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Breeder and Batch-Dependent Variability in the Acquisition and Performance of a Motor Skill in Adult Long-Evans Rats

Amber J. O'Bryan¹, Rachel P. Allred², Monica A. Maldonado¹, Lawrence K. Cormack^{1,2}, and Theresa A. Jones^{1,2}

¹Neuroscience Institute, University of Texas at Austin, TX, USA

²Psychology Department, University of Texas at Austin, TX, USA

Abstract

Reaching tasks are popular tools for investigating the neural mechanisms of motor skill learning and recovery from brain damage in rodents, but there is considerable unexplained variability across studies using these tasks. We investigated whether breeder, batch effects, experimenter, time of year, weight and other factors contribute to differences in the acquisition and performance of a skilled reaching task, the single pellet retrieval task, in adult male Long-Evans hooded rats. First, we retrospectively analyzed task acquisition and performance in rats from different breeding colonies that were used in several studies spanning a three year period in our laboratory. Second, we compared reaching variables in age-matched rats from different breeders that were trained together as a batch by the same experimenters. All rats had received daily training on the reaching task until they reached a criterion of successful reaches per attempt. We found significant breeder-dependent differences in learning rate and final performance level. This was found even when age-matched rats from different breeders were trained together by the same experimenters. There was also significant batch-to-batch variability within rats from the same breeder trained by the same experimenter. Other factors, including weight, paw preference and the experimenter, were not as strong or consistent in their contributions to differences across studies. The breeder and batch effects found within the same rat strain may reflect genetic and environmental influences on the neural substrates of motor skill learning. This is an important consideration when comparing baseline performance across studies and for controlling variability within studies.

Keywords

motor skill learning; forelimb behavior; breed variation; Long-Evans rats; within-strain differences; reaching performance

1. Introduction

Reaching tasks are widely used as tests and manipulations of skilled forelimb function in rats. In one of the most commonly used tasks, the single pellet retrieval task, rats inside a chamber are trained to reach with one forelimb through a narrow opening to grasp and

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Corresponding Author: Theresa A. Jones, PhD, Seay Hall, 1 University Station A8000, University of Texas at Austin, Austin, TX 78712, Phone: (512) 475-7763, Fax: (512) 475-7765, tj@psy.utexas.edu.

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retrieve a palatable food pellet. Variations of this task have been used to investigate the neurobiology of skill acquisition and the nature of the motor engram [1–7] and as a dependent variable or behavioral manipulation in models of motor recovery from stroke and other types of brain injury [8–20]. The specific movements used to perform the task [21,22] and handedness for reaching [23,24] have been extremely well characterized in rats.

Studies of strain-dependent differences in qualitative and quantitative performance indicate that Long-Evans rats have different movement styles compared with Sprague-Dawley rats [25] and are more skillful in reaching tasks compared with Fischer-344 rats [26]. Long-Evans rats also have larger forelimb movement representation areas in motor cortex, and the cortical stimulation intensities needed to elicit movements are lower compared with Fischer-344 rats [26]. However, little research has been done to determine the sources of the considerable *within*-strain variability in performance of the reaching task that is evident in the literature, even in studies from the same laboratory [e.g., 8,13,15,27–30]. Knowing the sources of this variability is important for designing well-controlled experiments. Gholamrezaei and Whishaw [31] found that some reaching variability can be attributed to the task strategy used by the rat to obtain food pellets. This was found in age-matched rats from one breeding colony and that were trained by the same experimenter. Thus, differences in behavioral performance can occur within a rat strain even when controlling for several potential sources of variability. We have noticed considerable variability across our experiments in male rats of the same strain in their acquisition rates and asymptotic reaching performance, even when experimenters were the same and training conditions were similar, but when the suppliers (“breeders”) of the rats varied [32–39]. This suggests that further analysis across studies is needed to assess within-strain sources of variability in reaching performance, including the possibility that the originating breeding colony is a significant contributor to this variability.

The purpose of this study was to identify major sources of variability in performance on the single pellet retrieval task across studies using the same strain of male Long-Evans hooded rats, a popular outbred strain for rat behavioral studies. This study was done using a retrospective analysis of data generated in previous studies from our laboratory on the reaching performance of rats acquired from three different breeding colonies (Table 1). This analysis included one study in which age matched rats from two different breeders were trained together at the same time by the same experimenters. This within-laboratory comparison permits assessment of variability across studies that have closely matched training parameters and conditions. It also avoids variability due to differences in laboratory environments, which has been found to be a major source of variability in behavioral measures in inbred mouse strains, despite standardization of testing procedures and many environmental variables [40].

2. Materials and Methods

2.1. Animals

Data were analyzed from 324 male Long-Evans hooded rats that were used in several previous or ongoing studies (Table 1). These studies examined reaching performance in CNS injury models, but only pre-injury performance data were included in the present study. Rats were obtained at one month of age from Charles Rivers Laboratories, Inc. (Crl:LE, n=99), Harlan Sprague Dawley, Inc. (HsdBlu:LE, n=165), or the Animal Resources Center (ARC) breeding colony at the University of Texas at Austin (n=60), which was established from Harlan stock rats with male rats backcrossed every two years. Hereafter, we refer to these Long Evans rats as CRL, HSD and ARC, respectively. Rats from the same study and breeder were housed in polycarbonate cages in pairs or triplets, were on a 12:12h light/dark cycle and were given water *ad libitum*. Rats were tamed by weekly gentle handling.

Beginning a few days before training, rats were restricted to 14–15g of chow per animal given at the end of each day to ensure that they were not sated at the time of reach training. Weekly weight changes during the shaping and training periods are reported in Table 1. Based on dates of birth supplied by the breeding colonies, approximate ages at the onset of training were 90–150 days in ARC, 75–90 days in CRL and 120–180 days in HSD rat studies (see Table 1 for details). The protocols for these studies were approved by The University of Texas at Austin Animal Care and Use Committee.

2.2. Shaping and Training on the Single Pellet Retrieval Task

In the studies analyzed, rats had been trained to a performance criterion on the single pellet retrieval task (Fig. 1) prior to the onset of other experimental manipulations. We examined various aspects of this “baseline” performance across experiments. The task was adapted from Whishaw and others [24, 41] and Greenough et al. [2], as previously described [37]. The reaching task apparatus was a Plexiglas chamber (26cm long × 34cm high × 16cm wide) with a tall narrow window (1cm wide × 23cm high) that was located in the middle of the 16cm wide wall. The animals were trained to reach with one forelimb through the window in order to retrieve a banana flavored pellet (Bio-Serve, Inc., Frenchtown, NJ) placed in a well 1cm from the window opening and on a 3cm high shelf. In order to prevent the animal from scraping the food pellet into the reaching chamber, a 2mm diameter metal rod formed a lip on the shelf in front of the window.

In a habituation and shaping period, rats were permitted to use either forelimb to retrieve centrally placed pellets. Previous research has shown that most rats develop a preferred limb for performing unilateral reaching tasks [23,24] and the preferred limb was determined during the shaping period. Most rats began reaching through the window on the second or third shaping session. Once the animal made 15 out of 20 reaches with the same forelimb in a shaping session, this limb was defined as the animals’ preferred limb and shaping ceased. When rats did not show a distinct limb preference during shaping, the limb making the majority of reaches was assigned as a side of training (or randomly assigned if evenly split). The laterality was calculated as the number of reaches made with the preferred, or assigned, forelimb divided by total number of reaches made during the last shaping session.

Once a preferred limb was established, rats began training with this limb. A Plexiglas wall (34cm high) was inserted into the reaching chamber ipsilateral to the preferred limb and pellets were placed, one at a time, in a well positioned contralateral to the limb (aligned with the edge of the reaching window). This configuration effectively enforced the use of the preferred or assigned forelimb for reaching. For each trial, a pellet was placed on the shelf outside of the reaching window and rats were permitted to make up to five attempts to retrieve it. A successful reach was one in which the pellet was grasped, retrieved and eaten. Unsuccessful reach attempts were ones in which the pellet was missed, knocked from the well or dropped. A trial ended when there was a successful reach, or when rats were unsuccessful by the fifth reach attempt. Animals were prompted to turn around and reset before starting each new trial by placing a pellet at the back of the cage. In most studies, rats were administered 30 trials per training session. The exceptions were two studies in which 60 trials per session were administered [37, 38].

2.3. Data Analyses

2.3.1. Reaching Performance Dependent Variables—The percentage of successful reaches per training session was calculated using the formula: (successful reaches/total reach attempts) × 100. This measure was used both to determine the rate of acquisition (“learning rate”) and to assess the performance on the first day of training (Day 1), excluding shaping days. Learning rate was defined as the number of training sessions to attain a criterion of

successful reaches per reach attempt. This was calculated as the reciprocal of days to criterion to normalize for a positively skewed distribution in days to criterion. The criterion was 50% successful reaches/reach attempt in ARC and HSD rat studies and 40% successful reaches/attempt in CRL rat studies. (The different criterion for the CRL rats, which was based on asymptotic performance in previous studies with them, is considered below). An additional measure was used to assess the performance on the last day of training. The percentage of successful reaches made on the first reach attempt (out of the 5 permitted per trial) or “hits”, was calculated using the following formula: (success on first attempt/trial number) $\times$ 100. The % hits/trial was used to further distinguish proficiency levels in rats that were performing similarly on the first performance measure (% successful reaches/attempt), i.e., because they had reached the criterion on it.

2.3.2. Independent Variables—Two variables of major interest were breeder (HSD, CRL and ARC) and batch, which was considered to be a cohort of rats undergoing training together by the same experimenter(s). Breeder effects were assessed across studies as well as within a study in which aged-matched rats from two breeders (ARC and CRL) were trained together by the same experimenters [37]. Batch effects were assessed by comparing rats from the same breeder that were trained at different time points by the same experimenter(s). In addition to the analyses of breeder and batch effects, other variables that were considered for their influence on reaching performance included the experimenter performing the training, the time of year of the shipping and training, age at the onset of training, onset weight, percent weight change from onset to the end of reach training, degree of laterality and the number of trials (30 or 60) per day of training.

2.3.3. Overview of Inferential Analyses—Analysis of variance (ANOVA) was used to investigate breeder and batch effects on reaching performance. Post-hoc Bonferroni corrected contrasts were used to further analyze reaching data as warranted by ANOVA results. ANOVAs and Chi-square analyses were also used to compare between studies and experimenters for differences in training parameters and rat characteristics. Stepwise multiple regression analysis was used in an exploratory manner to assess how some of these parameters contributed to the variance in reaching performance. Breeder source was included in this analysis using two dichotomous dummy variables (i.e., ARC, not HSD; HSD, not ARC; not ARC, not HSD). Finally, in the study directly comparing rats from two breeders[37], repeated measures ANOVA was used to assess reaching performance over days of training. SPSS (SPSS, Inc.) was used for all statistical analyses.

3. Results

3.1. Breeder-dependent differences in skilled reaching performance across studies

To analyze whether the source of rats made a significant contribution to variability in reaching, we pooled data from previous studies using rats from one of the three breeders (ARC, HSD or CRL). We then tested for breeder-dependent differences in reaching performance on Day 1, learning rate and the % hits/trial (successes on the first reach attempt) on the last day of training.

As shown in Figure 2, there were significant differences in the reaching variables among rats from different breeders. In one-way ANOVA, there were significant breeder-dependent differences in the % successful reaches/attempt on Day 1 ($F_{2,324}=47.73, P<0.001$), learning rate ($F_{2,324}=41.56, P<0.001$) and % hits/trial on the last training day ($F_{2,324}=87.47, P<0.001$). Overall, CRL rats were significantly slower to learn and performed less well than the rats from the other breeding colonies. HSD rats had the fastest acquisition rates and best performance and ARC rats were intermediate.

Note that the CRL rat studies had a lower criterion of success (40% successful reaches/attempt) than the studies using ARC and HSD rats (50%). Thus, CRL rats took longest to reach criterion despite having a lower criterion level. They also performed least proficiently on the first day of testing. However, this lower criterion complicates the measure of the % hits/trial (success on the first attempt). Five reach attempts were permitted per trial in all studies. Therefore, to achieve the 50% successful reaches/attempt criterion, ARC and HSD rats were required to have at least one trial with a hit to balance every trial without a successful reach by the second attempt. In contrast, to achieve the 40% criterion, CRL rats needed to be successful only by the third attempt in most trials and a balancing hit was required only when there were more than 8 trials in which a success wasn't had by the 4th attempt. To assess whether this difference in performance requirements contributed to the breeder-dependent differences, an additional analysis was performed in which any success by the second attempt of the trial was considered to be a hit in the CRL rats, because it approximates the need for success on the first attempt in the ARC and HSD rats. Comparing this more liberally defined hit in CRL rats with the original hit in ARC and HSD rats, there continued to be significant breeder-dependent differences in the % hits/trial among the three breeder sources ($F_{2,324}=26.46$, $P<0.001$). Posthoc analysis revealed that CRL rats performed less well compared to both ARC and HSD rats (P 's <0.016).

3.2. Other characteristics that varied among rats from different breeding colonies

Across studies, several characteristics varied among rats from different breeding colonies (Table 2). Rats from the three breeding colonies were significantly different in onset age (ANOVA, $F_{2,324}=317.02$, $P<0.001$), reflecting study design differences. They also differed in onset weight ($F_{2,324}=122.58$, $P<0.001$) and weight change during training ($F_{2,324}=105.52$, $P<0.001$). On average, CRL rats were used at a younger age, and weighed less, and HSD rats were oldest and weighed most. ARC rats had less weight change over the training period than the rats from the other breeders. There were also differences in the prevalence ($\chi^2_{2,n=325}=53.06$, $P<0.001$) and in the strength of limb preferences as measured by the laterality index ($F_{2,324}=3.51$, $P=0.03$). HSD rats had the least prevalent limb preferences and CRL rats had the most prevalent, as well as strongest, limb preferences (Table 2). However, among rats showing any limb preference, there were no major breeder-dependent differences in the proportions of left versus right preferences ($\chi^2_{2,n=325}=0.69$, $P=0.41$). Rats preferring the left limb made up 47% of ARC, 51% of CRL and 46% of HSD sample populations. There were also no differences in the mean number of shaping days across studies using rats from different breeders ($M=3.1$ to 3.2 ; ANOVA, $P=0.92$).

To explore how the differing characteristics, along with breeder, related to variance in reaching performance, we used stepwise regression analysis (which assesses the contribution of each predictor variable to the variance and keeps or removes the variable accordingly). Age was not considered in the regression analyses because of an unequal age distribution across rats from different breeding colonies. Of rats that were 2.5–3 months of age, all were CRL ($n=108$), whereas none of the rats 4–6 months of age ($n=217$) were CRL rats. (Note that a comparison of age-matched CRL and ARC rats is presented in Section 3.4). We analyzed performance on Day 1, learning rate and hits/trial on the last day. Breeder was found to be a significant contributor to the variance in reaching performance, as measured by Day 1 (adjusted $R^2=0.18$; $F_{2,324}=37.55$, $P<0.001$), learning rate (adjusted $R^2=0.20$; $F_{2,324}=37.55$, $P<0.001$) and hits/trial (adjusted $R^2=0.14$; $F_{2,324}=41.36$, $P<0.001$). However, onset weight (β 's=0.12, -0.004, 0.02, P 's=0.08, 0.95, 0.78), % weight change (β 's=-0.061, -0.006, -0.07, P 's=0.35, 0.93, 0.29) and laterality (β 's=-0.05, 0.04, -0.06, P 's=0.33, 0.48, 0.22) were not found to be significant contributors to the variance in Day 1 performance, learning rate or the % hits/trial on the last day, respectively. Thus, across studies, breeder was identified as the main contributor to the variance in the reaching performance measures.

3.3. Experimenter Effects on Training

Overall, the experimenter performing the training did not appear to result in major differences in reaching performance within rats from the same breeder. In the ARC-bred rats, among experimenters MA, RA and AO/BB, there were no differences in performance on Day 1 (ANOVA, $P=0.31$), learning rate ($P=0.79$), or the % hits/trial ($P=0.96$). The HSD-bred rats also showed no experimenter-dependent differences in performance on Day 1 ($P=0.35$) or the % hits/trial ($P=0.13$). However, there was a significant difference in the learning rate in the HSD-bred rats between two experimenters (RA versus MA; $F_{1,165}=4.74$, $P=0.031$). The learning rate for HSD rats trained by RA was 0.77 ± 0.03 , and 0.65 ± 0.05 for those trained by MA. However, this experimenter effect did not generalize to rats from another breeder. For ARC-bred rats, there were no differences between rats trained by RA versus MM in performance on Day 1 (ANOVA, $P=0.44$), learning rate ($P=0.70$), or the % hits/trial ($P=0.80$). The experience of the experimenter is considered in Section 3.5.

3.4 Breeder-dependent differences in age-matched rats trained by the same experimenters

To analyze breeder effects while controlling for variability due to age at onset of training, trainer, time, training context, and batch effects, we additionally compared daily performance of a subset of ARC and CRL rats that were trained in the same time period in the same room by the same experimenters (ARC3 and CRL5, Table 1) [37]. The two experimenters (AO and BB) alternated rats each day to ensure that the CRL and ARC rats were trained equally by each experimenter. These rats were reported by the suppliers to be of nearly identical ages (1 month old upon arrival), they arrived in the laboratory within days of each other and they were housed in the same vivarium. The rats from different breeders did not differ in laterality. They did differ in onset weight and weight change (CRL>ARC), but this difference was in the opposite direction of that found in the comparison across studies (CRL<ARC, Table 2).

As shown in Figure 3A, ARC and CRL rats trained together as a batch by the same experimenters also had significant differences in reaching performance. Rats from both breeders improved over days of training, but ARC rats performed better overall compared with CRL rats. In repeated measures ANOVA for % successful reaches/attempt, there was a significant main effect of breeder ($F_{1,15}=5.52$, $P=0.03$) between age-matched CRL and ARC rats. There was also a significant day of training effect ($F_{8,15}=5.96$, $P<.001$), but no significant breeder by day interaction effect ($F_{8,15}=0.64$, $P=0.81$). As shown in Figure 3B, ARC rats performed better than CRL rats in the % hits/trial. In repeated measures ANOVA for the % hits/trials over days trained, there was a significant breeder by day interaction effect ($F_{8,120}=2.06$, $P=0.045$), but no significant main effects of breeder ($F_{1,15}=2.69$, $P=0.12$) or day ($F_{8,120}=0.66$, $P=0.73$). Post-hoc analysis indicates that the ARC rats had greater % hits/trial on day 7 of training compared to the CRL rats ($P=0.002$). Thus, breeder-dependent differences in reaching performance were evident when there was control for several of the characteristics that varied between breeders in the cross-study analysis.

3.5 Batch effects on reaching performance within rats from the same breeder

A batch was considered to be a set of rats undergoing a course of shaping and training by the same experimenter(s) in the same time span. Batch effects were assessed by comparisons of reaching performance across batches of rats from the same breeder (Table 1).

3.5.1 Batch effects within trainers—There were 7 batches of CRL rats trained by the same experimenter, AO, inclusive of one trained by AO/BB (CRL5). As shown in Figure 4, there were significant differences in reaching performance across these different batches of CRL rats. In one-way ANOVAs, there were significant differences in % success/attempt on Day 1 ($F_{6,98}=5.76$, $P<0.001$), learning rate ($F_{6,98}=5.80$, $P<0.001$) and % hits/trial

($F_{6,98}=3.53$, $P=0.005$). Post-hoc analyses revealed that, on average, CRL3 acquired the task fastest and also had the greatest success on Day 1 compared with several other batches (Fig. 4C). The longitudinal pattern of the batch effects is inconsistent with a linear relationship between reaching performance and the experience of the experimenter (AO) in administering the reaching task. That is, there were both decreases and increases in reaching performance across batches as time and experimenter experience progressed.

Among these CRL batches, most received 60 trials/day (CR1–6), but two received 30 trials/day (CRL6 and 7). As described in the next section, performance measures were found to vary with training intensity. However, if the two batches receiving the lower training intensity are excluded from the analysis of batch effects, significant effects remained for each measure (success on Day 1: $F_{4,70}=5.51$, $P=0.003$); learning rate: $F_{4,70}=5.55$, $P=0.001$; % hits per trial: $F_{4,70}=4.65$, $P=0.002$).

It was also possible to assess batch effects within trainers in HSD rats, in which there were four batches trained by the same experimenter (HSD1 and 3–5; Table 1). In contrast to batch effects in CRL rats, in HSD rats trained by the same experimenter (RA), there was considerably less variability between batches, and there were no significant batch effects in the three reaching measures tested ($F'_{3,129}=0.20$ – 0.99 , P 's= 0.90 – 0.45 ; data not shown).

3.5.2. Batch effects related to training intensity—Studies, and thus, batches, of CRL and ARC rats varied in training intensity. Among CRL rats, those receiving 60 trials (CRL 6–7) had a faster learning rate (0.45 ± 0.04) than those receiving 30 trials (0.28 ± 0.04) and this was significant in one-way ANOVA ($F_{1,98}=4.61$, $P=0.034$). In addition, the number of hits per trial on the last training day was significantly different (60 trial batches: 50.33 ± 1.91 ; 30 trial batches: 42.74 ± 1.47 ; $F_{1,98}=6.28$, $P=0.014$). Because there were also differences among the batches that received 60 trials, as reported above, it is possible that these results reflect batch effects unrelated to training intensity. There were far fewer ARC-bred rats receiving 60 trials (ARC3, $n=9$) compared with those receiving 30 trials (ARC1–2, $n=51$). In this limited comparison, ARC-bred rats that received 60 trials on the single pellet retrieval task did not have a significant difference in learning rate compared to ARC-bred rats that received 30 trials ($F_{1,59}=0.32$, $P=0.58$). Furthermore, those receiving 60 trials had fewer hits per trial compared to the ARC-bred rats that received 30 trials ($F_{1,59}=13.28$, $P=0.001$). This suggests that either training intensity effects vary with breeder and/or experimenters, or that the differences found in CRL rats reflect batch effects unrelated to training intensity.

3.5.3. Batch effects related to seasons—Because laboratory context, shipping conditions and other factors may vary with seasons, the CRL batches considered above were sorted based on the time of year in which they began behavioral training. As shown in Table 3, batches were combined into those trained in fall (training onset in August or September, $n=21$), winter (January, $n=22$), spring (March or May, $n=33$) and summer (June, $n=23$). (Note that rats were shipped at 1 month of age, i.e., 1.5–2 months prior to training.) Using a one-way ANOVA, there were no significant differences in the learning rate ($F_{3,98}=1.25$, $P=0.30$) or performance on Day 1 ($F_{3,98}=1.78$, $P=0.16$) among seasons. However, there was a significant difference in hits per trial on the last day of training ($F_{3,98}=5.28$, $P=0.002$). Post-hoc analyses revealed that animals trained during the fall season (and shipped in summer) had fewer hits/trial compared to those trained in the other seasons (P 's <0.05).

4. Discussion

We found significant breeder-dependent differences in male Long Evans hooded rats in their performance on the single pellet retrieval task. Rats obtained from Charles Rivers Laboratories (CRL) had the least proficient performance, as measured by performance on

the first and last days of training and by learning rate. This was found in comparisons with rats from Harlan Sprague Dawley (HSD) and those bred at the University of Texas Animal Resources Center (ARC). Breeder-dependent effects were found even when the same experimenters trained age-matched rats from different breeding colonies together (CRL versus ARC). There were also significant "batch" effects in reaching performance in CRL rats. That is, CRL rats trained by the same experimenter, but at different times, had significant differences in reaching performance.

Outbred rats, by definition, are genetically diverse, and breeding strategies are intended to maintain this diversity across generations. Nevertheless, it is likely that different breeding colonies of Long Evans rats have some consistent genetic variation across them and that this variation contributes to differences in reaching performance. Since the ARC rats were bred from HSD stock rats, there should be less genetic distance between these two breeding colonies compared to CRL rats. Thus, to the extent that the present results can be explained by genetic differences between breeding colonies, it could be expected that HSD and ARC rats would perform more similarly on behavioral measures compared with CRL rats. The pattern of results is generally consistent with this, in that HSD rats outperformed both CRL and ARC rats on all three reaching variables. However, ARC rats also performed better than CRL rats, suggesting significant genetic divergence between these colonies, or the contribution of other sources of variability to reaching performance.

Multiple studies indicate that genetic differences contribute to behavioral differences, such as learning, motor activity [40,42], maternal behavior and vocalization [43,44]. This is an important consideration because some genetic variations can occur even in inbred strains and could contribute to overall differences in performance between studies. Genetic variations between and within strains may also result in differences in the neural changes underlying learning. Learning the skilled reaching task is linked with structural and functional changes in the motor cortex, including long-term potentiation [7], increases in synapses [5] and dendrites [2] and reorganization of movement representations [4,5]. Breeder-dependent differences in reaching performance might not be reflected in obvious macroanatomical brain differences. Gholamrezaei and Whishaw [31] found no differences in cell numbers, AChE density, pyramidal tract size, brain weight or measures of motor cortex thickness between high and low-performing Long-Evans rats. However, *between* strain comparisons have linked reaching performance differences with variation both in the baseline topographic representation of movement in the motor cortex (the "motor map"), and its change in response to motor skill learning [26]. Thus, it is possible that there are differences between breeding colonies in the baseline functional organization of the motor cortex, and in the structural and functional changes in synaptic connectivity thought to underlie its reorganization during learning [3,4,5,7].

In a previous study, Crabbe et al. [40] found that differences in performance among several inbred strains of mice were independent of the originating breeding colony. That is, adult inbred mice shipped from different suppliers (and undergoing different shipping times and environments) performed similarly to mice bred in the laboratory, as long as the shipped mice received adequate acclimation time. Based on these findings, it could be predicted that the CRL rats would perform similarly to the rats from other breeders, provided that the CRL rats were given ample time to acclimate. However, all of the CRL rats analyzed in this study were allowed adequate acclimation time (1.5–2 months) and CRL rats still performed least proficiently. Furthermore, ARC rats required no shipping and performed less well than HSD rats. Thus, the present study indicates that the originating breeding colony can play an important role in performance, at least among this outbred strain of Long Evans rats.

Though shipping and acclimation cannot explain the breeder-dependent effects found in the present study, the season of shipping may have contributed to batch effects in CRL rats. In one of the three reaching variables (hits/trial), CRL rats shipped in the summer and trained during the fall performed less well than those shipped and trained at other times of the year. In HSD rats, neither batch nor seasonal effects were found, but this was a more limited analysis because there were no HSD rats shipped and trained on the same schedule as CRL and there were fewer HSD batches trained by the same experimenter to compare. Thus, these results raise the possibility that fluctuations in temperature during shipping, or other season-dependent differences, could contribute to batch-related differences in reaching performance.

More study is needed to determine the influence of training intensity on the rate of learning of the skilled reaching task. CRL rats receiving 60 trials/day had a faster learning rate compared with those trained on 30 trials/day. However, it is possible that this was due to batch-related differences unrelated to training intensity, because there was just as notable batch-to-batch variability in learning rate among CRL rats receiving the same training intensity. Furthermore, there was no clear performance improvement with greater training intensity in ARC rats.

We also failed to find consistent experimenter-dependent differences in reaching performance. There were no significant experimenter effects in reaching performance in the ARC-bred rats. In the HSD rats, the experimenters performing the training may have contributed to differences in learning rate, but the same experimenters did not differ when the ARC-bred rats they trained were compared. Furthermore, batch effects in CRL rats could not be attributed to the effects of trainer experience. Thus, at least among these experimenters, the individual administering the task was not a major factor in reaching performance variability. This hardly indicates that reaching performance is invulnerable to such effects. Another recent retrospective analysis investigating factors contributing to the variance in skilled reaching found significant effects of experimenter experience, as well as effects of the rat's age and the day of the week of training [45]. Crabbe et al. [40] also found experimenter-dependent differences when inbred strains of mice were tested on a battery of cognitive and motor tasks. It may be that protocol standardization in the studies included in the present analyses effectively countered experimenter effects, or that variability due to breeder and batch obscured such effects.

There were breeder-related differences in both age and weight at the onset of training, reflecting study design differences, but these were not found to be major contributors to the variance in reaching performance. In the larger cross-study analysis, CRL rats were the youngest and weighed the least on average, and HSD rats weighed the most. Rats that began training at later ages (i.e., 5-months old) also had more stable weights compared to younger animals. However, stepwise multiple regression analysis failed to indicate a major relationship between weight or weight change and reaching performance. In addition, when aged matched CRL and ARC rats from the same study were directly compared, CRL rats still had the worst performance on single pellet retrieval task. Furthermore, in this age-matched comparison, CRL rats weighed *more* than ARC rats (532 versus 467g), i.e., showing weight differences in the opposite direction of the larger analysis, but similar behavioral differences.

It could be that the greater weight in age-matched CRL rats reflects an accelerated maturation that contributes to poorer performance, because older rats, at least sometimes, have less robust neural plasticity in response to manipulations of behavioral experience [46–48]. However, this could not explain the breeder-dependent differences in the cross-study analyses, given that CRL rats were used at the youngest ages on average. That is, if younger

rats learn faster and better, CRL rats would be expected to perform slightly better than the rats from other breeders, and the opposite was found. However, it is important to note that the analysis of age effects in this study was limited because all of the rats were relatively young adults (2.5–6 months old). Previous research indicates that younger adult rodents (6 months old) perform better in learning and memory tasks than older rodents (12–22 months old) [49–52]. Compared to younger rats, aged rats had more errors and required more time to learn and complete an elevated obstacle course [52]. In mice, motor cortical reorganization during motor skill learning varies with [53]. Also, aged rats (21 months old) show increased latencies and errors on a spatial learning task compared to younger, 6-month old rats [51]. Aged male mice (25-months old) also have reduced performance of spatial and olfactory reference memory tasks compared to 5-month old mice [49].

The development of handedness has long been thought to confer advantages in unimanual task performance [54,55], but differences in laterality did not appear to explain the breeder-dependent effects of this study. The degree of laterality in skilled reaching has previously been found to be positively correlated with skill level in rats [24]. Therefore, it could be predicted that the HSD rats, since they were the most proficient in the reaching task, would have the strongest laterality. However, the HSD rats had the weakest laterality, whereas the CRL rats (the worst reachers) had the strongest. Although this appears to conflict with previous findings, it is important to note that sufficient assessment of the relationship between skill and laterality requires both larger populations and more sensitive assays of laterality than that used in the present study. It is also possible that the robust breeder-dependent variability in performance obscured any influence of laterality in the comparison across breeders.

Only male rats were included in the present study, but female rats are frequently used in studies involving skilled reaching. Some of our previous studies of female CRL [56] and ARC [16] rats are suggestive of breeder dependent differences in reaching performance similar to those presently found in male rats, but a larger sample of studies, or direct comparison, is needed for more definitive analysis of the generalization of the breeder-dependent effects to female rats. Female Long-Evans rats show some subtle estrous-stage dependent differences in reaching performance [56] and there are sex-differences in approach and body orientation during reaching, but reaching performance has been found to be quantitatively and qualitatively similar in female and male rats [57,58]. However, findings of sex differences in the effects of stress on reaching performance [58] indicate that sex can interact with other contributors to variance in reaching performance.

The present analysis was limited to measurements of reaching performance, and did not assess differences in reaching strategies employed by rats. A recent study by Gholamrezaei and Whishaw [31] found that rats from the same breeding colony adopted different reaching strategies and that the strategy adopted contributed to differences in performance. Rats that adopted a “goal-directed” strategy of reaching learned the task better, needed fewer gestures for reaching and had more successful reaching performance compared to rats using a “stimulus-response” strategy for reaching. This raises the possibility that there might be breeder- and batch-dependent variation in reaching strategies that help explain the present findings. Thus, additional analysis of reaching strategies among the rats from different breeders could provide important insight into observed reaching differences.

In addition to the single pellet retrieval task, there are other types of reaching tasks used to test forelimb function and study motor skill learning, including the Montoya staircase task, [59,60], tray reaching tasks [19,61], and the pasta matrix reaching task [62]. Like the single pellet retrieval task used in the current study, these reaching tasks also require fine motor skill learning with the forelimb, including skillful reach-to-grasp movements. It seems

reasonable to suspect that the present findings of breeder and batch dependent differences in performance of the single pellet retrieval task generalize to these other reaching tasks.

While some research has found rat strain-dependent differences in reaching movements [25] and in motor cortical representational maps and thresholds following motor skill training [26], the current study is at least one of the first to systematically investigate *within*-strain differences in rats from different breeding colonies. Its results suggest that breeder and batch effects on skilled reaching exist within the outbred Long-Evans hooded rat strain and should be carefully controlled for and considered in studies of motor skill function with this strain. For example, disparate samples of rats trained in different batches or with different breeding histories could confound experimental comparisons. Further investigation is required to clarify the influence of training intensity, to determine if breeder-dependent differences in reaching styles underlie these results and to determine if similar breeder and batch effects generalize to female rats and to other behavioral tasks.

Research Highlights

- There is considerable variability in performance of a skilled reaching task among Long-Evans rats even with carefully standardized methodology.
- The originating breeding colony was found to be a major factor underlying this variability.
- There were also considerable "batch effects" among rats obtained from the same breeding colony.
- The experimenter, shipping, and baseline rat characteristics were not as important in contributing to variability in reaching performance.

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Figure 1.

A successful reach on the single pellet retrieval task. Sequential photographs of a rat (A) aiming for, (B) beginning to grasp, (C) retrieving and (D) releasing a food pellet it into the mouth. On each trial, rats were permitted 5 reach attempts to retrieve one pellet. Day 1 performance and learning rates were assessed based on the % successful reaches per attempt, as measured in 30–60 trials/day. The % of trials in which the first reach attempt was successful (a "hit") was used to further distinguish proficiency levels on the last training day.

Figure 2.

Reaching performance in male Long-Evans rats was dependent upon the originating breeding colony. HSD rats performed the best, ARC rats were intermediate and CRL rats performed the worst as assessed by (A) % successful reaches/attempt on the first day of training, (B) learning rate (1/days to criterion) and (C) the % of trials in which the first reach attempt was successful (“hits”). Data are means±S.E.M. (* $P < 0.016$)

Figure 3.

Performance on the single pellet retrieval task in aged-matched ARC and CRL rats, trained at the same time and by the same experimenters. (A) The ARC rats had greater reaching performance overall, as measured by the % successful reaches/attempt, compared to CRL rats. (B) In % hits/trial, there was a significant group by day interaction (see text for details). Data are from ARC3 and CRL5 batches (Table 1). Data are means $\pm$ S.E.M. (* $P < .05$)

Figure 4.

Differences among batches of CRL rats in the (A) % successful reaches/attempt on the first day of training, (B) learning rate and (C) % hits/trial. There were significant overall batch effects in reaching performance on Day 1 and learning rate. (See text for details.) CRL3 had greater % successful reaches/attempt on day 1 and learned significantly faster than CRL5–7 (* P 's < 0.004). Dates are months and year of training onset. Data are means $\pm$ S.E.M.

Baseline Characteristics and Training Parameters in Studies using Long-Evans Rats from Three Breeding Colonies

Table 1

Study Citation	Breeder	n	Onset Age (days)	Weight, M±SE		Shaping Days M±SE	Laterality Index M±SE	Training Trials/Day	Batches		Trainer Initials
				Onset (g)	% Change				n	I.D.	
[35] [†]	ARC	24	120	471±8	-2.1±0.04	3.0±0.2	0.83±0.04	30	1	ARC1	MM
[32]	ARC	27	150	467±10	4.7±1.9	3.0±0.2	0.82±0.05	30	1	ARC2	RA
[37] [*]	ARC	9	90	448±12	5.0±1.5	4.9±0.1	0.86±0.07	60	1	*ARC3	AO/BB
[38] [†]	CRL	63	75-90	383±4	5.3±0.6	2.9±0.2	0.92±0.02	60	4	CRL1-4	AO
[37] [*]	CRL	8	90	532±20	-2.2±1.5	3.8±0.4	0.80±0.06	60	1	*CRL5	AO/BB
[39] [†]	CRL	28	90	411±5	8.7±0.8	4.0±0.2	0.95±0.02	30	2	CRL6-7	AO
[33]	HSD	20	120	475±6	-5.4±0.6	4.2±0.4	0.78±0.09	30	1	HSD1	RA
[36]	HSD	36	120	490±7	-8.5±2.6	2.9±0.2	0.90±0.03	30	1	HSD2	MM
[33]	HSD	38	180	491±7	-1.9±0.6	2.8±0.1	0.77±0.05	30	1	HSD3	RA
[34]	HSD	71	150	487±4	-3.67±0.3	3.2±0.1	0.87±0.02	30	2	HSD4-5	RA

ARC, Animal Resources Center at the University of Texas at Austin, CRL, Charles Rivers Laboratory, HSD, Harlan Sprague Dawley, I.D., batch identification, M, means, SE, standard error of the mean,

* same study,

[†] abstract

Table 2

Characteristics of Rats from Different Breeders that Varied Between Studies

Breeder	Onset Age (days)	Onset Weight (g)	% Weight Change	Limb Preference % of Rats	Laterality Index
ARC	129±2.8 ^{*†}	469±6.3 ^{*†}	1.5±1.14 ^{*†}	82 ^{*†}	0.86±0.03 [*]
CRL	87±0.63 [†]	392±3.3 [†]	6.3±0.52 [†]	98 [†]	0.92±0.01 [†]
HSD	147±1.4	488±2.7	4.5±0.62	63	0.84±0.02

Data are $M \pm SE$ ^{*} $P < 0.01$ significantly different from CRL[†] $P < 0.01$ significantly different from HSD

Table 3

Seasonal Differences in Reaching Performance in CRL rats

	Training Onset			
	Fall Aug–Sept	Winter Jan	Spring March–April	Summer June
Batch(es)	CRL1, 4	CRL2	CRL3, 5, 6	CRL7
% Success on Day 1	25.5±3.4	28.9±4.0	22.4±3.5	17.6±2.6
Learning Rate	0.35±0.05	0.49±0.08	0.39±0.07	0.32±0.04
% Hits/Trial	36.2±2.0	45.7±1.7*	44.1±1.6*	43.2±1.5*
Shipping Season	Summer	Fall	Winter	Spring
Temperature (C°)[†]	28.3–28.6	15.4	8.6–15.9	18.9

Data are $M \pm SE$.* $P < 0.05$ significantly different from Fall batches (Aug–Sept)[†] Average monthly outdoor temperature in Austin, TX, USA, during shipping (<http://www.srh.noaa.gov/ewx/?n=ausclidata.htm>, 2010)