Published in final edited form as:

Neurobiol Learn Mem. 2012 October ; 98(3): 291–302. doi:10.1016/j.nlm.2012.09.004.

Skill learning induced plasticity of motor cortical representations is time and age-dependent

Kelly A. Tennant^{a,1,*}, DeAnna L. Adkins^b, Matthew D. Scalco^c, Nicole A. Donlan^c, Aaron L. Asay^c, Nagheme Thomas^d, Jeffrey A. Kleim^d, and Theresa A. Jones^{a,c}

^aInstitute for Neuroscience, University of Texas at Austin, Austin, TX 78712, USA

^bDepartment of Neurosciences, Medical University of South Carolina, Charleston, SC 29403, USA

^cDepartment of Psychology, University of Texas at Austin, Austin, TX 78712, USA

^dSchool of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ 85287, USA

Abstract

Movement representations in the motor cortex can reorganize to support motor skill learning during young adulthood. However, little is known about how motor representations change during aging or whether their change is influenced by continued practice of a skill after it is learned. We used intracortical microstimulation to characterize the organization of the forelimb motor cortex in young and aged C57/BL6 mice after short (2–4 weeks) or long (8 weeks) durations of training on a skilled reaching task or control procedures. In young mice, a short duration of reach training increased the area of proximal forelimb movement representations at the expense of distal representations. Following a longer training duration, ratios of proximal to distal movements returned to baseline, even with ongoing practice and skill maintenance. However, lingering changes were evident in thresholds for eliciting distal forelimb movements, which declined over the longer training period. In aged mice, movement representations and movement thresholds failed to change after either duration of training. Furthermore, there was an age-related loss of digit representations and performance decrements on other sensorimotor tests. Nevertheless, in quantitative measures of reaching success, aged mice learned and performed the skilled reaching task at least as well as younger mice. These results indicate that experience-driven topographical reorganization of motor cortex varies with age, as well as time, and is partially dissociable from behavioral performance. They also support an enduring capacity to learn new manual skills during aging, even as more youthful forms of cortical plasticity and sensorimotor function are lost.

Keywords

skilled reaching; intracortical microstimulation; aging; forelimb; motor cortex; map plasticity

© 2012 Elsevier Inc. All rights reserved.

*Address correspondence to: Kelly A. Tennant, PhD, Medical Sciences Building, University of Victoria, PO Box 1700 STN CSC, Victoria, BC V8W 2Y2, Canada, tennantk@uvic.ca, Phone: +1 250 853 3964, Fax: +1 250 472 5505.

¹Present address: Division of Medical Sciences, University of Victoria, Victoria, BC V8W 2Y2, Canada

Disclosure Statement

The authors have no conflicts of interest to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Age-related decreases in manual dexterity (Carmeli et al., 2003; Smith et al., 1999), compounded by decreases in speed and strength (Ranganathan et al., 2001b) may result in an increased risk of injury and trouble performing tasks of daily living (Incel et al., 2009). This contributes to a loss of independence and may also be an early predictor of cognitive decline (Kluger et al., 1997). However, in healthy subjects, motor training can attenuate the loss of dexterity associated with aging. Training on a skilled finger movement task improved the ability of older adults to control pinch force, hand steadiness, and manual speed, compared to untrained older adults (Ranganathan et al., 2001a). A better understanding of healthy aging effects on forelimb motor function and motor cortical organization could lead to the development of strategies to extend youth-like motor ability and manual dexterity into old age.

Age-related changes in motor performance are associated with functional alterations in several brain regions. Transcranial magnetic stimulation (TMS) and functional magnetic resonance imaging (fMRI) studies indicate greater activation of premotor, supplementary motor, sensory and cognitive areas in the aged brain during motor task performance (Heuninckx et al. 2005, 2008; Hutchinson et al., 2002; Talelli et al., 2008; Ward and Frackowiak, 2003). The increase in cognitive and sensory activation is thought to reflect an increased reliance on sensory cues and more cognitive control during movement, which may allow older adults to produce motor behavior that is more similar to that seen in young adulthood (Heuninckx et al. 2005, 2008).

Rats and mice provide useful models for studying age-related changes in manual skills due to their high degree of forelimb dexterity (Whishaw and Coles, 1996; Whishaw et al., 1998). In the somatosensory cortex, the topographical complexity of the forelimb representation of rats degrades as they age (Coq and Xerri, 2000) and this coincides with impairments in forelimb and hindlimb walking behavior (David-Jürgens et al., 2008; Spengler et al., 1995). The somatosensory map maintains some complexity (Coq and Xerri, 2001) and walking behavior more closely resembles that of young animals (David-Jürgens et al., 2008) when rats are raised in enriched environments beginning immediately after weaning. In contrast to the somatosensory cortex, little is known about how the organization and plasticity of the rodent motor cortex change during aging.

In young adult mice, motor cortical dendrites add new spines within the first hour after training on a novel skilled reaching task. The total spine density returns to baseline when a skill has been practiced for ~2 weeks, but there is selective survival of new spines formed during early skill acquisition (Xu et al., 2009). In young adult rats, Kleim et al. (1998) found that training on a skilled reaching task increases representation areas for movements necessary for task performance at the expense of representations of lesser-used movements. Molina-Luna et al. (2008) demonstrated that the areal extent of the forelimb movement representations in rats increases during acquisition of a motor skill and returns to baseline after 8 days without training, but the ability to perform the task is maintained. It is unknown why the map returns to baseline without a loss of motor skill: whether this results from the cessation of practice or is reflective of a typical stage of motor cortical reorganization that occurs even with continued practice. Studies of humans with extensive motor skill practice show conflicting results on this question as well. For example, several studies have shown that professional musicians (Elbert et al., 1995; Gaser and Schlaug, 2003), professional badminton players (Pearce et al., 2000), and blind Braille readers (Pascual-Leone et al., 1993) have a larger area of motor cortex corresponding to the trained hand compared to novices. However, Pascual-Leone et al. (1995) showed a decrease in motor representations

in Braille readers after a weekend of not practicing, and others have shown that professional musicians show less activation in motor areas compared to novices (Jäncke et al., 2000; Krings et al., 2000). Additionally, two recent studies have shown that the auditory receptive fields of rats undergo expansion at early stages of training on an operant conditioning task (Takahashi et al., 2011), a tone discrimination task, or after nucleus basalis stimulation with tone pairing (Reed et al., 2011) followed by shrinkage in later stages of training. Thus, the characteristics and dynamics of cortical map plasticity, especially as they relate to long-term training, are complex and insufficiently understood. More information is needed on the characteristics of motor cortical plasticity, even in young adult brains, to understand how changes in these characteristics contribute to motor behavior across the lifespan.

The main purpose of the current study was to investigate age-related changes in the baseline organization of motor cortex and its response to motor skill learning and practice. An additional goal was to determine the effect of extended practice on the maintenance of map reorganization after it has occurred. Young and aged mice were trained on one of two behavioral tasks, the Pasta Matrix Reaching Task or a dexterous food (pasta) handling task, or served as untrained controls. To determine the effect of training duration on the organization of motor cortex, young and aged mice underwent intracortical microstimulation (ICMS) procedures after either a short duration (2-4 weeks) or a long duration (8 weeks) of training. Our results indicate that the organization and plasticity of the forelimb motor cortex is altered in the aged brain, but the ability to learn a skilled motor task is maintained. Additionally, in young mice, the reorganization of movement representations during skill learning is transient even with ongoing skill practice.

2. Methods

2.1. Subjects

A total of 82 young (3-5 months old) and 43 aged (18-20 months old) well-handled male C57BL/6 mice were used. All young mice were purchased from Jackson Laboratories (Bar Harbor, ME). Aged mice were either retired breeders obtained from the Transgenic Mouse Facility at UT-Austin or Jackson Laboratories ($n = 26$), or virgin mice obtained from Jackson Laboratories at 1 month of age which were allowed to age in our colony ($n = 17$). The two groups of aged mice did not differ significantly in behavioral or intracortical microstimulation (ICMS) evoked motor map results and were combined for all analyses. Young mice and aged mice raised in our colony were typically housed in groups of four. Aged retired breeders were housed singly before arrival in our colony, and were maintained in single housing to prevent aggressive behavior characteristic of non-littermate males. All mice received standardized housing supplementation (a small piece of PVC pipe, a cardboard roll, small wooden objects, and nesting material) and were kept on a 12:12 h light/dark cycle. Animals were maintained on scheduled feeding, given at the end of each daily training session to prevent satiation at the time of reach training. Aged animals received slightly more food ($3\text{--}3.5$ g/day) than young animals ($2.5\text{--}3$ g/day) to account for their larger size (30.8 ± 0.6 versus 28.4 ± 0.2 g in young mice). A total of 6 animals (4 young and 2 aged) died during the ICMS procedure before a complete forelimb map was resolved. Behavioral data from these animals are included in the study. ICMS data from 27 of the young animals were used in a previously published paper describing the general organization of the mouse motor cortex (Tennant et al., 2011a), and some behavioral data from the subset of animals tested on the Capellini Handling Test were included in a previously published methods article (Tennant et al., 2010). Animal use was in accordance with a protocol approved by the University of Texas at Austin Animal Care and Use Committee.

2.2. Design of behavioral experiments

Both young and aged animals were randomly assigned to one of three training conditions: Reach Training, Pasta Handling, or Untrained Control conditions. Per each of these conditions there were two training durations: Short Duration (2-4 weeks) or Long Duration (8 weeks), for a total of twelve groups. Approximately equal numbers of mice from each behavioral condition were trained together to control for batch effects.

The reaching task used was the Pasta Matrix Reaching Task (Ballermann et al., 2001; Tennant and Jones, 2009). This task is similar to the pellet reaching tasks that have been shown to reorganize motor representations in the rat (Kleim et al., 1998). However, a difference between pellet reaching tasks and the Pasta Matrix Reaching task is that animals gain experience handling the retrieved food (capellini pasta), which itself has been found to result in spine plasticity in the motor cortex (Xu et al., 2009). Thus, the Pasta Handling condition was included to determine whether dexterous food handling could alter the motor map. Untrained control mice received bits of pasta too small to handle (pasta “niblets”) in the same training context.

The different durations of training were used to determine if short and long-term practice of a motor skill resulted in differences in reorganization of the forelimb motor cortical representations. The duration of training was determined in individual mice based on evidence that the task had been well learned, as defined by the transition from a rapid learning phase to more gradual improvements. The onset of the gradual learning phase was considered to have occurred at the first time point in which there was minimal performance improvement relative to the preceding training block of 3 days, as defined by a slope of < 0.2 in the success levels across training blocks. Short-duration mice were trained for at least 7 and no more than 14 days after reaching the gradual learning phase. Long-duration mice were trained for ~45 days after the onset of the gradual learning phase. ICMS experiments were conducted at the end of the short duration of training (shortly after the task was “mastered”) or at the end of the long duration of training (after the task had been mastered for some time).

2.3. Motor skill training tasks

Daily training on the Pasta Matrix Reaching Task involved training mice to reach for and break small pieces of vertically oriented, uncooked capellini pasta (3.2 cm in height and 1 mm diameter; DeCecco brand, Fratelli De Cecco di Filippo Fara San Martino S.p.A., Italy), arranged in a matrix distal and lateral to a centrally located aperture in a reaching chamber (Farr and Whishaw, 2002; Tennant and Jones, 2009). In order to successfully retrieve a pasta piece, the mouse must break the pasta by grasping and pulling forward. Mice first underwent a short shaping period in order to determine limb preferences. Mice were then trained to reach only with the preferred limb by filling with pasta only the half of the matrix contralateral to this limb. In the natural rodent posture for reaching through a narrow window, the snout is maintained in the reaching window during the reach-to-grasp sequence, such that midline is roughly aligned with the center of the window (Whishaw and Pellis, 1990; Whishaw, 1996; Farr and Whishaw, 2002). Reaching from this position requires the limb to be first extended to midline to access the window, and then further extended contralaterally to contact pasta pieces. Thus, filling only the half of the matrix contralateral to the preferred/trained limb with pasta makes it virtually impossible to successfully retrieve food pieces with the non-trained/non-preferred limb. Daily training sessions consisted of up to 100 reaches or 15 min, whichever occurred first. Recorded data included the number of pasta pieces successfully broken, the total number of reach attempts, the area of the matrix that the mouse cleared of pasta, the time taken to make 100 reach attempts (to a maximum of 15 min.), and the number of days until the gradual learning phase was reached. A total of

26 young mice (n=15 short duration, n=9 long duration) and 13 aged mice (n=8 short duration, n=5 long duration) were trained on this task. Data are presented in three day blocks of training to simplify the presentation.

To control for effects of training environment across conditions, daily pasta handling and control procedures consisted of animals being placed into reaching chambers without a pasta matrix in front of the reaching aperture. At the same time, another mouse was being trained on the Pasta Matrix Reaching Task. Pasta handling and untrained control animals were yoked to this reach trained mouse and remained in the testing chamber for the same amount of time. For the pasta handling condition, mice received a number of pasta pieces equivalent in length and numbers to those retrieved by the reach trained mouse to which they were yoked. Untrained controls also received the same amount of pasta as the reach trained mouse, but the pieces were broken into bits too small to handle (pasta “niblets”), which mice retrieved from the cage floor with the mouth and tongue. A total of 19 young mice (n=8 short duration, n=11 long duration) and 15 aged mice (n=9 short duration, n=6 long duration) received daily pasta handling and a total of 16 young mice (n=8 short duration, n=8 long duration) and 13 aged mice (n=8 short duration, n=5 long duration) served as untrained controls. No data on pasta handling was collected during daily training sessions, but a more detailed analysis of pasta handling behavior, the Capellini Handling Test, was conducted on the subset of mice tested on the sensorimotor battery.

2.4. Other sensorimotor tasks

To probe aging effects on sensorimotor function, a subset of mice in the short-duration training conditions (n=8 young, 7 aged) were additionally tested at once-weekly intervals on the Ladder Rung Walking Test (Farr et al., 2006; Metz and Whishaw, 2002; Tennant and Jones, 2009), the Bilateral Tactile Stimulation Test (Schallert et al., 1982; Schallert and Whishaw, 1984; Tennant et al., 2009), and the Capellini Handling Test (Allred et al., 2008; Tennant et al., 2010; Xu et al., 2009). Animals were tested on multiple occasions to capture performance across the time span of the reach training period. Additionally, because practice on the Capellini Handling Test has previously been shown to elicit plasticity in the motor cortex (Xu et al., 2009), only animals from pasta handling and reach training conditions were tested on the sensorimotor battery so that these groups were similarly practiced in pasta handling and any additional learning effects would not confound the ICMS results of control animals. The limited testing on this sensorimotor battery had no discernible influence on the organization of motor representations, as determined by comparisons within training conditions of mice that did or did not receive the test battery (t statistics= -1.02 - 0.40 , p -values= 0.17 - 0.65).

2.4.1. Capellini Handling Test—Mice were placed into a Plexiglas cylinder on top of a front-surface mirror, and were videotaped while eating 3-4 pieces of 2.6 cm long uncooked capellini pasta at an angle that permitted view of both paws in the mirror. Slow-motion video analysis was used to measure the total time to eat each pasta piece and the number of adjustments made with each paw. Mice eat the pasta by moving the pasta piece into the mouth with the paws, which are typically used in an asymmetrical manner with one paw placed near the mouth (the guide limb) and the other holding the pasta further down the piece in a full-paw grasp (the grasp limb; Tennant et al., 2010). An adjustment was counted each time the paw or digits released and regripped the pasta piece. Particular attention was paid to the number of “superfluous adjustments”, defined as adjustments that did not result in advancement of the pasta piece into the mouse. Often, these appear as repeated fluttering movements of the digits, a behavior that increases during aging (Tennant et al., 2010). Other atypical eating patterns that were counted included dropping the pasta piece during eating, keeping the paws together when the pasta piece is long, or apart when the pasta piece is

short, switching the positions of the guide and grasp limbs, and failing to contact the pasta with one paw. Atypical eating patterns are described in more detail by Allred et al. (2008) and Tennant et al. (2010). The mean time to eat, mean number of adjustments with each paw and mean incidence of atypical behaviors were calculated by averaging across trials.

2.4.2. Ladder Rung Test—This task measures coordinated forelimb use during locomotion (Farr et al., 2006; Tennant and Jones, 2009). Mice were videotaped while walking across an elevated horizontally oriented ladder. The home cage was placed at one end of the ladder and the mouse was placed at the other end and allowed to walk across the ladder and into the home cage. Three ladder crossings per testing day were videotaped. Slow-motion video analysis was used to count incidences of forepaw slips between or off a rung (errors) and the total number of steps with each paw. The number of errors per crossing, the total number of steps, the number of errors per step, and the average step length (in rungs) were calculated.

2.4.3. Bilateral Tactile Stimulation Test—This task measures sensorimotor asymmetries in paw use (Schallert et al., 1982; Schallert & Whishaw, 1984; Tennant and Jones, 2009). The mouse was placed into a shallow transparent plastic container with an open top and allowed to habituate for 1 min. The mouse was picked up and lightly restrained by the scruff while a ~1.25 cm long piece of 3 mm wide tape (crepe art tape, Office Depot, Delray Beach, FL) was placed onto the ventral side of each forepaw. The mouse was then placed back into the container and allowed to remove each piece of tape using its mouth. The latency to contact and remove each piece of tape was recorded for five trials per day, allowing 30 seconds of rest between each trial. The removal time for each stimulus was calculated by subtracting the contact time from the total removal time in order to separate the motor component from the sensory component of the test.

2.5. Intracortical microstimulation (ICMS) mapping

Detailed methods of the ICMS procedure are described in Tennant et al. (2011). Animals were anesthetized with an initial cocktail of ketamine (150 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) that was supplemented with additional ketamine to maintain an anesthetic plane suitable for ICMS. Isoflurane (0.5-1 % in oxygen) was used if necessary to bridge between ketamine boosters. The mouse was placed into a mouse stereotaxic frame (Stoelting, Wood Dale, IL), lidocaine (2 mg/kg, s.c.) was injected into the scalp, and a midline incision was made. The cisterna magna was punctured to drain cerebrospinal fluid (which reduces cortical upwelling), and the skull and dura overlying the motor cortex were removed. The craniotomy was then filled with warm (37° C) silicone oil to prevent drying. Using a digital surgical microscope, a picture of the cortical surface was taken and overlaid in Canvas software with a grid consisting of 250 × 250 μm grid intersection spacing. Sites of stimulation were at grid intersections with the exception of minor adjustments in placement needed to avoid penetrating surface vasculature. Intracortical penetrations with a glass microelectrode (tip diameter of 20-25 μm) with a platinum wire were made using a hydraulic micropositioner at a depth of 790-800 μm (corresponding to deep layer V). Penetrations were made throughout the cortex until the entire extent of the forelimb representation was resolved, including the caudal and rostral aspects of the forelimb motor map. At each site, a 40 ms train of 13 200 μs monophasic cathodal pulses was delivered at 350 Hz from an electrically isolated, constant current stimulator (BAK Electronics, Mount Airy, MD) at a rate of 1 Hz. Stimulation began at 0 μA and was rapidly increased until a visible movement was evoked, up to a maximum of 60 μA. Following the standard method of determining movement thresholds in primates and rodent ICMS studies (e.g. Kleim and Nudo, 1998; Teskey et al., 2007; Young et al., 2011; Tandon et al., 2008), if a movement was evoked at or below 60 μA, the threshold current was determined by gradually

decreasing the stimulation until the movement stopped. The lowest amount of stimulation required to evoke movement was determined to be the threshold current. If no movement was seen at 60 μ A, the site was considered non-responsive. In addition to facilitating comparisons across studies, the purpose of this method is to minimize the current delivery, in time and intensity, needed to determine whether a movement is present at a given site. When stimulation at a site produces movements of two or more different forelimb joints and/or other body parts, it also permits quick determination of the movement that is elicited at the lowest stimulation current. The site is considered responsive to the movement elicited at the lowest threshold. An approach that minimizes current delivery is important for high resolution ICMS because excess stimulation can alter borders of movement representations (Nudo et al., 1990). Electrode penetrations were made in a systematic manner across the cortex until the entire extent of the caudal and rostral aspects of the forelimb map were resolved. At the end of the ICMS procedure, animals were euthanized with an overdose of sodium pentobarbital (175 mg/kg, i.p.). The areal extent of individual movement representations, distal representations (digit and wrist) and proximal representations (elbow) were calculated by multiplying the number of stimulation sites producing a given movement by the area of a grid square (0.0625 mm²).

In mice, movement representations of the shoulder tend to show a distinct aggregation, usually at the caudal border of the CFA, in contrast to digit, wrist and elbow representations which intermingle in CFA (Tennant et al., 2011a). In animals trained for a short duration, shoulder was not included as part of the forelimb during ICMS experiments and was not mapped in entirety. However, because the shoulder area may be activated during reaching, we began to map the shoulder representation in its entirety for the long-duration trained animals. To enable comparisons across training conditions, shoulder representations were omitted from all analyses in the current study. However, relative proportions of movement subtypes can be found in Supp. Table 1 (exclusive of shoulder) and Supp. Table 2 (inclusive of shoulder for long duration trained groups only).

2.6. Statistics

All statistical analyses were conducted using SPSS software. To test for age and training condition effects in performance over time on the Pasta Matrix Reaching Task, we performed repeated-measures analyses of variance (ANOVAs). Age by training day effects were tested in two analyses: one inclusive of all training conditions was performed for training days 1-14 (i.e., the short duration training period). A separate ANOVA inclusive of long duration groups was performed for training days 15-60 (the long duration training period). Two non-repeating reaching performance variables were also analyzed: the number of days until the gradual learning phase and the final success level. Two-tailed t-tests were performed to determine the effect of age on days until the gradual learning phase and the effect of duration on final success level between age-matched groups. Two-tailed t-tests were also used to compare performance between young and aged animals on the Ladder Rung Task, the Bilateral Tactile Stimulation Task, and the Capellini Handling Task, with performance averaged over time to simplify the analysis. Correlations were conducted to analyze relationships between the ratio of proximal to distal forelimb representations and skilled reaching success or stimulation thresholds.

To test for age effects on the baseline organization of the forelimb representation, t-tests and chi-square analyses were conducted to compare aspects of the CFA and RFA of young and aged mice. To test for training condition effects on the organization of forelimb representations, we conducted *a priori* planned comparisons on ICMS data collected from the CFA and RFA. Planned comparisons were designed to test whether the ratio of proximal to distal forelimb representations was affected by 1) short duration reach training (controls vs. reachers) or 2) continued reaching practice for a long duration (short duration vs. long

duration; controls vs. long duration). Each of these comparisons was conducted separately for young and aged animals. To determine if pasta handling experience had an effect on forelimb motor cortical representations, we conducted an ANOVA comparing all pasta handlers (short and long duration) vs. all controls. One-way ANOVAs were used to compare differences in movement thresholds between training conditions and a bivariate correlation was used to probe the relationship of movement thresholds to the ratio of proximal to distal forelimb representations. Supplementary and post-hoc analyses used Bonferroni-corrected *t*-tests to further characterize group differences.

3. Results

3.1. Effect of age on acquisition and performance of motor tasks

3.1.1. Pasta Matrix Reaching Task—To determine how the motor map reorganizes in response to motor skill learning and extended practice throughout the adult lifespan, we trained young and aged mice daily on a skilled reaching task, altering the total number of days of training. The Pasta Matrix Reaching task was chosen because it involves the development of novel, skillful movement sequences of the forelimb. In order to successfully perform the task, mice learn to break and retrieve small pieces of vertically oriented dry capellini pasta arranged in a matrix placed directly outside of a slit in a Plexiglas reaching chamber. The performance of this task over time was characterized by an initial rapid learning phase, in which there were substantial increases in successful retrievals in each successive block of training, followed by a gradual learning phase in which improvements across successive training blocks were more subtle (Fig. 1A,B). The first time point in which there was minimal change (slope < 0.2) in successful retrievals relative to the preceding training block was defined as the onset of the gradual learning phase.

We found that young and aged mice had similar rates of learning as defined by reaching success over days of training (Fig. 1B), the number of days until the gradual learning phase (Fig. 1C), and the final success level following short duration training (Fig. 1D). Young and aged mice reached the rapid-to-gradual learning transition point at approximately the same time, with young mice taking an average of 11 ± 1 days to reach the transition point compared to 12 ± 1 days for aged mice ($t(43) = 0.25$, $p = 0.80$). While young and aged animals initially had similar rates of learning, aged animals surpassed the success rate of young animals with ongoing training (Fig. 1B). During the short duration portion of training, there was a significant main effect of day due to the increased success as training progressed ($F(13,546) = 45.61$, $p < 0.001$), but no main effect of age ($F(1,42) = 1.78$, $p = 0.19$) or age by training day interaction ($F(13,546) = 1.20$, $p = 0.28$). In the long duration training period, there was no main effect of age ($F(1,14) = 1.33$, $p = 0.27$), but there was a significant age by training day interaction ($F(44,616) = 1.83$, $p = 0.001$), reflecting that the aged mice surpassed the young mice in the later stages of training. Aged mice in the long duration training condition also performed at a much greater success rate at the end of the training period than aged animals in the short duration training condition ($t(16) = -5.68$, $p < 0.001$; Fig. 1D), whereas the performance of young mice did not significantly differ between the time points ($t(25) = -1.92$, $p = 0.07$). These results indicate that aged animals were able to successfully learn and perform the skilled reaching task as well as young animals, and increasing the duration of training led to additional performance gains in aged mice.

3.1.2. Capellini Handling Test—Successful performance of the Pasta Matrix Reaching Task involves the ability to dexterously manipulate retrieved pasta pieces during eating. The Capellini Handling Test, a test of coordinated dexterous forepaw use during pasta eating, allows measurement of skillful adjustments of the forepaws and atypical eating patterns (Tennant et al., 2010). This test was used in a subset of mice to probe for age differences in

forepaw dexterity. When all types of forepaw adjustments were considered together (Fig. 2A), aged mice had a greater number of adjustments during pasta handling than young mice ($t(13)=-5.12$, $p<0.001$). Though a greater number of adjustments can indicate greater dexterity (Allred et al., 2008), further analysis showed that aged mice make more adjustments that do not result in the advancement of the pasta piece into the mouth (“superfluous adjustments”, $t(13)=-5.80$, $p<0.001$; Tennant et al., 2010). However, even when these superfluous adjustments are subtracted from the total number of adjustments, aged mice still made more adjustments when compared to young mice ($t(13)=-2.81$, $p=0.02$). Aged mice also took significantly longer to consume each pasta piece ($t(13)=-8.35$, $p<0.001$; Fig. 2B) and to make each adjustment ($t(13)=-2.24$, $p=0.04$; Fig. 2C). Analysis of atypical handling behaviors (Fig. 2D) showed that aged mice drop the pasta piece during eating significantly more often than young mice ($t(13)=-1.65$, $p=0.03$). (Drop time is not counted in total eating time.) However, aged and young mice did not differ significantly in the incidence of other atypical handling behaviors that were measured (paws together when long, paws apart when short, guide and grasp limb switch, and failure to contact with one limb). These other atypical behaviors had a low incidence (< 0.5 times per piece, on average) in both young and aged mice, and there were no age differences in the frequency of their occurrence.

3.1.3. Other sensorimotor tests—On the Ladder Rung Walking Test, a test of coordinated paw use during locomotion, aged mice were significantly slower in the time taken to cross a horizontal ladder ($t(13)=-3.57$, $p=0.003$; Table 1). Their number of errors per step failed to reach significance compared with younger mice ($t(45)=-9.25$, $p=0.06$; Table 1). Aged animals took more steps while crossing the ladder ($t(46)=-2.44$, $p=0.02$), but the mean step length of young mice (6.35 ± 0.06 , measured in number of ladder rungs crossed) was not significantly different from that of aged mice (5.73 ± 0.06 ; $t(13)=1.72$, $p=0.11$).

In the Bilateral Tactile Stimulation Test, small pieces of tape were placed on the ventral surface of both paws and removed by the mouse. Aged mice showed a significant delay in the time taken to contact either stimulus compared with younger mice ($t(478)=-3.57$, $p=0.0003$; Table 1). Age-dependent differences in the amount of time to completely remove the stimuli did not reach significance ($t(476)=-1.72$, $p=0.09$; Table 1).

3.2. Effect of age on the organization of the forelimb area of motor cortex

The forelimb motor map of young adult mice is composed of a large caudal forelimb area (CFA) and a smaller rostral forelimb area (RFA), similar to that of the rat, with the notable exception of the much larger relative area of digit representations in the CFA of mice (Tennant et al., 2011a). In order to assess aging effects separately from reach training effects, we only compared young and aged mice that had no reach training: control ($n=16$ young and $n=13$ aged) and pasta handling ($n=19$ young and $n=15$ aged) groups were combined for the analysis because there were no significant differences in the areal extent of elbow, wrist, or digit representations between them (young CFA: t ’s= -1.89 - 1.86 , p ’s= 0.07 - 0.60 ; aged CFA: t ’s= -2.20 - 1.25 , p ’s= 0.06 - 0.57 ; young RFA: t ’s= -0.79 - 1.79 , p ’s= 0.09 - 0.44 ; aged RFA: t ’s= -0.55 - 0.58 , p ’s= 0.57 - 0.59).

There were no age-related differences in the absolute area of the CFA (young: 1.49 ± 0.08 mm²; aged: 1.48 ± 0.12 mm²), but there were some differences in its internal organization. Aged mice had a significantly smaller area of the CFA composed of digit representation compared to young mice ($t(62)=2.47$, $p=0.02$; Fig. 3A). There were no significant differences in the size of the elbow ($t(62)=-1.39$, $p=0.17$) or wrist ($t(62)=-1.22$, $p=0.23$) representations. Aged mice tended to have a slightly larger ratio of proximal (elbow) to

distal (digit and wrist) forelimb representation in the CFA (young: 0.44 ± 0.06 ; aged: 0.61 ± 0.08), but this difference failed to reach statistical significance ($t(62)=-1.71$, $p=0.09$).

The smaller RFA of the mouse motor cortex is not always discernible (Tennant et al., 2011a), and thus the analysis of RFA maps included fewer animals than that of the CFA. The percentage of animals with a discernible RFA was similar between age groups (75 % vs. 69 %, aged vs. young; $\chi^2(1, N=49) = 0.24$, $p = 0.62$). Among mice with an evident RFA, the RFA of aged animals was significantly larger in area than that of young animals (0.24 ± 0.03 vs. 0.16 ± 0.02 mm², respectively; $t(44)=-1.97$, $p=0.05$). This size difference was due in part to the significantly larger digit representation in the aged RFA compared to the young RFA ($t(44)=-2.29$, $p=0.03$; Fig. 3B). This is in contrast to the digit representations in the CFA which were larger in young mice compared to aged mice. The size of the elbow representation in RFA was similar between age groups ($t(44)=-0.19$, $p=0.85$). The size of the wrist representation in RFA was not significantly different between age groups ($t(44)=-1.41$, $p=0.17$).

3.3. Effect of behavioral training on the forelimb motor maps of young and aged mice

Previous research has shown that, following motor skill training, the overall map area can remain unchanged while there is internal reorganization of the movement subtypes (e.g., enlargement of distal at the expense of proximal forelimb representations; Kleim et al., 1998). An increase in the representation area of one movement type typically accompanies a decrease in area of one or more other movement types. In rats, areas that are smallest at baseline (i.e. distal forelimb representations) increase at the expense of the areas that are largest at baseline (i.e. proximal forelimb representations; Kleim et al., 1998). As a result, ratios of proximal to distal forelimb representations have been useful for quantifying reorganization of movement representations during motor learning (Kleim et al., 1998). Because we have previously shown that mice typically have a small elbow representation at baseline (Tennant et al., 2011a) and based on preliminary results, we hypothesized that the elbow representation would expand at the expense of distal representations. Thus, we quantified ratios of proximal to distal movements.

There were no significant differences between training groups in the relative proportions of individual or total forelimb representations in the CFA of young or aged mice (Supp. Tables 1 and 2). In young mice, motor cortical maps examined after a short duration of training were found to have a significantly higher ratio of proximal to distal forelimb representations compared to controls ($F(1,22)=4.59$, $p=0.04$, short duration vs. controls; Fig. 4A). After a longer duration of training, proximal to distal ratios tended to decrease in comparison to those found after a short duration of training ($F(1,18)=3.99$, $p=0.06$, short duration vs. long duration) to levels that were not significantly different from controls ($F(1,18)=0.66$, $p=0.43$, controls vs. long duration; Fig. 4A). The size of the proximal to distal ratio did not correlate with the final success level in short or long duration trained young mice ($r(13)=0.34$, $p=0.22$ and $r(9)=0.56$, $p=0.12$, respectively). Pasta handling alone had no effect on the proximal to distal ratio compared to controls ($F(1,35)=0.65$, $p=0.43$).

In contrast to the effects found in young mice, short duration reach training in aged mice had no significant effect on proximal to distal ratios ($F(1,15)=0.03$, $p=0.87$, short duration vs. controls; Fig. 4B) and ratios were similar in mice with a long and short duration of training ($F(1,12)=0.34$, $p=0.57$). The size of the proximal to distal ratio tended to correlate with the final success level in short or long duration trained aged mice, but did not reach significance ($r(8)=0.69$, $p=0.06$ and $r(3)=0.77$, $p=0.07$, respectively). Pasta Handling had no effect on proximal to distal forelimb ratios compared to controls ($F(1,27)=0.10$, $p=0.75$).

The effects of reach training in CFA tended to be mirrored in RFA (Supp. Table 3), but there were no significant differences in comparisons between training conditions.

3.4. Movement thresholds

Movement thresholds were unaffected by training condition in the short duration period. However, after a longer duration of reach training in young mice, the movement thresholds of distal movements were lower than those of short duration trained mice. That is, at a time when map proportions appear to return to control levels, reach training effects were apparent in the ability to elicit wrist and digit movements at lower stimulation intensities (Fig. 5; see Supp. Table 4 for mean threshold values). In young mice, there was a significant effect of training condition on movement thresholds of the wrist ($F(2,25)=12.00$, $p<0.001$) and digit ($F(2,26)=7.71$, $p=0.002$), but no effect on elbow movement thresholds ($F(2,23)=2.57$, $p=0.10$). The difference in distal (wrist and digit) movement thresholds of long duration vs. short duration reach trained mice was significantly greater than in controls (wrist: $t(16)=2.82$, $p=0.01$; digit: $t(17)=3.22$, $p=0.005$; Fig. 5). Additionally, mice that received long duration *pasta handling* practice had lower thresholds for eliciting digit movements than short duration pasta handlers ($t(18)=2.93$, $p=0.009$). In young mice, the ratio of proximal to distal representations was positively correlated with movement thresholds for the wrist ($r(51)=0.28$, $p=0.03$) and digit ($r(45)=0.28$, $p=0.03$), but not the elbow ($r(47)=-0.04$, $p=0.78$). That is, larger relative areas of distal representations were associated with lower thresholds for these movements.

In aged mice, there were no significant effects of training duration on movement thresholds of the elbow ($F(2,13)=0.71$, $p=0.51$), wrist ($F(2,13)=1.08$, $p=0.37$), or digit ($F(2,13)=1.83$, $p=0.20$) in the CFA. Average thresholds for each forelimb movement type (elbow, wrist, digit) did not significantly differ between young and aged mice (t statistics= -0.20 - 1.61 , p -values= 0.11 - 0.84) and there were no correlations between movement thresholds and ratios of proximal to distal forelimb representations in the aged motor cortex (r 's= -0.14 - 0.11 , p 's= 0.42 - 0.81).

4. Discussion

We found that training young adult, but not aged, mice on a novel skilled reaching task is associated with the reorganization of movement representations in motor cortex. Continued practice beyond what was necessary to initially learn the task resulted in reductions in movement thresholds, but the reorganization of movement representations was not maintained. Though training-related map reorganization was not detected in aged mice, there was no decrement in the ability of aged mice to acquire the reaching skill, and in fact, aged mice eventually surpassed performance levels of young mice. These results suggest that plasticity of motor map topography is likely to reflect a transient stage of the cortical changes that accompany skill learning in young adults, and unlikely to reflect an essential mechanism for skill learning in aged brains.

4.1. Transience of motor map reorganization despite extended skill practice

A novel finding of this study is that, in young adult mice, motor map reorganization reverts to baseline even with ongoing practice of an established skill. A previous study by Molina-Luna et al. (2008) showed a transient enlargement of the rat motor cortex in response to motor learning that returned to baseline after an 8 day period of no training, though the ability to perform the skill was retained. The current study expands on these results, confirming that a short duration of reach training resulted in reorganization of forelimb representations, and additionally showing that even when mice were allowed continuous daily practice in reaching over a longer period of time, the motor map organization still

returned to baseline. Taken together, both Molina-Luna et al. (2008) and the current study suggest that motor map reorganization is necessary for acquisition of a skill, but that the maintenance of the reorganized state is not necessary for maintenance of the skill.

There are at least two possibilities for why the topographical reorganization of motor cortex is transient. The first is that after a longer duration of training, the reorganized region of motor cortex is no longer required for performance of the task and the map resets to a baseline organization. However, this possibility conflicts with studies that have shown loss of motor skill following lesions of the sensorimotor cortex (Adkins et al., 2004; Gharbawie et al., 2007; Kleim et al., 2003) as well as findings of the long-term maintenance of new spines generated early in learning (Xu et al., 2009). A more likely possibility is that ICMS is revealing a transient stage in the long-term reorganization of the motor cortex that subserves the maintenance of the skill. Auditory cortical representations undergo a similar transient reorganization during perceptual training (Reed et al., 2011; Takahashi et al., 2011). In addition, a similar phenomenon is detected in human motor cortex during motor learning with transcranial magnetic stimulation (TMS) mapping. Motor skill practice results in reorganization of the motor cortex that resets to baseline following a period without practice, but reappears when practice is reinstated (Classen et al., 1998; Pascual-Leone et al., 1995). Furthermore, areas that are active during the early stage of learning differ from those active at later stages of learning, as detected with fMRI (Floyer-Lea and Matthews, 2005; Shadmehr and Holcomb, 1997; Tracy et al., 2003). Long-term, highly skilled practice, like that of professional musicians, has been shown to be associated with a decrease in activation of motor areas compared to amateur musicians (Jäncke et al., 2000; Krings et al., 2000). Together with these findings, the present results suggest that while motor cortex reorganization is a characteristic of early stages of motor skill learning, later stages of learning are likely to rely on other processes within the motor cortex, as well as elsewhere in the brain.

Skilled learning-induced plasticity in the forelimb movement representation is thought to reflect the recruitment of neurons in the cortical territory devoted to producing novel movement sequences necessary for performing a newly learned motor skill (Keller, 1993). The interconnected nature of the motor representation is thought to provide the flexibility necessary to modify the existing network to accommodate this behavioral change (Sanes and Donoghue, 2000). In support of this, the representations of proximal and distal movements partially overlap within the forelimb representation area as well as with body representations that border the forelimb area (Tennant et al., 2011a). The representation areas within the motor cortex are interconnected via layer II/III cortico-cortico connections, and plasticity of these connections is a proposed mechanism of motor map reorganization (Riout-Pedotti et al., 2007).

The process underlying reorganization of the motor map may be partially mediated by an LTP-like mechanism. Motor skill training results in a long-term, but ultimately also transient, increase in the synaptic modification range that allows for additional LTP in the motor cortex (Monfils and Teskey, 2004; Riout-Pedotti et al., 2007). This potentiation, along with a transient net increase in spines seen during the early stages of motor skill learning (Xu et al., 2009), may result in a shift in the ability to elicit trained movements with ICMS stimulation. There may be a prolonged time period in which the processes of synaptic pruning and potentiation are still ongoing and the resetting of the motor map may coincide with the full establishment of the circuitry underlying the ability to continue to perform the skill proficiently. That is, the reset map organization is unlikely to indicate a reversal of the plasticity, and it very well may indicate that the plastic changes are being more permanently incorporated into the motor cortical network, possibly through both the potentiation and stabilization of new spines and synapses. For example, Kleim et al. (2002) showed that the

number of synapses per neuron increased only in areas of the motor cortex that had reorganized in rats that were trained on a skilled reaching task.

Our results show that although the training-induced reorganization of the CFA in young animals was not fully maintained after the short duration period, movements of the wrist and digit became easier to induce. It is possible that lower movement thresholds are associated with increased synaptic efficacy (Monfils et al., 2005), and that the lower thresholds seen after extended training are due to an area-specific lasting synaptic potentiation that is maintained after the map has returned to control levels. It is interesting to note that Molina-Luna et al. (2008) found no difference in movement thresholds measured immediately after a period of training and those measured after an additional 8 day period without training. Thus, while ongoing practice did not result in the maintenance of map reorganization, the decrease in movement thresholds is suggestive of synaptic change that endures longer than motor map reorganization, at least when a skill is continually practiced. This is supported by studies of professional musicians that show less activation of primary motor areas compared to amateurs (Jäncke et al., 2000; Krings et al., 2000), suggesting that when a task has been extensively practiced, fewer neurons within the motor cortex need to be active in order to produce the same movement.

It is possible that the standard ICMS parameters used in the present study, and by Molina-Luna et al., (2008), were simply insensitive to long-term changes in motor map organization. In rats, a different form of ICMS from the one used in this study, long-duration ICMS, produces complex, multi-joint movements which approximate reaching and grasping movements (Ramanathan et al., 2006). Reorganization of complex movement representations was seen following focal electrolytic lesions of the CFA and rehabilitative training, but not following short duration motor skill training in intact animals. It is possible that following long-term practice on a skilled motor task, the organization of complex movement representations may differ between animals trained for long and short durations. Long-duration ICMS may also be useful for further probing the role of the RFA in motor learning in future studies. The small size of the RFA may not allow for large-scale reorganization, but it may be possible to detect more subtle changes in RFA reorganization within complex movement representations.

4.2. Age-related dissociation of motor skill learning and motor map plasticity

Our results suggest that while the ability to learn and perform skilled motor tasks is retained in aged animals, there is a dissociation between behavioral performance and cortical reorganization. Aged mice were able to learn a new motor skill in a similar time frame and with a similar success rate to young mice. With extended training, the performance of aged mice even surpassed that of young mice. However, in contrast to young mice, skill acquisition was uncoupled with a detectable reorganization of forelimb movement representations in the motor cortex of aged mice. At baseline, the aged mouse motor cortex also had significantly less cortical area in the CFA dedicated to digit movements compared to young mice, and there were age-related decrements evident in the sensorimotor test battery. In the forelimb somatosensory cortex of rats, an age-related loss of map complexity is related to impairments in walking behavior (Spengler et al., 1995). Because the areal extent of a motor cortical representation tends to be related to the dexterity of that particular body part (Monfils et al., 2005), the loss of digit representation during aging may reflect a loss of dexterity in the digits. This could lead to the presumption that aged mice would have a more difficult time learning fine motor skills with the forelimbs due to the loss of dexterity, but their adept acquisition of a challenging reaching task in the present study fails to support this.

The possibility that the primary motor cortex is simply uninvolved in this type of motor skill learning in aged animals seems to be contradicted by findings that lesions of this area impair reaching performance in aged mice (Tennant et al., 2011b). It seems more likely that its involvement is simply not reflected in the reorganization of movement representations as detected using the ICMS methods of this study. This hardly rules out that there is an age-related recruitment of other brain areas for the acquisition of motor skills. In the human motor system, the aged brain is able to compensate for the slowed processing capabilities of primary motor areas by recruiting additional areas of the brain, including contralateral motor areas and ipsilateral sensory and cognitive areas, during performance of a motor task (Heuninckx et al., 2008). This is thought to enable most aged individuals to perform at the same level as their young counterparts during interlimb coordination tasks. Tasks requiring a greater degree of dexterity, such as single digit or nondominant hand use, are associated with greater recruitment of additional brain areas (Hutchinson et al., 2002).

Although older adults may be able to perform tasks with similar success levels to younger adults, their movements tend to be slower, especially on skilled tasks utilizing fine movements of the hand and digits, further evidence that compensatory strategies may be in place to allow for better motor performance (Smith et al., 1999). Consistent with this, analysis of dexterous forepaw use during pasta handling showed that aged mice eat more slowly and make fewer forepaw adjustments per second, compared to young mice. The handling strategy also differs between young and aged mice. Aged mice made more adjustments per pasta piece than young, but many of these adjustments failed to efficiently advance the pasta piece into the mouth, whereas young mice efficiently advance the pasta into the mouth with relatively few adjustments.

Another age difference was that the RFA was larger overall and had a significantly larger digit representation in aged mice compared with young mice. Aspects of the anatomical organization of the rodent RFA resemble both the primate premotor and supplementary motor areas (Jurgens, 1984; Matelli et al., 1986; Neafsey and Sievert, 1982), as well as the supplementary motor area of the porcupine (Lende and Woolsey, 1956). The RFA, like premotor and supplementary motor areas, may play a role in modulating forelimb motor function. Damage to the premotor cortex in humans results in deficits in learning and performing sequential motor movements (Dovern et al., 2011) and ischemic lesions of the RFA in rats have been shown to produce slight, transient deficits in skilled reaching ability (Gharbawie et al., 2007). Thus, although aged mice have less cortical area in the CFA corresponding to digit movements, the increased digit representation in the RFA may allow for better motor planning and performance of sequential motor movements, which could potentially compensate for a loss of digit dexterity. If so, because no training-related changes were found in RFA, this involvement must not require a major reorganization of its topographical representation of movement.

4.3. Summary and remaining questions

The current study shows that training-induced motor map reorganization is transient even in conditions of continued task practice in the young brain and that motor map plasticity is altered in aging. In healthy aged mice, the decline in motor function on some sensorimotor tasks was not reflected in the ability to learn a new motor skill, but we failed to see motor cortical reorganization following skill learning. This suggests that motor skill learning, motor performance, and motor cortical plasticity must be at least somewhat dissociable. Given the transience of the topographical reorganization and the delayed change in movement thresholds in young adults, and the differences in learning time courses of young and aged animals, it is impossible to rule out that our failure to see reorganization of the aged motor map was due to mapping after the changes had already occurred, or that they were still to come. Alternatively, given that motor learning in older humans involves more

non-primary motor and non-motor areas compared to young adults, as discussed above, as well as evidence from the human literature showing dysfunction of the aged primary motor cortex (Inuggi et al., 2011; Siedler et al., 2010; Todd et al., 2010), it could be that the motor cortex does not play as large a role in motor learning in aged animals as it does in young animals. Other brain areas that are candidates for motor skill learning-induced plasticity may include the somatosensory cortex, striatum, thalamus, internal capsule, cerebellum, spinal cord, and cognitive areas. It remains to be determined what neural changes underlie the resetting of motor map organization, whether learning an additional motor task would accelerate its resetting and normalize distal forelimb movement thresholds, and what training parameters, if any, are sufficient to produce reorganization of forelimb representations in aged animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dr. Cam Teskey for comments on preliminary results, Dr. Tim Schallert for advice on behavioral testing, and Jorge Villafana for help with behavioral analysis. This work was supported by the National Institute of Neurological Disorders and Stroke at the National Institutes of Health (NS056839 to TAJ) and the National Institute on Aging at the National Institutes of Health (F31AG034032 to KAT).

References

- Adkins DL, Voorhies AC, Jones TA. Behavioral and neuroplastic effects of focal endothelin-1 induced sensorimotor cortex lesions. *Neuroscience*. 2004; 128:473–486. [PubMed: 15381277]
- Allred RP, Adkins DL, Woodlee MT, Husbands LC, Maldonado MM, Kane JR, Schallert T, Jones TA. The vermicelli handling test: a simple quantitative measure of dexterous forepaw function in rats. *J Neurosci Methods*. 2008; 170:229–244. [PubMed: 18325597]
- Ballermann M, Metz GA, McKenna JE, Klassen F, Whishaw IQ. The pasta matrix reaching task: a simple test for measuring skilled reaching distance, direction, and dexterity in rats. *J Neurosci Methods*. 2001; 106:39–45. [PubMed: 11248339]
- Carmeli E, Patish H, Coleman R. The aging hand. *J Gerontol A Biol Sci Med Sci*. 2003; 58:146–52. [PubMed: 12586852]
- Classen J, Liepert J, Wise SP, Hallett M, Cohen LG. Rapid plasticity of human cortical movement representation induced by practice. *J Neurophysiol*. 1998; 79:1117–23. [PubMed: 9463469]
- Coq JO, Xerri C. Age-related alteration of the forepaw representation in the rat primary somatosensory cortex. *Neuroscience*. 2000; 99:403–11. [PubMed: 11029533]
- Coq JO, Xerri C. Sensorimotor experience modulates age-dependent alterations of the forepaw representation in the rat primary somatosensory cortex. *Neuroscience*. 2001; 104:705–15. [PubMed: 11440803]
- David-Jürgens M, Churs L, Berkefeld T, Zepka RF, Dinse HR. Differential effects of aging on fore- and hindpaw maps of rat somatosensory cortex. *PLoS One*. 2008; 3:e2299. [PubMed: 18523551]
- Dovern A, Fink GR, Saliger J, Karbe H, Koch I, Weiss PH. Apraxia impairs intentional retrieval of incidentally acquired motor knowledge. *J Neurosci*. 2011; 31:8102–8108. [PubMed: 21632932]
- Elbert T, Pantev C, Wienbruch C, Rockstroh B, Taub E. Increased cortical representation of the fingers of the left hand in string players. *Science*. 1995; 270:305–307. [PubMed: 7569982]
- Farr TD, Liu L, Colwell KL, Whishaw IQ, Metz GA. Bilateral alteration in stepping pattern after unilateral motor cortex injury: a new test for analysis of skilled limb movements in neurological mouse models. *J Neurosci Methods*. 2006; 153:104–113. [PubMed: 16309746]
- Farr TD, Whishaw IQ. Quantitative and qualitative impairments in skilled reaching in the mouse (*Mus musculus*) after a focal motor cortex stroke. *Stroke*. 2002; 33:1869–1875. [PubMed: 12105368]

- Floyer-Lea A, Matthews PM. Distinguishable brain activation networks for short- and long-term motor skill learning. *J Neurophysiol.* 2005; 94:512–518. [PubMed: 15716371]
- Gaser C, Schlaug G. Brain structures differ between musicians and non-musicians. *J Neurosci.* 2003; 23:9240–9245. [PubMed: 14534258]
- Gharbawie OA, Karl JM, Whishaw IQ. Recovery of skilled reaching following motor cortex stroke: do residual corticofugal fibers mediate compensatory recovery? *Eur J Neurosci.* 2007; 26:3309–3327. [PubMed: 18028116]
- Gholamrezaei G, Whishaw IQ. Individual differences in skilled reaching for food related to increased number of gestures: evidence for goal and habit learning of skilled reaching. *Behav Neurosci.* 2009; 123:863–874. [PubMed: 19634947]
- Heuninckx S, Wenderoth N, Debaere F, Peeters R, Swinnen SP. Neural basis of aging: the penetration of cognition into action control. *J Neurosci.* 2005; 25:6787–6796. [PubMed: 16033888]
- Heuninckx S, Wenderoth N, Swinnen SP. Systems neuroplasticity in the aging brain: recruiting additional neural resources for successful motor performance in elderly persons. *J Neurosci.* 2008; 28:91–9. [PubMed: 18171926]
- Hutchinson S, Kobayashi M, Horkan CM, Pascual-Leone A, Alexander MP, Schlaug G. Age-related differences in movement representation. *Neuroimage.* 2002; 17:1720–8. [PubMed: 12498746]
- Incel NA, Sezgin M, As I, Cimen OB, Sahin G. The geriatric hand: correlation of hand-muscle function and activity restriction in elderly. *Int J Rehabil Res.* 2009; 32:213–8. [PubMed: 19293723]
- Inuggi A, Amato N, Magnani G, Gonzalez-Rosa JJ, Chieffo R, Comi G, Leocani L. Cortical control of unilateral simple movement in healthy aging. *Neurobiol Aging.* 2011; 32:524–538. [PubMed: 19327869]
- Jäncke L, Shah NJ, Peters M. Cortical activations in primary and secondary motor areas for complex bimanual movements in professional pianists. *Cog Brain Res.* 2000; 10:177–183.
- Jurgens U. The efferent and afferent connections of the supplementary motor area. *Brain Res.* 1984; 300:63–81. [PubMed: 6733468]
- Keller A. Intrinsic synaptic organization of the motor cortex. *Cereb Cortex.* 1993; 3:430–441. [PubMed: 8260811]
- Kleim JA, Barbay S, Cooper NR, Hogg TM, Reidel CN, Remple MS, Nudo RJ. Motor learning-dependent synaptogenesis is localized to functionally reorganized motor cortex. *Neurobiol Learn Mem.* 2002; 77:63–77. [PubMed: 11749086]
- Kleim JA, Barbay S, Nudo RJ. Functional reorganization of the rat motor cortex following motor skill learning. *J Neurophysiol.* 1998; 80:3321–3325. [PubMed: 9862925]
- Kleim JA, Bruneau R, Calder K, Pocock D, VandenBerg PM, MacDonald E, Monfils MH, Sutherland RJ, Nadar K. Functional organization of adult motor cortex is dependent upon continued protein synthesis. *Neuron.* 2003; 40:167–176. [PubMed: 14527441]
- Kleim JA, Nudo RJ. Functional reorganization of the rat motor cortex following motor skill learning. *J Neurophysiol.* 1998; 80:3321–5. [PubMed: 9862925]
- Kluger A, Gianutsos JG, Golomb J, Ferris SH, Reisberg B. Motor/psychomotor dysfunction in normal aging, mild cognitive decline, and early Alzheimer's disease: diagnostic and differential diagnostic features. *Int Psychogeriatr.* 1997; 9(Suppl 1):307–316. [PubMed: 9447451]
- Krings T, Töpper R, Foltys H, Erberich S, Sparing R, Willmes K, Thron A. Cortical activation patterns during complex motor tasks in piano players and control subjects. A functional magnetic resonance imaging study. *Neurosci Lett.* 2000; 278:189–193. [PubMed: 10653025]
- Lende RA, Woolsey CN. Sensory and motor localization in cerebral cortex of porcupine (*Erethizon dorsatum*). *J Neurophysiol.* 1956; 19:544–563. [PubMed: 13377203]
- Matelli M, Camarda R, Glickstein M, Rizzolatti G. Afferent and efferent projections of the inferior area 6 in the macaque monkey. *J Comp Neurol.* 1986; 251:281–298. [PubMed: 3021823]
- Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *J Neurosci Methods.* 2002; 115:169–179. [PubMed: 11992668]
- Molina-Luna K, Hertler B, Buitrago MM, Luft AR. Motor learning transiently changes cortical somatotopy. *Neuroimage.* 2008; 40:1748–1754. [PubMed: 18329289]

- Monfils MH, Plautz EJ, Kleim JA. In search of the motor engram: motor map plasticity as a mechanism for encoding motor experience. *Neuroscientist*. 2005; 11:471–483. [PubMed: 16151047]
- Monfils MH, Teskey GC. Skilled-learning-induced potentiation in rat sensorimotor cortex: a transient form of behavioural long-term potentiation. *Neuroscience*. 2004; 125:329–336. [PubMed: 15062976]
- Neafsey EJ, Sievert C. A second forelimb motor area exists in rat frontal cortex. *Brain Res*. 1982; 232:151–6. [PubMed: 7055691]
- Pascual-Leone A, Cammarota A, Wassermann EM, Brasil-Neto JP, Cohen LG, Hallett M. Modulation of motor cortical outputs to the reading hand of Braille readers. *Ann Neurol*. 1993; 34:33–37. [PubMed: 8517678]
- Pascual-Leone A, Wassermann EM, Sadato N, Hallett M. The role of reading activity on the modulation of motor cortical outputs to the reading hand in Braille readers. *Ann Neurol*. 1995; 38:910–915. [PubMed: 8526463]
- Pearce AJ, Thickbroom GW, Byrnes ML, Mastaglia FL. Functional reorganization of the corticomotor projection to the hand in skilled racquet players. *Exp Brain Res*. 2000; 130:238–243. [PubMed: 10672477]
- Ramanathan D, Conner JM, Tuszyński MH. A form of motor cortical plasticity that correlates with recovery of function after brain injury. *PNAS*. 2006; 103:11370–11375. [PubMed: 16837575]
- Ranganathan VK, Siemionow V, Sahgal V, Liu JZ, Yue GH. Skilled finger movement exercise improves hand function. *J Gerontol A Biol Sci Med Sci*. 2001; 56:M518–522. [PubMed: 11487606]
- Ranganathan VK, Siemionow V, Sahgal V, Yue GH. Effects of aging on hand function. *J Am Geriatr Soc*. 2001; 49:1478–1484. [PubMed: 11890586]
- Reed A, Riley J, Carraway R, Carrasco A, Perez C, Jakkamsetti V, Kilgard MP. Cortical map plasticity improves learning but is not necessary for improved performance. *Neuron*. 2011; 70:121–131. [PubMed: 21482361]
- Rioult-Pedotti MS, Donoghue JP, Dunaevsky A. Plasticity of the synaptic modification range. *J Neurophysiol*. 2007; 98:3688–3695. [PubMed: 17913995]
- Sanes JN, Donoghue JP. Plasticity and primary motor cortex. *Annu Rev Neurosci*. 2000; 23:393–415. [PubMed: 10845069]
- Schallert T, Upchurch M, Lobaugh N, Farrar B, Spirduso WW, Gilliam P, Vaughn D, Wilcox RE. Tactile extinction: distinguishing between sensorimotor and motor asymmetries in rats with unilateral nigrostriatal damage. *Pharm Biochem Behav*. 1982; 16:455–462.
- Schallert T, Whishaw IQ. Bilateral cutaneous stimulation of the somatosensory system in hemidecorticate rats. *Behav Neurosci*. 1984; 98:518–540. 1984. [PubMed: 6539617]
- Seidler RD, Bernard JA, Burutolu TB, Fling BW, Gordon MT, Gwin JT, Kwak Y, Lipps DB. Motor control and aging: links to age-related brain structural, functional, and biochemical effects. *Neurosci Biobehav Rev*. 2010; 34:721–33. [PubMed: 19850077]
- Shadmehr R, Holcomb HH. Neural correlates of motor memory consolidation. *Science*. 1997; 277:821–825. [PubMed: 9242612]
- Smith CD, Umberger GH, Manning EL, Slevin JT, Wekstein DR, Schmitt FA, Markesbery WR, Zhang Z, Gerhardt GA, Kryscio RJ, Gash DM. Critical decline in fine motor hand movements in human aging. *Neurology*. 1999; 53:1458–1461. [PubMed: 10534251]
- Spengler F, Godde B, Dinse HR. Effects of ageing on topographic organization of somatosensory cortex. *Neuroreport*. 1995; 6:469–473. [PubMed: 7766845]
- Talelli M, Ewas A, Waddingham W, Rothwell JC, Ward NS. Neural correlates of age-related changes in cortical neurophysiology. *Neuroimage*. 2008; 40:1772–1781. [PubMed: 18329904]
- Tandon S, Kambi N, Jain N. Overlapping representations of the neck and whiskers in the rat motor cortex revealed by mapping at different anaesthetic depths. *Eur J Neurosci*. 2008; 27:228–37. [PubMed: 18093166]
- Tennant KA, Adkins DL, Donlan NA, Asay AL, Thomas N, Kleim JA, Jones TA. The organization of the forelimb representation of the C57BL/6 mouse motor cortex as defined by intracortical microstimulation and cytoarchitecture. *Cereb Cortex*. 2011; 21:865–876. [PubMed: 20739477]

- Tennant KA, Asay AL, Allred RP, Ozburn AR, Kleim JA, Jones TA. The vermicelli and capellini handling tests: simple quantitative measures of dexterous forepaw function in rats and mice. *J Vis Exp.* 2010; (41):pii. 2076. doi: 10.3791/2076.
- Tennant KA, Jones TA. Sensorimotor behavioral effects of endothelin-1 induced small cortical infarcts in C57BL/6 mice. *J Neurosci Methods.* 2009; 181:18–26. [PubMed: 19383512]
- Tennant, KA.; Kerr, AL.; Adkins, DL.; Donlan, NA.; Thomas, N.; Kleim, JA.; Jones, TA. Motor rehabilitative training and plasticity of the forelimb motor cortical representations in an aged mouse model of focal cortical ischemia; Program No. 590.06. 2011 Neuroscience Meeting Planner; Washington, DC: Society for Neuroscience. 2011; Online
- Teskey GC, Young NA, van Rooyen F, Larson SE, Flynn C, Monfils MH, Kleim JA, Henry LC, Goertzen CD. Induction of neocortical long-term depression results in smaller movement representations, fewer excitatory perforated synapses, and more inhibitory synapses. *Cereb Cortex.* 17:434–42. [PubMed: 16547346]
- Todd G, Kimber TE, Ridding MC, Semmler JG. Reduced motor cortex plasticity following inhibitory rTMS in older adults. *Clin Neurophysiol.* 2010; 121:441–447. [PubMed: 20071228]
- Tracy J, Flanders A, Madi S, Laskas J, Stoddard E, Pyrros A, Natale P, DelVecchio N. Regional brain activation associated with different performance patterns during learning of a complex motor skill. *Cereb Cortex.* 2003; 13:904–910. [PubMed: 12902389]
- Ward NS, Frackowiak RS. Age-related changes in the neural correlates of motor performance. *Brain.* 2003; 126:873–888. [PubMed: 12615645]
- Whishaw IQ. An endpoint, descriptive, and kinematic comparison of skilled reaching in mice (*Mus musculus*) with rats (*Rattus norvegicus*). *Behav Brain Res.* 1996; 78:101–11. [PubMed: 8864042]
- Whishaw IQ, Coles BL. Varieties of paw and digit movement during spontaneous food handling in rats: postures, bimanual coordination, preferences, and the effect of forelimb cortex lesions. *Behav Brain Res.* 1996; 77:135–148. [PubMed: 8762164]
- Whishaw IQ, Sarna JR, Pellis SM. Evidence for rodent-common and species-typical limb and digit use in eating, derived from a comparative analysis of ten rodent species. *Behav Brain Res.* 1998; 96:79–91. [PubMed: 9821545]
- Xu T, Yu X, Perlik AJ, Tobin WF, Zweig JA, Tennant K, Jones T. Rapid formation and selective stabilization of synapses for enduring motor memories. *Nature.* 2009; 462:915–919. [PubMed: 19946267]
- Young NA, Vuong J, Flynn C, Teskey GC. Optimal parameters for microstimulation derived forelimb movement thresholds and motor maps in rats and mice. *J Neurosci Meth.* 2011; 196:60–9.

Highlights

1. Plasticity of the young motor map is transient despite long-term skill practice.
2. Aged mice can learn a skilled reaching task in a similar way to young mice.
3. The organization and plasticity of motor representations is altered with aging.
4. Reaching behavior and motor map plasticity are at least partially dissociable.
5. Motor map reorganization seems to be a transient stage in motor learning.

\$watermark-text

\$watermark-text

\$watermark-text

Figure 1.

Acquisition of skill reaching in young and aged mice. (A) A schematic of the area of pasta cleared from the pasta matrix on various days throughout training by a representative young mouse in the long duration training group. The rapid improvements in successful pasta retrieval between Days 1-7 versus the subtle differences between Days 15, 30 and 45 are representative of the rates of improvement over the short duration versus the long duration of skill training. (B) Reaching success over 3 day training blocks in young and aged animals. The solid lines represent the combined averages of short and long duration trained animals. The dotted lines represent only the long duration trained animals. $*p < 0.05$, young vs. aged. The vertical light gray line indicates the transition point between the rapid and gradual learning phases. Data are the number of pasta pieces successfully retrieved. (C) The average number of days until young and aged animals transitioned from a rapid learning phase to more gradual improvements. (D) Final success level, measured as the average number of successful retrievals averaged over the last 3 training days. Data are the same as presented in A, but presented here to highlight the differences in final success rates between the short and long duration groups. $*p < 0.001$, aged short duration vs. long duration. All data are means $\pm$ S.E.

Figure 2.

Behavioral performance on the Capellini Handling Test. (A) Average number of adjustments per paw per pasta piece. (B) Average time to eat each pasta piece. (C) Average time, in seconds, per adjustment, calculated by dividing the time to eat by the number of adjustments. (D) Average occurrence per piece of the two most commonly seen atypical eating behaviors. Drop = dropping the pasta piece during eating, Super. Adj. = superfluous adjustments; forepaw adjustments that do not result in advancement of the pasta into the mouth. * $p < 0.05$. All data are means $\pm$ S.E.

Figure 3.

Aging results in a decrease in the digit representations in the caudal forelimb area (CFA) and an enlargement of the rostral forelimb area (RFA). The motor maps of young and aged mice with no reach training experience were compared in order to access the effect of aging on the baseline forelimb motor representation. (A) Areal extents of elbow, wrist, and digit representations in young and aged animals in the CFA. (B) Areal extents of elbow, wrist, and digit representations in young and aged animals in the RFA. (C,D) Representative maps from a young mouse (C) and an aged mouse (D), indicating CFA and RFA. * $p < 0.05$, Young vs. Aged. All data are means $\pm$ S.E. Scale bar equals 500 μ m.

Figure 4.

Transient training-induced reorganization of movement representations are found in the CFA of young adult, but not aged, mice following a short duration of reach training. (A,B) Ratios of proximal to distal forelimb representations within the CFA across groups with various levels and durations of behavioral experience in (A) young mice and (B) aged mice. (C) A representative map from a short duration reach trained young adult. (D) A representative map from a long duration reach trained young adult. The latter had no discernible RFA. $*p < 0.05$, young short duration reach trained animals compared to young controls. ns=non-significant. All data are means $\pm$ S.E. Scale bar equals 500 μ m.

Figure 5.

Distal forelimb movement thresholds decreased within the CFA of young animals following long duration behavioral training. Data are presented as the difference between long and short duration thresholds, in μA . * $p < 0.01$ vs. Controls, † $p < 0.001$ Pasta Handlers vs. Reachers. All data are means $\pm$ S.E.

Table 1

Age effects on other sensorimotor tests

	Ladder Rung Test		Bilateral Tactile Discrimination Test	
	Time (sec)	Errors/Step	Contact Time (sec)	Removal Time (sec)
Young (n=8)	15.00 ± 1.80	0.04 ± 0.00	8.59 ± 0.57	5.79 ± 0.56
Aged (n=7)	25.57 ± 2.61 *	0.06 ± 0.01	14.16 ± 1.10 *	7.67 ± 0.66

All data are means ± S.E.

*
 $p < 0.05$ vs. young.