Differential Corticostriatal Plasticity during Fast and Slow Motor Skill Learning in Mice

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Summary

Background: Motor skill learning usually comprises "fast" improvement in performance within the initial training session and "slow" improvement that develops across sessions. Previous studies have revealed changes in activity and connectivity in motor cortex and striatum during motor skill learning. However, the nature and dynamics of the plastic changes in each of these brain structures during the different phases of motor learning remain unclear.

Results: By using multielectrode arrays, we recorded the simultaneous activity of neuronal ensembles in motor cortex and dorsal striatum of mice during the different phases of skill learning on an accelerating rotarod. Mice exhibited fast improvement in the task during the initial session and also slow improvement across days. Throughout training, a high percentage of striatal (57%) and motor cortex (55%) neurons were task related; i.e., changed their firing rate while mice were running on the rotarod. Improvement in performance was accompanied by substantial plastic changes in both striatum and motor cortex. We observed parallel recruitment of task-related neurons in both structures specifically during the first session. Conversely, during slow learning across sessions we observed differential refinement of the firing patterns in each structure. At the neuronal ensemble level, we observed considerable changes in activity within the first session that became less evident during subsequent sessions.

Conclusions: These data indicate that cortical and striatal circuits exhibit remarkable but dissociable plasticity during fast and slow motor skill learning and suggest that distinct neural processes mediate the different phases of motor skill learning.

Introduction

Improving movement accuracy, speed, and coordination can be critical for survival. During the learning of a new motor skill there is "fast" improvement in motor performance within the first training session and "slow" improvement that develops across sessions [1]. Motor skill learning is impaired in disorders affecting cortico-striatal circuits, such as Parkinson's [2] and Huntington's diseases [3]. Previous studies investigated changes in activity and connectivity during motor learning in motor cortex [4–14] and striatum [14–20]. From some of these studies it is clear that both striatum and motor cortex are activated throughout the different phases of motor learning (reviewed in [1, 19]). However, the nature and dynamics of the plastic changes occurring during the different phases of motor learning remain uncertain. Moreover, it remains unclear whether the plastic processes that occur in motor cortex and striatum during each stage of learning are similar or different and whether fast and slow motor skill learning are mediated by different gradations of similar ongoing processes or distinct processes altogether. Some of these questions are difficult to address with current imaging techniques, which provide valuable information about the overall activity in a particular brain region but do not differentiate between changes in number, type, or activity pattern of neurons. We therefore developed and implemented a method for multielectrode recordings in awake-behaving mice by using microwire arrays, which can be used to investigate those issues. Using this method, we simultaneously recorded the activity of neuronal ensembles in motor cortex and dorsal striatum to investigate the nature of the plastic changes in each structure during fast and slow motor skill learning in mice. We employed the accelerating rotarod task, which allows us to investigate the neural correlates of motor skill learning in the absence of the associative and working memory components of other motor learning tasks. We found that a high percentage of striatal and cortical neurons are modulated during the performance of this highly repetitive motor task and that there is extensive but differential functional rearrangement of cortical and striatal circuits during the fast and slow phases of motor skill learning.

Results

Method for Multielectrode Recordings in Awake-Behaving Mice

We implanted arrays of tungsten isonel-coated microwires into the brains of adult mice and investigated the ability to isolate single neurons from dorsal striatum and motor cortex (Figures 1C and 1D) with different array designs and recording modes (see Figure S1). With the specific parameters we probed, we observed no advantage in using triangulation to isolate single units (Figure S1D). We therefore opted to employ single-channel recording mode in all the experiments described hereafter.
Figure 1. Method for Chronic Simultaneous Recording of Single Neuron Activity in Awake-Behaving Mice

(A) Example of three independent units recorded in one channel in single-channel mode. Left, waveforms of the spikes of the three units recorded online and ISI histograms. Center, 2D projection of the clusters correspondent to each unit based on analysis of the first three principal components of the waveforms recorded. Right, 3D representation of the same clusters displaying the three principal components along the three axes. Note that the green (U1) and the blue unit (U2) clusters are grouped along different axes. The yellow unit cluster (U3) has smaller amplitude and is closer to the noise cluster (in gray). In the cases where we recorded more than one unit from the same channel, we always made sure that clusters of smaller amplitude cells did not represent smaller spikes of the same cell during bursting; for example, by ensuring that the smaller spike occurred both before and after larger spikes.

(B) Stability of the recordings during a session. Example of a unit recorded 4 months after surgery. Left, waveforms of the spikes, ISI histogram, and 3D display of the cluster of the unit—obtained from the first two principal components (x and y axes)—throughout the time of the recording session (z axis). The waveform (center) and the respective cluster of the unit based on the first three PCs (right) remained stable throughout the recording session.

(C) Histological verification of the localization of the electrodes. Shown is an overview of the cortex and dorsal striatum on the right hemisphere at +0.5 Bregma. The plane displays the track of one of the electrodes of an array implanted in motor cortex. The electrode tip position pointed by the arrow in the high-magnification picture indicates that the electrode tip was positioned in layer 5 of motor cortex.

(D) The plane displays the track of one of the electrodes of an array implanted in dorsal striatum. The electrode tip position pointed by the arrow in the high-magnification picture indicates that the electrode tip was positioned in dorsal striatum.

Under these conditions, we were able to simultaneously record many single units per mouse (~18 with 32 electrodes per mouse), occasionally several with the same electrode (Figure 1A), in both cortex and dorsal striatum (~0.5 units per electrode in striatum and ~0.7 in cortex). Units were selected online by using a waveform template algorithm and then isolated offline based on waveform, amplitude, and interspike interval histogram by using an offline sorting algorithm (see Supplemental Data for detailed description and criteria). We observed that the waveform recordings across a session were generally very stable (Figure 1B), allowing us to follow single units through time. Also, the implants seemed to be well tolerated by the brain; single-unit recordings continued for several months after surgery (Figure 1B). This indicates that our methodology is appropriate for conducting long-term longitudinal studies in behaving mice.

Fast and Slow Motor Skill Learning in the Accelerating Rotarod Task

In the present study, we trained seven implanted, isogenic mice of hybrid genetic background (B6129SF1/J) on an accelerating rotarod (4 to 40 rpm in 300 s). We implanted 32 microwires in each mouse (16 in each hemisphere). Four animals were implanted in both dorsal striatum and motor cortex, and three other animals were implanted bilaterally in dorsal striatum. In the accelerating rotarod task, animals learned a novel and highly stereotypical sequence of movements that allowed them to maintain equilibrium on a rotating rod accelerating at a constant rate. Animals were trained in a single session of 10 trials per day for three consecutive days. Each trial (running period) was preceded by an intertrial interval (resting period) of 300 s, during which the animal remained at the bottom of the apparatus without walking (except for occasional turning around). Animals showed significant learning during the first day (Figure 2A, effect of training trial F9,60 = 2.98, p < 0.05; posthoc trial one versus trial ten, p < 0.05), demonstrated by an increase in latency to fall from the rotating rod. During the second day, there was still a significant difference between the first and last trial of the session (planned comparison, F1,12 = 18.8, p < 0.05), although there was no overall significant effect of training trial on the latency to fall from the rotarod (F9,60 = 0.92, p > 0.05). During the third day, animals had reached a plateau in which neither an overall effect of trial (F9,60 = 0.19, p >
Figure 2. Fast and Slow Motor Skill Learning in the Accelerating Rotarod Task

(A) Animals showed fast learning during the first training day, which slowed down during day 2 and reached a plateau during the third day.

(B) Across days, there was a significant change in the latency to fall during the early trials (one and two) of every session. We observed a significant improvement from day 1 to day 2 but no further improvement from day 2 to day 3.

(C) Across days, there was no significant improvement in the latency achieved during the late trials (nine and ten) of every session indicating that the most substantial improvement occurred during the first training day.

Differential Corticostriatal Plasticity during Fast and Slow Motor Skill Learning

We next investigated if corticostriatal neural activity changed during motor skill learning. To assess whether the task-related neurons modified their involvement in the task throughout a session, we compared the activity profile of each neuron during early trials (the first two trials) and late trials (the last two trials) of each session (see Experimental Procedures, Figures 4 and 5B). Overall, we observed that many task-related neurons changed their activity profile during the first session (45% in stratum and 35% in motor cortex). In both stratum and motor cortex there was a dramatic increase in the number of task-related neurons during the first session (51% to 90% in stratum, $F_{1,12} = 20.9, p < 0.05$; 66% to 89% in motor cortex, $F_{1,6} = 8.56, p < 0.05$), which remained...
Figure 3. Modulation of Neural Activity during Running on the Accelerating Rotarod

(A–H) Each panel displays the raster plots and corresponding peri-event time histogram (PETHs) for a particular neuron throughout one session. The beginning of each running period is aligned at time zero and marked by a red square. The end of the running period is marked by a blue circle. In each graph, trials are presented top to bottom, just as they were presented during training. Dorsal striatum neurons are
Figure 4. Plasticity in Corticostriatal Neuronal Activity during Learning in the Accelerating Rotarod Task

For each neuron a continuous-rate histogram of the entire session plus PETHs for the first two trials (black line), the last two trials (red line), and the individual trials (one, two, nine, and ten) are displayed. Red squares indicate the onset of the trial and blue circles the end of the trial.

(A) Example of a cortical neuron that did not change its firing rate during running at the beginning of the session but gradually started increasing its firing rate during each running period and became task-related by the end of the session. Note that during trial two the neuron already increased its firing rate during running, but by trials nine and ten the increase in firing rate starts abruptly after time 0.

(B) Example of a striatal neuron that was task related at the beginning of the session and became less task related throughout the session. This neuron gradually decreased its firing rate during each running period, stabilizing during the last two trials.

(C) Striatal neuron that became task related throughout a session by gradually (on average, with some trial to trial variation) decreasing its firing rate during the rest period.

(D) Striatal neuron that became task-related with training. This neuron increased its firing rate during both the resting and running periods from the beginning to the end of the session and became task related early during the session (note trial 2).

unchanged for the rest of the training days (striatum: day 2, 76% to 80%, $F_{1,7} = 0.13$; day 3, 92% to 73%, $F_{1,8} = 1.68$; motor cortex: day 2, 83% to 79%, $F_{1,5} = 0.45$; day 3, 78% to 78%, $F_{1,6} = 0.00$; $p > 0.05$) (Figure 5B). Neurons changed their firing modulation depth (and therefore their task relatedness) by either changing their firing rate during the running period (Figure 4A) or by changing their firing rate during the resting period (Figure 4C). In some neurons we observed both phenomena concomitantly (Figure 4D). In agreement with this increase in the number of task-related neurons, during this first session, the number of recruited neurons (i.e., neurons that were not task related during the early trials and became so by the late trials) was significantly larger than the number of dismissed neurons (i.e., neurons that were initially task related and became less involved by the end of the session) in both striatum and cortex (Figure 5C, 42% recruited and 3% dismissed in striatum,

presented on the left column and motor cortex neurons on the right column. (A and B) Example of a striatal and a cortical neuron that abruptly increase their firing rate during the running period. (C and D) Example of a striatal and a cortical neuron that abruptly decrease their firing rate during the running period. (E) Example of a striatal neuron with a rather high firing rate at the onset of each trial, which gradually decreases its firing rate toward the end of the trial. (F) Example of a cortical neuron that gradually increases its firing rate during the running period. (G and H) Example of a striatal and a cortical neuron that transiently changed their firing rate at the beginning of the running period and then gradually decreased it throughout the trial.
Figure 5. Characterization of the Changes in Neural Activity in Striatum and Motor Cortex during Motor Skill Learning

(A) Percent of task-related neurons averaged across each session. In both striatum and motor cortex, the percent of task-related neurons did not change across days.

(B) Percent of task-related neurons during the early (black) and late (white) trials in each session. In both striatum and motor cortex, the percent of task-related neurons increased significantly during the first training day but did not change in subsequent days.

(C) Percent of recruited (black) and dismissed (white) neurons across all sessions. In both striatum and motor cortex, there were significantly more recruited neurons than dismissed during the first day. On days 2 and 3 fewer neurons changed their activity profile during the training session, and there was no significant difference between the number of recruited and dismissed cells.

(D) Percent of task-related neurons that increased (black) or decreased (white) firing rate during movement throughout training. In motor cortex, the difference between the two populations increased gradually throughout training and became significant early on the second day. In striatum, we observed more neurons increasing versus decreasing their firing rate all through training, and the difference between the two populations remained unchanged throughout training.

(E) Percent of velocity-correlated neurons (from all the neurons recorded) across days. In striatum, we observed an increase in velocity-correlated neurons across days, while in motor cortex we observed no change throughout training.

\[ F_{1,12} = 26.3, \text{ and } 29\% \text{ and } 6\% \text{ in motor cortex, } F_{1,6} = 8.74; p < 0.05. \] In subsequent training days 2 and 3, plasticity in the activity profile of neurons continued to a lesser extent (\(~27\% \text{ in striatum and } ~24\% \text{ in motor cortex}). However, during each of these sessions, the number of recruited neurons equaled that of dismissed neurons (Figure 5C, striatum: day 2, 13\% and 9\%, \( F_{1,12} = 0.25; \) day 3, 7\% and 25\%, \( F_{1,12} = 1.73. \) Motor cortex: day 2, 12\% and 16\%, \( F_{1,6} = 0.09; \) day 3, 9\% and 10\%, \( F_{1,6} = 0.008; p > 0.05). \) It is important to note that the fact that some animals had rather short first trials did not make a crucial contribution to the pattern of changes observed (example in Figure 4A, see Experimental Procedures). Interestingly, when we average the number of task-related neurons by using all ten trials in a given session, this value did not change from day 1 to day 3 in either the striatum (60\%, 58\%, and 53\% on days 1, 2, and 3, respectively, \( F_{2,18} = 0.18, p > 0.05 \)) or in motor cortex (62\%, 40\%, and 64\% on days 1, 2, and 3, respectively, \( F_{2,6} = 1.42, p > 0.05 \) (Figure 5A), indicating once more that the increase in number of task-related neurons occurred specifically during the first session. Together, these results indicate that during the fast phase of learning, the majority of the plastic changes corresponded to an expansion of the task-related neural circuitry, which occurred in parallel in both striatum and motor cortex.

Next, we investigated whether in addition to the rapid recruitment of neurons observed specifically during the first session, slower-evolving plastic changes would be observed across sessions, paralleling the slow improvement in motor performance. We observed an increase in "velocity-correlated" neurons across days in the striatum (Figure 5E, main effect \( F_{2,18} = 2.6, \) day 1 versus day 3 \( F_{1,12} = 7.34, p < 0.05, \) day 1 = 33\%, day 2 = 47\%, and day 3 = 56\% of all the neurons recorded). This increase in the striatum was gradual and became signifi-
Cortical and Striatal Neuronal Ensemble Plasticity during Motor Skill Learning

It has been previously shown that neuronal ensemble activity in motor cortex can increasingly predict behavioral outcome with motor training [22]. We therefore investigated whether plasticity at the neuronal ensemble level during motor skill learning occurred in striatum and motor cortex. By using the neuronal ensemble activity from trials nine and ten of each day (similar length across all 3 days, Figure 2C), we generated a linear model to estimate the rotarod velocity throughout a trial [23, 24]. We used this model (same neuronal ensemble and constant neuronal weights) to estimate the rotarod velocity in early (one and two) and late trials (seven and eight) of each session (Figure 6A). We then calculated how well the model estimated the rotarod velocity during these trials by correlating the estimated velocity with the actual velocity. Different correlation values (R²) between early and late trials indicated that the ability of the model to estimate the rotarod velocity varied during the session; i.e., that the relative contribution of the different neurons in the ensemble probably changed from early to late trials. Therefore, different correlation values between early and late trials suggested that the neuronal ensemble activity changed throughout the session, while similar values signified that the neuronal ensemble activity remained relatively stable. We observed considerable variation in neuronal ensemble activity within the first session in both striatum and motor cortex (Figure 6A, early versus late: striatum: 0.23 versus 0.66, F₁₁₂ = 6.6; motor cortex, 0.14 versus 0.49, F₁₁₂ = 6.9; Figure 6C: striatum, 0.54, t₆ = 3.6; motor cortex 0.50, t₆ = 2.8; p < 0.05 for all comparisons). This variation became less evident during day 2 and even smaller during day 3 (Figure 6A, early versus late, striatum: day 2, 0.47 versus 0.68; day 3, 0.62 versus 0.6; motor cortex: day 2, 0.3 versus 0.7; day 3, 0.84 versus 0.65; Figure 6C, striatum: day 2, 0.28; day 3, 0.12; motor cortex: day 2, 0.16; day 3, 0.06; p > 0.05 for all comparisons).

The pattern of plasticity in neuronal ensemble activity throughout training paralleled the pattern of behavioral changes (Figure 2) as well as the pattern in single neurons (Figure 4) and neuronal population (Figure 5) plasticity. Thus, some of the changes observed at the single neuron and population level may have contributed to the changes observed in neuronal ensemble activity. However, it is interesting to note that the average firing rate (Hz) during the running period of the population of neurons that constituted the ensemble did not change between the early and late trials described above or across sessions (Figure 6D, early versus late, striatum: day 1, 4.5 versus 4.9; day 2, 6.2 versus 5.9; day 3, 7.2 versus 7.2; motor cortex: day 1, 4.1 versus 5.6; day 2, 5.3 versus 5.9; day 3, 5.6 versus 6; p > 0.05 for all comparisons). Moreover, the firing-rate modulation (i.e., the relationship of the firing rate during running versus resting for a particular neuron during each trial, see methods) of the neurons that constituted the ensemble did not change between early and late trials or across sessions (Figure 6E, early versus late, striatum: day 1, 0.25 versus 0.3; day 2, 0.31 versus 0.29; day 3 0.24 versus 0.3; motor cortex: day 1, 0.19 versus 0.23; day 2, 0.2 versus 0.22; day 3, 0.17 versus 0.21; p > 0.05 for all comparisons).

Discussion

In this study, we measured for the first time the simultaneous activity of neuronal ensembles in primary motor cortex and dorsal striatum during motor skill learning in mice. For this purpose, we developed a methodology to reliably record neuronal activity from multiple brain areas of awake-behaving mice. Our data showed that corticostriatal circuits undergo rapid and extensive re-
Figure 6. Neuronal Ensemble Plasticity in Striatum and Motor Cortex during Motor Skill Learning

(A) Correlation between the estimated and actual rotarod velocity (R²) during early (one and two) and late (seven and eight) trials based on a linear model generated from neuronal data of the ninth and tenth trials of each session. R² values depicted are normalized to the average R² of the fitting trials.

(B) Examples of estimated velocity versus actual rotarod velocity (in rotations per second) during a trial.

(C) Quantification of the changes in neuronal ensemble activity throughout a session (see Experimental Procedures). Overall, we observed considerable variation in neuronal ensemble activity during the first session in both striatum and motor cortex, which became less evident during day 2 and indistinguishable during day 3. The pattern of neuronal ensemble plasticity paralleled the pattern of behavioral, single neuron, and neuronal population changes.

(D) The average firing rate during running of the neurons that constituted the ensemble did not change within sessions or across sessions.

(E) The average firing-rate modulation (see Experimental Procedures) did not change within sessions or across sessions.

Enrollment of task-related neurons during the first session, indicating that there is a fast expansion of the task-related neural circuitry both in striatum and cortex during the fast phase of skill learning (day 1). In subsequent days, there was a continuous turnover of the task-related neurons, but the percentage of recruited versus dismissed neurons reached a balance. Across days, during slow motor skill learning, we continued to observe changes in the firing profiles of the neurons, suggesting that besides the first recruitment phase, cortical and striatal neuronal ensembles continued to change in parallel with further refinement of the movement [20]. Importantly, these slow changes were dissociable between these structures. We observed an increase in velocity-correlated neurons throughout training in striatum, but not in motor cortex. Conversely, we observed a significant increment in the number of neurons increasing versus decreasing firing rate during running in motor cortex, but not in dorsal striatum. The changes at single neuron and neuronal population level were paralleled by changes in neuronal ensemble activity. Thus, during the first session, we observed considerable variation in the activity of both striatum and motor cortex neuronal ensembles, which became less evident during subsequent sessions.

The plastic changes we observed were specific to the acquisition of a motor skill and did not arise just from general motor-related activity, because both at the single neuron (Figure 5) and the neuronal ensemble level (Figure 6), the plastic changes observed during day 3 (after the animals have reached a behavioral plateau) were less pronounced than and different from the changes observed during days 1 and 2. The parallel expansion of the task-related neural circuitry in both motor cortex and striatum during the fast phase of learning could represent a rapid way to improve motor perfor-
mance by incrementing the computational space available to control the motor response; and is in agreement with models positing recurrent loop architectures involving cortex and striatum. With time, during slow learning across sessions, refinement of the firing patterns in each structure could facilitate further improvement of the movement. For example, the increase in velocity-correlated neurons in striatum could reflect improvement in action selection, while the enhancement in the proportion of neurons increasing versus decreasing firing rate in motor cortex may facilitate action performance in this highly constrained motor task, which involves single-axis improvement in performance. However, these hypotheses are not easily testable through the present task due to the difficulty in precisely measuring the kinematics and force applied during the execution of the movement and could perhaps be addressed by using tasks that allow better characterization of these parameters [8] or by using a modified version of the present task.

It is interesting to note that throughout training, even during the phase of increase in number of task-related neurons, the task-related firing rate modulation of the population of neurons (Figures 6C and 6D) remained unaltered. This suggests that in parallel to the experience-dependent plastic changes observed, homeostatic mechanisms either at the cellular [25, 26] or network level may preserve the overall level of firing of large neural circuits.

It has been previously shown that motor cortex is essential for the initial stages of motor skill learning [11], which is consistent with our observation that there is a significant expansion of the number of task-related neurons in motor cortex during the initial stages of motor skill learning. However, there is recent evidence that in motor cortex, synaptogenesis and reorganization of motor representations occur during the late, but not early, phase of motor skill learning [27]. In combination with these previous studies, our data suggests that neural activity changes mediated by processes like synaptic plasticity may precede structural changes and underlie early motor skill learning. In agreement with recent studies in conditional visuomotor learning [20], the plasticity we observed tended to consist of changes in the differential firing rate during the running and resting periods, suggesting that changes in synaptic weights rather than ongoing sustained recurrent activity accompanied the improvement in performance.

The striatum has been postulated to be activated at different stages of motor skill learning, depending on the behavioral paradigm used [19]. Our results show activation and plasticity of both cortical and striatal neurons throughout training. However, besides the initial recruitment phase, differential changes in neuronal activity patterns, which may be difficult to detect in imaging studies (e.g., velocity correlation, profile of firing rate modulation) seem to be the most evident. These ongoing changes could reflect a continuous rearrangement of the motor representation to allow further refinement of the subject’s movement [20, 28]. Also, substantial changes observed even in the absence of training, for example between the late trials of day 1 and the early trials of day 2 (Figure 5E, motor cortex), suggest that

The neuronal representations continue to change during the consolidation of the learned skill, either after training [29, 30] or during the following sleep cycle [31–34]. Our results show that the performance of this motor skill involves activation of a high proportion of the neurons both in striatum and motor cortex. These data suggest that performance of this novel, highly repetitive sequence of movements on the rod may involve the activation of dense (nonsparse), distributed circuits in both striatum and motor cortex. All through training, we observed that about one-third of the striatal neurons decreased their firing rate during running while two-thirds increased it. It would be important to determine if these two types of neurons belong to the same or to different populations/circuits; for example, to the direct versus indirect striatal pathways [35, 36].

Conclusion

In the present study, we observed that corticostriatal circuits undergo substantial plasticity during motor learning. Furthermore, this plasticity differs between fast and slow motor skill learning. During fast, within-session learning, motor cortex and dorsal striatum change their activity in concert. During slow, across-session learning, however, the activity changes in M1 and striatum differ. Importantly, these changes occur in the absence of any alterations in the overall firing rate of the neuronal population. These findings suggest that distinct neural processes mediate fast and slow motor skill learning. It remains to be investigated whether these distinct neural processes develop in series or in parallel. These data may also provide interesting starting points to analyze the motor deficits observed in mouse models of neurodegenerative disorders, such as Parkinson’s and Huntington’s diseases.

Experimental Procedures

Animals

All procedures were approved by the Duke Institutional Animal Care and Use Committee and performed in accordance with National Institutes of Health guidelines. We used F1 isogenic hybrids (2–7 months old) resulting from the cross between C57BL/6J female and 129S1/SvImJ male (Jackson labs). Nine mice were used for the experiments leading to the establishment of the technique and seven other animals for the motor skill learning study (three implanted bilaterally in dorsal striatum and four in both dorsolateral striatum and motor cortex in separate hemispheres).

Behavior

A computer-interfaced rotarod with capability to accelerate from 4–40 rpm in 5 min was used (ENV-575M, MedAssociates). After each 300 s resting period at the bottom of the rotarod, mice were placed on the rod, which was then activated to start moving. Each trial ended when mice fell off the rod or when mice ran for 300 s (end of acceleration, 40rpm). Immediately at the end of the trial the rod was stopped. The beginning and end of the running period were signaled to the MAP recording system (Plexon Inc., Texas) as events. A custom-made pulley system balanced the weight and torque of the wires during running and allowed the wires to accompany the mouse during the fall.

Surgery

Two 1 mm² craniotomies were made bilaterally; 0.5 mm rostral to bregma; up to 1.8 mm laterally for motor cortex and 2 mm laterally for dorsal striatum; microwire arrays were lowered ~0.9–1.2 mm
from the surface of the brain for motor cortex (electrodes aimed at layer 5 of primary motor cortex) and 2–2.2 mm for dorsal striatum [37] while recording neural activity. Because we used a hybrid mouse strain, we used an extrapolation of the limits measured for C57Bl/6 and 129/Sv for the definition of our motor cortex boundaries (−2.3 to +0.56 AP; 1.0 to 2.4 ML) with the obvious inaccuracies that this extrapolation may cause. The onset of cortical layer IV was considered to mark the boundary between primary motor cortex and somatosensory cortex, and no electrodes were placed beyond this boundary.

In striatum the electrodes were aimed at the dorsolateral striatum (−0.5 bregma), which has been shown to receive projections from primary motor cortex. Final placement of the electrodes was decided based on the coordinates and the neural activity and confirmed histologically after electrolytic marking lesions, perfusion with 10% formalin, and brain fixation with 30% sucrose, 10% formalin, cryostat sectioning, and cresyl violet staining.

Data Analysis

Task-Related Neurons throughout a Session

We used a paired t test (α = 0.01) to compare firing rate during movement (running on the rotarod) and rest (intertrial interval).

Task-Related Neurons during the Early and Late Trials

To determine whether a neuron was task related during the first two trials of a session, we generated two firing-rate distributions: one during the rest period and the other during the movement period. We divided each period into 20 s bins (to include at least one complete rotation of the rod) and calculated the firing rate of the neuron in each bin. The initial two trials (early trials) were combined in one distribution to eliminate possible effects of short trials in the first session. Then we used analysis of variance (ANOVA) to determine whether the firing rate during the running period was significantly different than during the rest period (α = 0.01). The same analysis was used for the last two trials (late trials) in each session. We realize that each 20 ms bin is not an independent measure, and we are therefore overlooking one of the assumptions of ANOVA in this analysis. Repeated measures ANOVA cannot be used in this case because (1) it is a paired test, and therefore it would compare the first bin of 20 ms in the rest period with the first bin of 20 ms in the run period and so forth, and (2) the rest and running periods (and trials one and two) have a different number of bins.

We verified that by using either (1) α = 0.05 instead of 0.01 or (2) the first 100 s of data from the running period and 100 s from the resting period to define task-related neurons did not change the findings presented here.

Velocity-Correlated Neurons

Neurons whose firing rate during the running period were significantly correlated with the speed of the rotarod. The running period in each trial was divided into 20 s bins and firing rate was calculated for each bin. Data points from all ten trials were used to calculate the correlation coefficients of the neuron’s firing with the known rotarod velocity (α = 0.05). Note that the pattern of changes in velocity-correlated neurons observed in striatum (Figure 5 E) is not an artifact of some short trials during the first session. If this were the case, an equally low number of velocity-correlated neurons would be observed in both striatum and motor cortex during day 1, which is clearly not the case in our data (33% in striatum and 58% in motor cortex).

On-Off Neurons

Task-related neurons that displayed a persistent change in firing rate during the running period compared to the rest period and whose firing rate during the running period were not significantly correlated with the speed of the rotarod.

Ensemble Analysis

Neuronal ensembles were defined as the group of simultaneously recorded neurons in each session. A variation of previously used models [23, 24] was used to estimate the rotarod velocity throughout the trial. Running velocity was calculated as a weighted linear combination of neuronal activity by using a multidimensional linear regression

\[ y(t) = b + \sum_{u=-m}^{a} a(u)x(t-u) + \epsilon(t). \]

In this equation, \( x(t-u) \) is an input matrix of neuronal firing rates at time \( t \) and timelag \( u \), \( y(t) \) is a vector of velocity at time \( t \), \( a(u) \) is a vector of weights at timelag \( u \), \( b \) is a vector of y-intercepts, and \( \epsilon(t) \) is the residual error. We considered ten lags of 100 ms into the past (as in [23]). We used the data of the ninth and tenth trials (which were equal in length between the 3 days of training) to determine the weights for each neuron in the model. By using the same neuronal ensemble and model, we then estimated the rotarod velocity in early (one and two) and late trials (seven and eight) of each session.

We then calculated the correlation between the estimated and actual rotarod velocity (\( R^2 \)) in early and late trials. Differences in \( R^2 \) between early and late trials signified that the neuronal ensemble activity changed during the session; i.e., the optimal weights for each neuron changed during the session, while no change in \( R^2 \) between early and late trials implied stability of the neuronal ensemble activity throughout the session. For each animal, the \( R^2 \) of the estimation trials was normalized to the average \( R^2 \) of the fitting trials. We used the ratio \( (R^2_{est} - R^2_{fit})/(R^2_{fit} + R^2_{est}) \) for each animal to quantify the degree of change in neuronal ensemble plasticity throughout a session. We obtained similar results using ten lags of 200 ms or if we calculated the model using trials five and six (estimating for late trials nine and ten and by using the same length of trial in days 2 and 3 as in day 1), just trial nine (estimating for late trials eight and ten), or just trial ten (estimating for late trials nine and ten).

Firing-Rate Modulation

Firing-rate modulation for each neuron was calculated during the first 100 s of a particular trial as (firing rate \( t_{est} \) − firing rate \( t_{fit} \))/ (firing rate \( t_{est} \) + firing rate \( t_{fit} \)).

Statistical Procedures

All results were averaged per animal and statistics were performed on the values per animal. Single-factor ANOVA (α = 0.05) was used to investigate general main effects and in all planned comparisons. Two-factor ANOVA (α = 0.05) was used to determine main effects shown in Figures 5B–5D. Only when there was a main effect, we performed posthoc comparisons by using either Fisher’s PLSD or paired t test when comparing against 0.

Supplemental Data

Supplemental Data including Experimental Procedures detailing multielectrode array design and data collection accompanied by one figure and one table are available at http://www.current-biology.com/cgi/content/full/14/13/1124/DC1/.

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ningston’s, and Parkinson’s disease patients. J. Neurosci. 9, 582–587.
Supplemental Experimental Procedures

Multielectrode Array Design and Data Collection

The main design used in this study consists of an array of 16 S-isonel-coated tungsten microwire electrodes, a printed circuit board (PCB) connected to the microwire electrodes, and a high-density, miniature connector attached to the opposite side of the PCB [55].

We investigated the advantages and disadvantages of several array designs: (1) 16 single-ended wires spaced 250 μm apart (Figure 1A) (referred to as design a), (2) four groups of four microwires separated by 100 μm in a square arrangement (each group separated by 250 μm, Figure 1B) (referred to as design b), and (3) eight pairs of two adjacent electrodes (maximum 50 μm between wire centers in each pair, pairs separated by 250 μm, Figure 1C) (referred to as design c). We also tested two wire diameters: 50 μm, with impedance around 1.2 MΩ; and 35 μm, with impedance around 1.5 MΩ. The arrays and headstage were miniaturized (weight ~0.26 g per array), allowing mice to move freely. We implanted 32 microwires per mouse (an array of 16 microwires in each hemisphere) in the dorsal striatum and the motor cortex (Figures 1H and 1I). In order to test the different designs, we recorded in single-channel mode (for all designs), tetrode mode (for design b), or stereotrode mode (for design c) (by using MAP, Plexon, Inc.). We used differential recording in which one of the channels with no neural activity was used as a reference electrode. By using the single-channel recording mode, we were able to record well-isolated single units from single channels in all three designs (Figure 1D). With both designs b (tettrode mode) and c (stereotrode mode), we were unable to record simultaneous activity from the same unit in adjacent electrodes, even when very well-isolated single neurons could be recorded from each of the adjacent electrodes in single-channel mode (Figure 1E).

In this context, our results differ from previous studies using stereotrode and tetrode recording methods [S1]. Likely, this difference results from the fact that we used electrodes with an impedance four to five times higher than other studies, larger wire diameter, different array design geometry, and also that we recorded from sparsely distributed (in striatum two to four cells 35 μm² or 6–12 cells 50 μm² [S2]) and very small (~12 μm cell body diameter for striatal medium spiny neurons [S3]; ~15 μm for pyramidal cortical cells [S4]) neurons. Using the specific parameters described here, we observed no advantage in using triangulation to isolate single units (Figure S1D). This observation could be different when recording in other brain areas and/or when using other parameters. We therefore opted to employ single-channel recording mode in all the experiments described hereafter. In the striatum, we obtained better results with the 50 μm wires (both in number of units isolated and longevity of the recordings), while in motor cortex both wire diameters produced similar outcomes.

Single cell and multiunit activity were recorded with the MAP system (Plexon, Inc., Texas). The activity was initially sorted using an online sorting algorithm (Plexon, Inc., Texas). Only units that had a clearly identified waveform with a signal-to-noise ratio of at least 3:1, where no waveforms were dropped by the acquisition system (sampling rate of 40 KHz), were used. At the end of the recording, units were resorted offline based on waveform, amplitude, and interspike interval histogram using an offline sorting algorithm (Plexon, Inc.) [55]. Only units in which all the spikes had amplitudes well above the voltage threshold were considered (to ensure that no spikes were lost). When the waveform, amplitude, and interspike interval (ISI) did not allow unambiguous isolation (even with signal-to-noise ratio larger than 3:1), the signals were either discarded or labeled as multiunit activity. The criteria were the following: when the waveforms from a unit clearly resulted from two or more neurons that could not be discriminated, this unit was discarded. In the occasional cases where not all the spikes of a single-unit could be unequivocally isolated during offline sorting (due to the proximity between two clusters or proximity to the noise cluster), the unit was labeled as multiunit. The average number of multiunits per session was 11%, 13%, and 11% during sessions one, two, and three, respectively. Analyses of these units were conducted separately. The data were combined for the purpose of presentation since there were no significant differences in the results from the two groups. Although comparison of neuronal waveforms, interspike intervals (ISI) and firing activity during the task showed that sometimes we could record the same neuron in consecutive sessions, we adopted a more conservative treatment of the data: we assumed that each day we were sampling independent neurons. The animal-by-animal breakdown of neurons recorded during each session is in Table S1.

Supplemental References

Figure S1. Comparison of Different Arrays and Recording Modes to Record Multiple Single Neuron Activity in Awake-Behaving Mice (A–C) Different microarray designs. (A) 16 single-ended wires spaced 250 μm apart. (B) Four groups of four microwires separated by 100 μm in a square arrangement, each group separated by 250 μm. (C) Eight pairs of two adjacent electrodes. (D) Example of a striatal single-unit recorded in stereotrode mode. Although the signal recorded in channel 2 of the stereotrode is very prominent, no signal from this unit is captured in channel 1 of the stereotrode (note that threshold is very close to zero). Also, the isolation quality of the cluster of this single unit is similar when the unit is recorded in stereotrode or single-channel mode.

Table S1. Animal-by-Animal Breakdown of the Neurons Recorded during Each Session

<table>
<thead>
<tr>
<th>Animal</th>
<th>Mouse 03</th>
<th>Mouse 09</th>
<th>Mouse 10</th>
<th>Mouse 11</th>
<th>Mouse 13</th>
<th>Mouse 14</th>
<th>Mouse 15</th>
<th>Total per session</th>
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<tbody>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Day 1</td>
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<td>5</td>
<td>8</td>
<td>5</td>
<td>10</td>
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<td>6</td>
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<tr>
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<td>25</td>
<td>6</td>
<td>9</td>
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<td>9</td>
<td>83</td>
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<tr>
<td>Motor cortex</td>
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