although this may explain some aspects of use-dependent motor recovery, it is not likely to account for the spontaneous and complete recoveries we observed in our task ([Figures 4 and 5](#)). This is because the spatiotemporally precise movement patterns we train have no general utility outside the context of our task and are not practiced during the post-lesion recovery period. That the skills, acquired over many weeks of training, were nevertheless expressed in their distinct pre-lesion forms on the very first day of post-lesion training ([Figure 5](#); [Movies S3 and S5](#)) suggest that they were consolidated and stored in unlesioned parts of the motor system.

The spontaneous post-lesion recoveries may therefore be better explained by subcortical circuits, including spinal circuits, spontaneously recovering their pre-lesion skill-related activity patterns ([Seitz et al., 1999](#)). The mechanisms that underlie such spontaneous recovery remain to be addressed, but homeostatic regulation of circuit dynamics ([Golowasch et al., 1999](#)) and augmentation of subcortical inputs to spinal circuits ([Zaimi et al., 2012](#)) are likely candidates.

Contrasts with Prior Studies on Motor Skill Learning

Previous studies have established the importance of motor cortex for learning and generating new and dexterous movements. Our study was not designed to delve further into this domain of motor control or motor learning. Rather, it was meant to dissociate learning and control processes to investigate

whether and how motor cortex contributes to the acquisition of skills that can be built from sequencing and coordinating sub-cortically generated actions.

We found that rats with motor cortical lesions were unable to learn our sequential lever-pressing task despite having no obvious motor control or motivational deficits that would prevent them from doing so (Figure 6). This inability was surprising, given that rodents can learn to perform skilled reaching tasks even after large motor cortical lesions (Gharbawie and Whishaw, 2006). The common explanation for such outcomes is that animals compensate for loss of dexterity by adapting their innate reaching movements to the imposed experimental constraints in non-dexterous ways (Darling et al., 2011; Gharbawie and Whishaw, 2006; Whishaw, 2000). But if rats can compensate for such motor deficits and learn to master skilled reaching tasks after motor cortical lesions, then why can they not learn our task, which, after all, does not depend on motor cortex for control?

Although our task differs from skilled reaching in several ways, one distinction that could potentially explain the differential effects of motor cortex lesions is that our task requires learning and consolidating a fairly complex and novel motor sequence with no prior ethological relevance and no clear a priori goal. Skilled reaching, in contrast, involves modifying naturally expressed reaching movements to achieve a clear goal (get food). One hypothesis to be tested is that motor cortex is essential for sequencing movements into new task-specific sequences but less important for modifying the details of existing and habitually expressed behaviors.

A Tutor Function for Motor Cortex?

Our results suggest that motor cortex functions as a tutor for subcortical motor circuits during learning, helping them acquire and consolidate new motor sequences. The facts that lesioned animals had great difficulty reliably executing longer IPIs (Figures 7B and 7C), persevered with unrewarded motor patterns, and were incapable of withholding lever-pressing after unrewarded IPIs (Figures 7F–7I) are consistent with motor cortex suppressing innate motor tendencies and reflexes (such as rapid tapping; Figure S1) in context-specific ways (Ioffe, 1973; Stoltz et al., 1999). Our results suggest that such cortical tutoring or modulation can lead, over the course of weeks of training, to the permanent reprogramming of sub-cortical circuits.

The extent to which this explains the essential contributions of motor cortex to the learning process remains to be parsed, and we note that other hypothetical functions of motor cortex, including adaptive shaping of motor variability (Chaisanguanthum et al., 2014; Wu et al., 2014) and general facilitation of sub-cortical plasticity, could also contribute to its tutor function. But regardless of how motor cortex shapes motor output during skill learning, our results show that the adaptive biases it contributes or enables become incorporated and consolidated in downstream circuits.

Intriguingly, the principle of a cortical brain region tutoring downstream control circuits during complex motor sequence learning has been demonstrated previously in songbirds (Andalman and Fee, 2009; Bottjer et al., 1984; Turner and Desmurget, 2010). During vocal learning, the lateral magnocellular nucleus of the anterior nidopallium (LMAN), a cortex-analogous brain area,

provides a consistent error-correcting premotor bias that is thought to drive permanent changes in vocal control circuits (Andalman and Fee, 2009). LMAN, similarly to motor cortex in our task, is essential for learning the complex motor patterns underlying song but not for executing them when they have been learned and consolidated (Bottjer et al., 1984). This suggests that the dissociation between learning and control in neural hardware, and the idea that one motor-related circuit shapes the control policies of another, reflect a general principle of how the brain implements complex motor learning (Graybiel, 2008; Turner and Desmurget, 2010).

Evolutionary Implications

Motor cortex is a mammalian addition to the basic vertebrate motor plan that has radically improved the capacity of animals to learn and execute new and dexterous movements (Nudo and Frost, 2009). In addition to its unique control function, our results suggest that motor cortex is utilizing phylogenetically older motor controllers to a larger extent than assumed previously. Motor cortex, as the master overseer of the animal's motor output, has access to contextual, sensory, and planning information from other neocortical areas that may not be independently available to subcortical motor circuits. Using this information to shape the control policies of lower-level motor circuits could be an efficient strategy for influencing and adapting motor output. Rather than reinventing control functionalities already present in the ancestral brain, mammals with a cortex may, instead, have evolved strategies to make better use of these pre-existing and robust control functions (Doyle and Cssete, 2011; Shmuelof and Krakauer, 2011).

Therefore, if a task can be solved by assembling individual actions from the animal's subcortical motor repertoire, then motor cortex could help in sequencing and coordinating those components, expanding the functionality of lower-level motor circuits in the process. When faced with tasks, such as skilled reaching, that cannot be efficiently solved by subcortical control strategies alone, motor cortex could contribute to movement generation directly through its projections to the spinal cord.

If such a division of control is indeed realized, it would imply that many complex motor skills are implemented across cortical and subcortical control networks (Drew et al., 2008). Interestingly, the one outlier rat (Figure 5C; Movie S4) that had difficulty performing the task after motor cortex lesions was also the only one to have adopted a strategy requiring precise digit control, and it was this deficit that seemingly affected its post-lesion performance (Movie S4). Skilled reaching behaviors are compromised in similar ways, with dexterous aspects being affected preferentially (Alaverdashvili and Whishaw, 2008), suggesting that the non-dexterous part of the species-typical reaching movements may be stored downstream of motor cortex. If controllers at different levels of the motor hierarchy work in synergy, then synchronizing them will be essential for robust and smooth performance (Drew et al., 2008). How this is implemented in neural circuitry is an intriguing problem that remains to be understood.

In contrast to most studies of motor cortex's role in skill learning that focus on plasticity in corticospinal controllers, our study was designed to dissociate learning and control

processes. Our new paradigm achieved this in a surprisingly clean way, revealing a function of motor cortex in guiding plasticity in downstream control circuits. Although the nature of motor cortex's interaction with downstream circuits during skill learning still remains to be understood, our new paradigm should provide a powerful means to dissect it.

EXPERIMENTAL PROCEDURES

Animals

Experimental subjects were male and female Long Evans rats 3–8-months old at the start of training (Charles River Laboratories). The care and experimental manipulation of the animals were reviewed and approved by the Harvard Institutional Animal Care and Use Committee.

Behavioral Training

A total of 38 rats were trained in our motor skill learning assay (Figure 2A). Training was done in custom-made behavioral boxes measuring approximately 12" x 12" x 12" (length x width x height). The ventilated boxes were supplied with a lever (14 cm above the floor), a reward spout for water delivery, a loudspeaker for generating the reward tone, and either a high-speed color camera (200 fps, Pike F-032C) or a webcam (30 fps, Agama V-1325R) for monitoring the lever-pressing behavior. The lever was placed behind a protruding slit (~1.5 cm wide, 5 cm tall, and protruding ~2 cm into the cage) made of two pieces of cut plastic or glass glued to the wall of the cage. This was done to ensure that the rat pressed the lever with its forepaws and not, for example, with its snout. The force required to press the lever was <0.1 N, and the deflection amplitude threshold required for registering a "press" was ~3 mm.

Animals were either transferred to the training boxes for ~1-hr training sessions 5 or 6 times a week ($n = 6$ rats) or kept in the training boxes for the duration of the experiments and received two 1-hr daily training sessions 7 days a week ($n = 32$ rats). The fully automated home cage training system that was used for the latter cohort has been described in detail (Poddar et al., 2013). We have shown previously that the learning rates as well as number of trials performed per day are similar whether animals are trained in home cages or transferred to behavioral boxes for experimental sessions (Poddar et al., 2013). Therefore, results from rats trained in the different contexts were pooled.

The lever-press sequences were shaped by rewarding water-restricted rats with water for approximations to the desired sequence (700 ms apart for all but two rats, which had targets of 500 and 600 ms, respectively). The predefined and automated training protocol was implemented in custom software (C#) (Poddar et al., 2013) and is described in the [Supplemental Experimental Procedures](#).

Kinematic Tracking

High-Speed Tracking of Forepaw Movements

To track and quantify the kinematics of the rats' forepaw movements as a function of learning, we tattooed the paws with colored ink (red and blue) under light anesthesia (1.5% isoflurane in carbogen) in four animals prior to the start of training. Animals were then trained in a behavioral box outfitted with a high-speed camera (200 fps). The box was placed in a lightproof enclosure lit with light-emitting diodes (LEDs) (Rayconn Electronics, catalog no. FLB6 RGB), ensuring constant illumination of the box over the course of training. Video acquisition was triggered on lever-presses, and custom-written software (Matlab) was used to automatically extract the centroid of the colored blob that corresponded to the forepaws (Movie S2) by thresholding the red and blue color channels in the movies (see also "Kinematic Analysis" in the "Data Analysis" section). In practice, it was only possible to continuously track the paw closest to the camera because the other paw was often obscured. For all four animals we tracked, the paw closest to the camera was involved in pressing the lever.

Tracking from Webcam Movies

To increase the number of animals in which the effects of 10-day control breaks and motor cortex lesions on paw kinematics could be assessed, we

also tracked the paws of animals whose interaction with the lever was captured with the lower frame rate webcams (30 fps). Only a subset of these animals was amenable to paw tracking. The reason for excluding individual rats from tracking were as follows: (1) The camera angle/position prevented us from seeing/tracing either paw (both obscured by the animal's body, $n = 2$); (2) inadvertent displacement of the camera during weekly cleaning of the cage preventing us from comparing movie-based paw tracking before and after a given manipulation ($n = 1$); and (3) for two rats, the plastic protrusion around the lever was opaque (in the other cases they were see-through), therefore obscuring the paw for much of the movement. In one of these cases we were able to instead track a recognizable part of the elbow region of the forelimb, but these data were not pooled with the other subjects because they tracked a different part of the rat's anatomy. Nevertheless, we present these data in Figure S3 because they show kinematics to be largely preserved, even after a cortical lesion larger than our standard ibotenic acid lesions (Figure 4B).

Motor Cortex Lesions

Rats received motor cortex lesions either before training ($n = 11$) or after the behavior reached criterion performance and had asymptoted in terms of CV ($n = 20$). The lesions in these cohorts were done following the same protocol as described below. Animals were anesthetized with 2% isoflurane in carbogen and placed in a stereotactic apparatus. Craniotomies were made over motor cortex. The location of the bregma was noted, and the lesions were induced relative to the bregma and the midline (Figure 4B). Two of the rats lesioned after asymptotic performance had been reached underwent aspirations of motor cortex as described previously (Whishaw, 2000). Although the forelimb areas of motor cortex were targeted, in one case, a larger-than-intended area was removed (Figure S3). We initially did aspirations because the collateral impact on downstream circuits is less than that for alternative lesioning methods (Glenn et al., 2005). In our hands, however, aspirations were not as accurate or reproducible in terms of lesion size and location, and we switched to inducing lesions by injecting 1% ibotenic acid, an excitotoxic agent, for the remainder of the rats (i.e., all but the initial two).

The injection sites were at (1.0 mm, 2.0 mm), (3.0 mm, 2.0 mm), and (1.0 mm, 4.0 mm) respectively, where the first coordinate is the anterior distance from the bregma and the second coordinate is the lateral distance from the midline. At each site, 82.8 nl, in increments of 9.2 nl every 10 s, was injected at two depths, 1.5 and 0.75 mm, with respect to the surface, for a total of 165.6 nl per site (Nanoject, WPI). We calibrated the lesions to ensure that they encompassed both the primary and secondary forelimb areas; i.e., the parts of motor cortex that, when electrically stimulated, induce movements of the forelimb (Bonazzi et al., 2013; Neafsey et al., 1986). To ensure that our protocol induced consistent lesions, we lesioned four untrained animals and sacrificed them 3 days post-lesion. We found that our drug injection protocol induced very reproducible lesions (Figure 4B shows the extent of the lesions in these four animals). We used this protocol for all subsequent lesions.

Following surgery and lesions, animals recovered in normal rodent cages for 5 (for the two aspirated rats) or 10 (for the ibotenic acid-lesioned rats) days before resuming behavioral training. We noted that general motor function, i.e., the ability to ambulate normally and to properly use the limb contralateral to the lesioned hemisphere, seemed to recover faster after aspirations than after ibotenic acid lesion, consistent with the excitotoxicity associated with ibotenic acid lesions lasting several days. This was also the main reason for the different recovery periods.

Bilateral motor cortex lesions in untrained rats were done in two stages for 6 of the 11 rats. After one hemisphere had been lesioned, animals recovered for 19–28 (21 ± 3 , mean \pm SD) days before undergoing lesions to the contralateral hemisphere. Animals then recovered for another 6–21 (11 ± 4) days before starting training. In the remainder of the rats ($n = 5$), we lesioned motor cortex bilaterally in the same surgery. Because there was no significant difference between one-stage and two-stage lesioned animals in terms of performance or learning, the results from these cohorts were pooled.

At the end of the experiment, animals were euthanized, perfused with 4% paraformaldehyde (PFA), and their brains were harvested for histology to confirm lesion size and location. The brains were sectioned into 100- μ m slices

and strained with Cresyl Violet. Quantitative assessment of lesion size was done by demarcating the lesions boundaries and then generating a map of the lesion using custom-written software that stitched together the marked slices (Figure 4B).

Data Analysis

Definition of Asymptotic Performance in Terms of Temporal Precision

We first estimated the CV around each trial by calculating the CV over a 500-trial sliding window. We then fit a linear regression to the CV curve every 400 trials using a 2,700-trial window size. Asymptotic performance was defined as the point at which four consecutive linear regression fits had slopes less than 1.5×10^{-5} /trial.

Calculation of Performance Metrics

For Figures 2 and 7 (learning), the CV was calculated across 100 trials, and the moving average was then low pass-filtered with a 300-trial boxcar filter. The distance from the target was calculated similarly using the absolute deviation from the target as the variable. For Figure 4 (pre/post manipulation), we used the same procedure as for learning but used a smaller moving window (25 trials) and boxcar filter (50 trials).

Criterion Performance

Animals were deemed to have reached criterion performance when, for a 3,000-trial sliding window, the CV was less than 0.25 and the mean of the IPI distribution was within 10% of the target.

Learning the Prescribed 1.2-s Inter-Trial Delay

According to our definition, the prescribed 1.2-s inter-trial delay was learned when, for a 3,000-trial sliding window, the median time to the next tap was larger than 1.2 s and this was maintained for at least another 3,000 trials. Intervals >5 s or those that occurred after rewarded trials were excluded (but included for the sliding window).

Kinematic Analysis of High-Speed Movies

Movie frames were converted to the hue saturation value (HSV) color representations, and, for each animal, a single HSV threshold was determined for isolating the tattooed paw. The threshold was chosen at a value that reliably (>90%) picked out the paw in the video. The location of the paw was defined as the centroid of the pixels above threshold. The trajectories were filtered using a running median filter (window size of 5 frames at 200 fps). All trajectories were aligned to the first lever-press and traced for 1,150 ms, starting 250 ms before the first lever-press. Because some of the paw trajectories were not traced properly, we excluded outliers in our dataset by calculating the principal components (PCs) of the traced trajectories in each session and then using the first three PCs to calculate the Mahalanobis distance for each trajectory. We excluded trajectories beyond the 90% confidence interval of the chi-square distribution (<10% of the tracings were therefore excluded).

Pairwise Correlations to Estimate Trial-by-Trial Variability

The trials were pre-selected by IPI (see text). The trial-by-trial variability (Figure 3D) was estimated as the average correlation coefficient of all pairs of trajectories that conformed to the selected IPI range. The correlation coefficient for a given pair was calculated as follows.

The horizontal and vertical paw displacements of the i -th trial ($X_i(t)$ and $Y_i(t)$, respectively) were mean-subtracted and concatenated to form one single trajectory vector as follows:

$$T_i = \begin{bmatrix} X_i(t) - \langle X_i(t) \rangle \\ Y_i(t) - \langle Y_i(t) \rangle \end{bmatrix}. \quad (\text{Equation 1})$$

The correlation coefficient of two trials, i and j , was then calculated as

$$R(T_i, T_j) = \frac{C(T_i, T_j)}{\sqrt{C(T_i, T_i)C(T_j, T_j)}}, \quad (\text{Equation 2})$$

where C is the covariance.

Correlation in the Mean Trajectory across Days and Conditions

The trials were pre-selected by IPI (see text). The mean trajectory of each session was used to calculate the correlation coefficient matrix (Figure 3C). The correlation coefficient across two sessions was calculated with the same equations as above, except here, $X_i(t)$ and $Y_i(t)$ were the average paw displacement in a session rather than in an individual trial.

Effects of Lesions on Paw Kinematics

To assess whether the effects of the lesions were above and beyond what could be expected from 10-day control breaks, we compared the root-mean-square (RMS) of the IPIs as follows. Let μ_{pre} be the mean IPI before a condition (window of 1,000 trials). The RMS before or after a condition was then calculated as follows:

$$RMS = \sqrt{\frac{1}{n} \sum_{i=1}^n (t_i - \mu_{pre})^2}, \quad (\text{Equation 3})$$

where $n = 300$ trials immediately preceding or following the condition (lesion/break), and t_i the IPI of the i -th trial. To compare across conditions, we calculated the RMS difference as $RMS_{Post} - RMS_{Pre}$ for each condition. We then performed a z test at the 5% significance level for each animal against the null distribution, which was constructed from the RMS differences across the 10-day control breaks in animals that underwent them.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and five movies and can be found with this article online at <http://dx.doi.org/10.1016/j.neuron.2015.03.024>.

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Supplemental Information

Motor Cortex Is Required for Learning but Not for Executing a Motor Skill

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Supplemental Information

1. Supplemental Figures 1-5
2. Supplemental Experimental Procedures

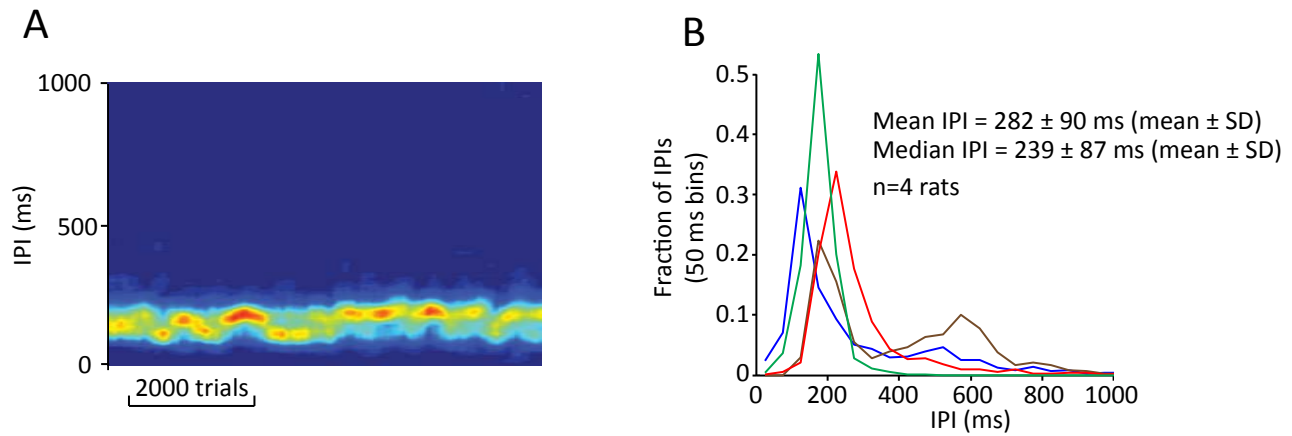


Figure S1 (Supplemental to Figure 2). Rats have a natural tendency to press the lever in quick succession. When the timing between successive lever-presses was not prescribed (all IPIs between 0-1.2 seconds equally rewarded), rats tended to have IPIs in the 200-300ms range. **A.** IPI density plot for a representative rat at asymptotic performance in this version of the task (green line in B). **B.** IPI histograms for four rats trained to press the lever twice within 1.2 seconds at asymptotic performance on the task (data from five sessions).

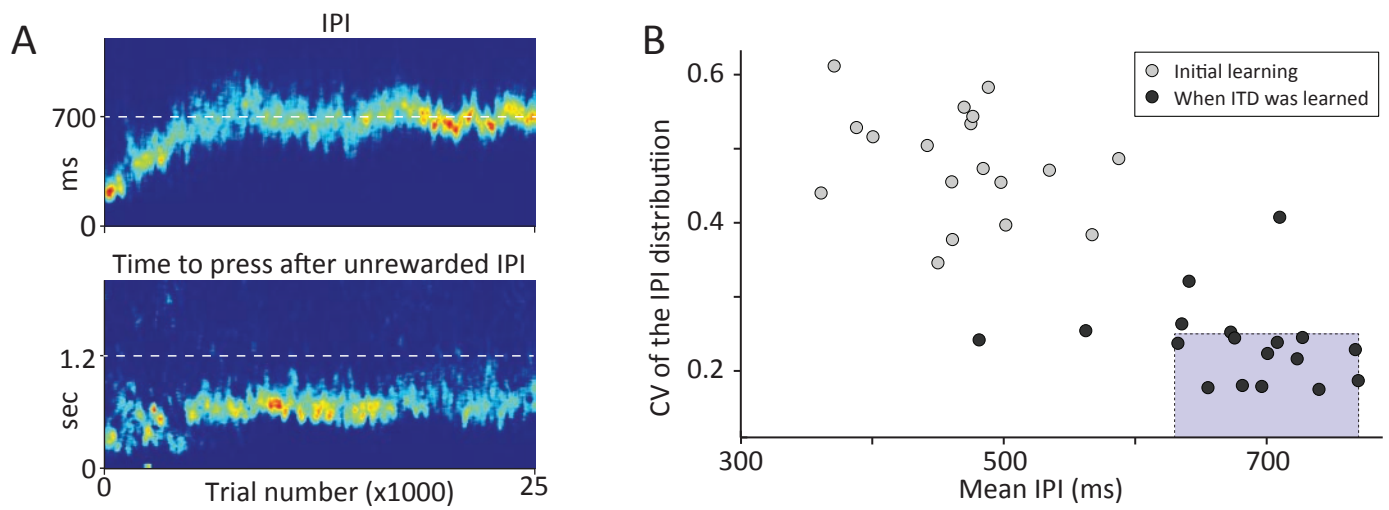


Figure S2 (Supplemental to Figure 2). Learning the target IPI (700 ms) is not contingent on having mastered the 1.2 second inter-trial delay. **A.** Example of a rat that reached criterion performance before learning to suppress extraneous lever-pressing after unrewarded IPIs. Top: Density plot of IPIs. Bottom: Density plot of time to lever press after unrewarded IPI. **B.** CV and mean of the IPI distribution for rats early in learning (first 1000 trials, grey dots), and over the last 1000 trials before they learned the 1.2 second inter-trial delay (ITD, Experimental Procedures, black dots). Blue shaded area denotes criterion performance. A majority of animals (12/18) reached criterion performance before they learned the inter-trial delay.

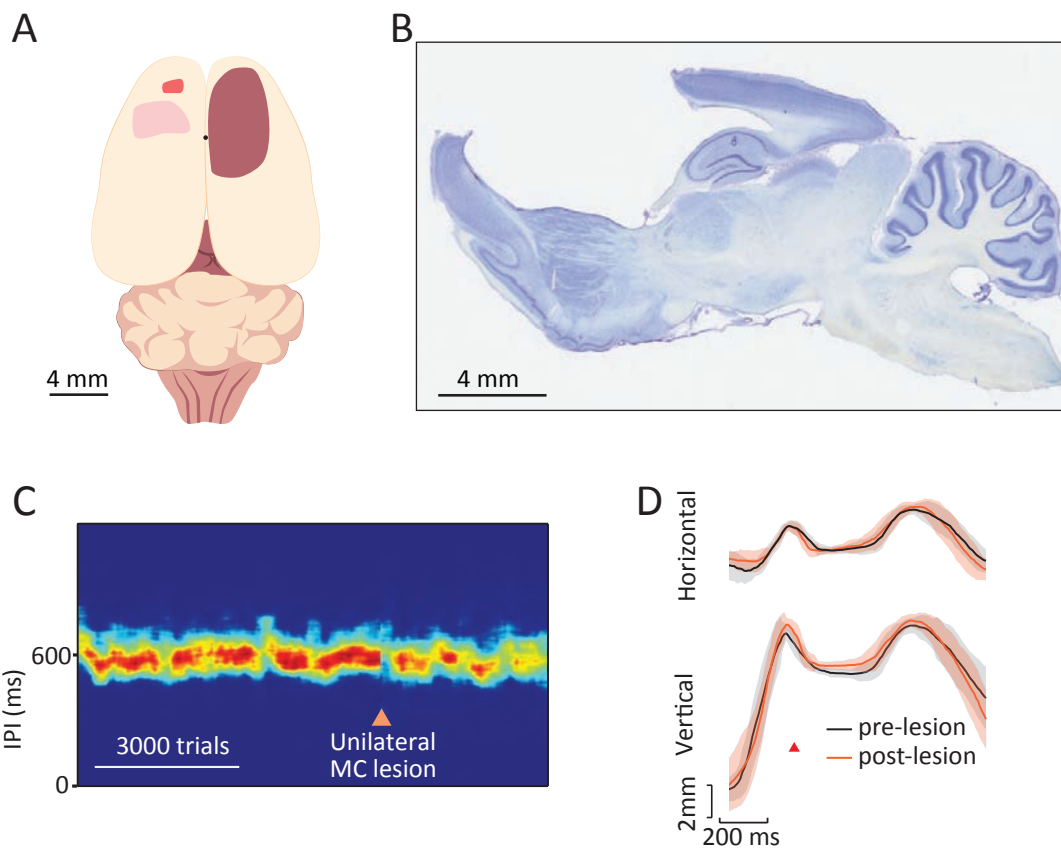


Figure S3 (Supplemental to Figure 4). Performance on the timed lever-pressing task was robust also to a large cortical aspiration that lesioned areas beyond motor cortex, including parts of cingulate, prelimbic, somatosensory and parietal association cortices, and also a part of the dorsomedial striatum. **A.** Schematic showing the approximate extent of the induced lesion (right hemisphere). Location of primary (pink) and secondary (red) forelimb motor cortex denoted on the left hemisphere. Black dot denotes the location of bregma. **B.** Saggital histological section of the lesioned brain 2.5 mm from the midline. **C.** IPI distribution before and after the lesion (IPI target 600 ms). Unlike the ibotenic acid lesioned animals, this rat resumed training 5 days after the lesion. **D.** Average forelimb trajectories (see Experimental Procedures) for IPIs in the 575-625 ms range on the day before (black) and the first day of training after the lesion (orange). Shaded regions denote standard deviation across trials.

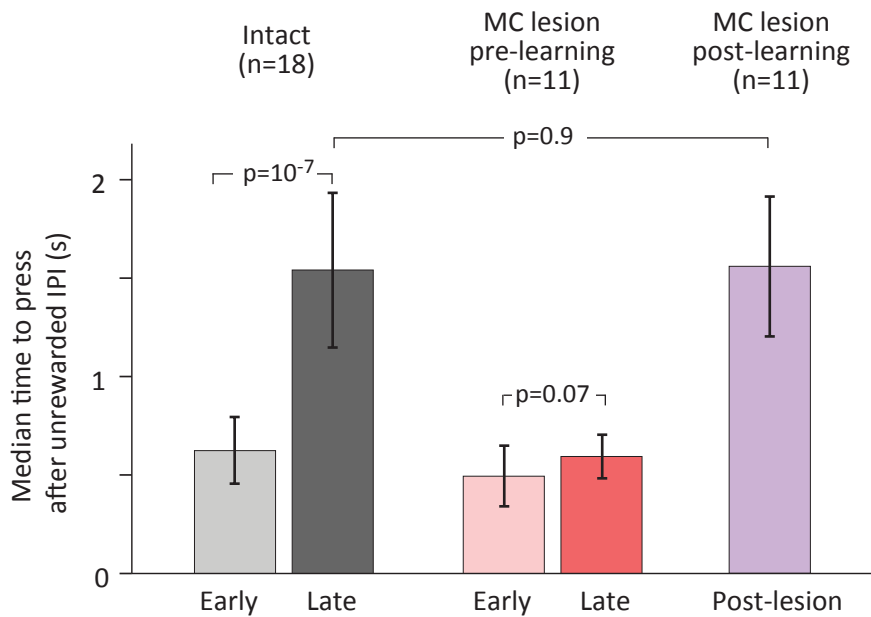


Figure S4 (Supplemental to Figure 7). Learning to suppress extraneous lever-presses in the prescribed 1.2 second inter-trial interval requires motor cortex. Once learned, however, motor cortical lesions do not affect this aspect of the acquired behavior. Graph shows median time to lever-press after unsuccessful IPIs averaged over 1000 trials at start of training (light bars) and after 50 training days (dark bars), for normal rats (grey) and rats with bilateral motor cortex lesions prior to training (red). Performance of rats with bilateral motor cortex lesions after training are shown in purple. Error bars denote standard deviation across animals. Paired t-test was used to test for significance within groups ('Intact' and 'MC lesion pre-learning' respectively), and unpaired t-test to compare across groups ('Intact' vs. 'MC lesion post-learning').

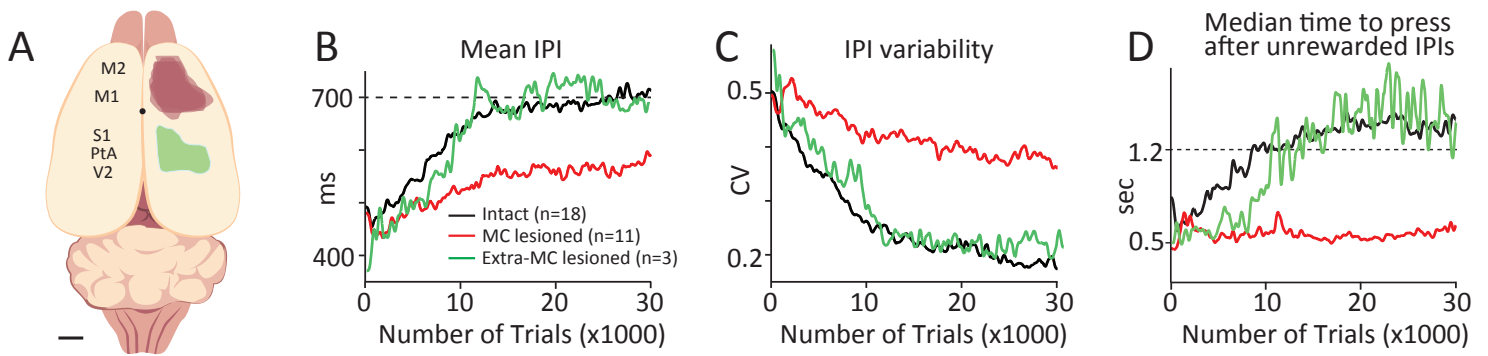


Figure S5 (Supplemental to Figure 7). Unlike motor cortical lesions, lesions to sensory/parietal cortex do not interfere with learning in our motor skill paradigm. **A.** Demarcations of the lesions based on histology. Brown blobs denote motor cortex lesions (same as in Figure 4B). Green blob denotes the extent of extra-motor cortical lesions (estimated from histology). These lesions were induced as for motor cortex, but translating the injection coordinates 5 mm posterior (Experimental Procedures). Abbreviations on the left denote relevant brain areas. M1- primary motor cortex; M2 - secondary motor cortex; S1 - Primary somatosensory cortex; PtA - Parietal association cortex; V2 - Secondary visual cortex. Scale bar is 2 mm. **B-D.** Learning curves as in Figure 7, showing the mean (B) and CV (C) of the IPI distributions, and the median times to press after unrewarded IPIs (D) as a function of training in the task. Data for intact and motor cortex lesioned animals as in Figure 7.

2. Supplemental Experimental Procedures

Training Protocol

1. Initial lever-press training

Rats were first conditioned to collect water from the reward spout in response to a tone (4 kHz pure tone for 0.25 seconds). They could then trigger the reward tone and subsequent reward delivery by pressing the lever. After 50 rewarded lever-presses, reward probability decreased to 80%, then, after another 50 presses, to 60%. The timed lever-press sequence training commenced after 50 lever-presses at 60% reward. This initial training phase, which took on average 5.9 ± 3.0 training days in unlesioned rats ($n=20$ rats) and 3.5 ± 2.7 in lesioned rats ($n=11$), was not included in the training times quoted in the manuscript.

2. Timed lever-press sequence training

During training sessions, rats self-initiated a trial by pressing the lever. If the inter-press interval fell outside the rewarded range, animals had to wait 1.2 seconds before initiating the next trial (inter-trial delay). Rats were initially rewarded for pressing the lever twice between 200 and 1100ms. Reward boundaries were then dynamically adjusted to shape the animal's behavior toward the target IPI while maintaining reward rates around 35%. Since animals initially exhibited mean IPIs that were shorter than the target, we first adjusted the lower bound (LB) until they reached a median IPI greater than the target. At this point LB was set to the target. As performance improved, the upper bound (UB), which had been at 1100 ms, was adjusted downward to increase the precision of the IPIs around the target.

Reward boundaries were updated based on the percentage of trials rewarded in the previous session (R). If R was between 30% and 40%, the boundaries remained unchanged. If R was below 30% and the median IPI below the target, LB was lowered by $I_1 = 10\text{ms}$. If R was below 30% and the median

IPI above the target, UB was increased by I_1 . If R was above 40% and the median IPI below target, LB (in ms) was shifted to:

$$LB(t) = \min(\min(\max(Q_1, LB(t-1) + I_1), LB(t-1) + I_2), T) \quad (\text{eq.5})$$

where t = session number, Q_1 = 1st quartile, T = target IPI, I_2 = 50 ms.

If R was above 40% and the median IPI above target and if

$$UB(t-1) - LB(t-1) > D \quad (\text{eq.6})$$

then UB (in ms) was shifted to:

$$UB(t) = \max(\min(Q_3, UB(t-1) - I_1), UB(t-1) - I_2) \quad (\text{eq.7})$$

else

$$UB(t) = UB(t-1) - I_1 \quad (\text{eq.8})$$

where Q_3 = 3rd quartile, D = 150ms.

To incentivize IPIs close to the target, rats received more water the closer their IPIs were to the target (see Figure 2C). The distance between the target and each of the boundaries was divided into 5 equal size bins with the reward amount increasing in increments of 20 μ l the closer the bin was to the target (Figure 2C).

Water consumption was continuously monitored and rats supplemented at the end of the day with 'free' water if failing to earn their daily required dose (5ml/100g of body weight) through training. Rats were weighed every two weeks to ensure that they were above 90% of their initial weight. All intact (i.e. unlesioned animals) that started training reached criterion performance and subsequent asymptotic performance in terms of CV (see Data analysis section for definition). All animals that started training in the task were included in the analysis. Before animals were reintroduced into the training box after lesions or control breaks, they were water deprived for ~ 12-16 hours to ensure motivation for the task.

Parietal/Sensory cortex lesions

Cortical lesions of the same size as in the 'motor cortex lesioned' cohort were induced in non-motor cortical regions to control for non-specific effects of cortical lesions on learning (Figure S5). These lesions used the following coordinates (-4.0, 2.0) (-2.0, 2.0) (-4.0, 4.0) and were done in two separate stages. The extra-motor cortical lesions extended to parts of parietal cortex and sensory cortex, and also included dorsal parts of the hippocampus below. The damage to hippocampus was because the coordinates for ibotenic acid injections were optimized for motor cortex which is significantly thicker than parietal and sensory cortex in rats.

Kinematic analysis from webcam videos

The paw trajectories from the web-cam videos were traced using custom-written software that involved manually (i.e. with a computer mouse) placing a marker at a well-defined part of the rat's paw in each frame of the video. To compare these tracings to the trajectories captured from high-speed videos, we resampled, using linear interpolation, the trajectories to match the frame rate of the high-speed videos (200 fps). The trials for which the forepaw was tracked were pre-selected based on IPI (see text), and a random set of 40 trials for each condition was selected and tracked. The tracking was done blind to condition (e.g. pre vs post-lesion). Using this manual tracking method, we could trace the paw trajectories in several additional rats pre-post lesion (n=3, n=7 total) and pre-post control break (n=4, n=8 total) (Figures 3G and 4B).