Learning of paw preference in mice is strain-dependent, gradual and based on short-term memory of previous reaches

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ABSTRACT

We study the dynamics of mouse paw-preference learning in unbiased symmetrical test chambers where mice can freely choose which paw to reach. When compared to non-learner model mice, three strains exhibit different degrees of learning within and between two training sessions separated by a one-week interval. While paw preference is probabilistic, positive autocorrelation between paw choices made by individual mice in a training session shows that bias in paw preference changes gradually with successive paw reaches and it is concordant between sessions. Within a session, the degree of positive autocorrelation between consecutive paw choices differs among strains and it decreases faster with increasing lag between reaches in stronger than in weak learners, suggesting that the degree of learning is the genetic variable in mice. We propose that strong biases in individual mice result from weak biases that appear by chance early in training and increase by a positive feedback mechanism due to learning from training. This explains how individual mice of each strain are strongly biased when there is almost no bias at the population level. Decrease of autocorrelation with lag shows that constitutive behaviours play a minor role and, hence, there is adaptability in paw preference to environmental changes. We conclude that paw-preference learning in mice is genetically dependent on past experience, changes gradually within training sessions, and is based on short-term memory; it also has long-term effects, provided time for memory consolidation.

Key words: paw preference, mouse, autocorrelation, null model, learning, probabilistic, adaptation, short and long term memory
INTRODUCTION

The probabilistic nature of the interaction between individuals and their environment is a source of diversity in complex traits (Den Nijs & Sterk 1980; van Baarlen et al. 2000). Phenotypic heterogeneity in morphology and behaviour is a key factor in adaptability and survival (Hamilton et al. 1990; Maynard Smith 1978; Mayr 1997, 2004). What determines the extent of phenotypic diversity within populations still requires much study (Mayr 1997, 2004), especially in behavioural traits such as human hand preference (McManus 2002; Tommasi 2009; Rodriguez et al. 2010). Genes responsible for influencing such behavioural traits are also largely unknown (Corballis 2009).

Paw preference of laboratory mice provides a practical model without the cultural biases and developmental asymmetries that affect human handedness (Medland et al. 2009). When mice reach for food, their paw preference is a quantifiable behavioural trait and the inbred strains allow replication and comparison of the trait among different genotypes under various experimental conditions (Biddle & Eales 2006). Simplicity of mouse pawedness should facilitate characterization of its underlying learning process.

FIGURE 1

Mouse paw usage was first assessed in unbiased or ‘U-World’ (UW) test chambers (Fig. 1) with a central food tube that does not hamper reaching with either forepaw (Collins 1968). Direction of paw preference is the right-paw entry (RPE) or the number of reaches with the right paw in a specified number of reaches. Populations of genetically
identical individuals in the form of inbred strains show wide phenotypic diversity in RPE values (Collins 1968; Signore et al. 1991; Takeda & Endo 1993; Biddle & Eales 1996).

In the UW, paw preference appears to be genetically neutral because most strains have an equal number of right and left-pawed mice (Collins 1968; Biddle & Eales 1996). Selection of parents with more biased paw usage in the C57BL/6J strain does not produce more left- or right-pawed offspring (Collins 1969). The reproducible and distinct shape of the RPE distributions of the inbred strains implies that genetic differences in this mouse trait are in the degree of lateralization, not in its direction (Collins 1985). In agreement, bidirectional selective breeding of genetically heterogeneous mice produced divergent lines of highly lateralized and weakly lateralized mice in a few generations and these lines can be maintained by random breeding without losing the selected behaviour (Collins 1985, 1991). Quantitative trait locus analysis detected a positive, but yet to be validated, association between variation in degree of lateralization and marker genes on mouse chromosome 4 (Roubertoux et al. 2003). Paw preference in mice has also been studied in other contexts, such as its dependence on the corpus callosum (Ward et al. 1987; Bulman-Fleming et al. 1992; Gruber et al. 1991; Biddle & Eales 1996), hippocampal “mossy fibre projections” (Lipp et al. 1996) and immune reactivity (Neveu et al. 1988; Neveu et al. 1991; Denenberg et al. 1991a, 1991b).

Paw preference and motor lateralization has been studied in other animals, such as cats (Wells & Millsopp 2009), horses (McGreevy & Thomson 2006) and dogs (Wells 2003; Poyser et al. 2006; Batt et al. 2008). One result, relevant to the present study, is that distributions of handedness scores from different tests of individual dogs (Poyser et al. 2006) show large variation in lateralization of the left- and right-paw preference, whereas
there is no bias at the population level (e.g., Wells 2003; Batt et al. 2008). This is also observed in several mouse strains (Biddle and Eales 1996, 2006).

Large sample size in these studies appears to be necessary (Ribeiro et al. 2010). For example, it is still unclear whether nonhuman primates exhibit significant bias in hand preference at the population level or simply express a sampling error that is inflated when individuals with small and unequal sample sizes make a disproportionate contribution to population-level measures (Palmer 2002, 2003). Resolution of these questions will aid in better understanding the evolutionary origin of human hand preference (Rogers 2009).

Here we focus on the role of learning in the paw preference behaviour of various mouse strains and ask what determines the degree of lateralization in an unbiased, symmetrical UW where mice freely choose the paw to reach. As in other animals (Magat & Brown 2009), a process of learning and memory regulates mouse paw preference in biased test chambers with the food tube placed to the right or left side (Biddle & Eales 1999). Kinetic analysis established that paw preference in biased worlds is an adaptive behaviour since mice show a directed response to changing environment and their future performance is genotypically dependent on past experience (Biddle & Eales 2006). Therefore, mice learn paw preference when training in biased worlds and different strains differ in how much the training affects the behaviour (Ribeiro et al. 2010).

Although the distributions of RPE scores of the inbred strains are reproducible, the RPE score of a mouse does not predict its strain of origin (Collins 1969). Paw preference in the UW was suggested, but not formally demonstrated to have a stochastic component (Collins et al. 1993). Recently, it was proposed that learning rate per successful reach of
individual mice is the genetically deterministic trait that “shapes” the different RPE distribution of each strain (Ribeiro et al. 2010).

We address three questions about paw-preference behaviour in the UW. Do mice learn a direction of paw preference in a UW and, if so, why, when use of either paw provides equal reward and involves the same effort? How does paw preference change with training in a UW: Is it a gradual change during a training session or is it detectable only when comparing tests after a period of memory consolidation? Finally, do mice have constitutive biases in the direction of paw preference and what is the relative importance of constitutive behaviour versus training in mice of different inbred strains? To address these questions, we examine the process of reaching in individual mice on a reach-to-reach basis during training sessions, compare results between training sessions, and compare the measurements with model null-learner mice with random paw choices.

METHODS

When referring to “memory”, we mean “implicit memory” or an unconscious form of memory. In tests of implicit memory, there is no conscious effort to retrieve recent experiences to perform the tests, which we believe to be the case here (Roediger 1990; Schacter 1987). When referring to “learning”, we imply “procedural learning”, i.e. a behavioural change by acquisition of implicit memory (Nissen & Bullemer 1987).

Measurements of paw preference were made in unbiased test chambers, described as U-world or UW test chambers. We assume that reaching with the right or left paw in a UW involves identical effort and that both possible choices for the mouse are equally
rewarded. This is necessary if one is to assess what aspect of the behaviour is constitutive and what aspect is due to learning because, in a UW setting, the mice ought to use a constitutive behaviour “unbiased” by environmental factors, such as would occur in right- or left-biased test chambers. A previous study suggested that mice change paw preference in the absence of a reward versus punishment stimulus in a UW (Ribeiro et al. 2010). Therefore, if learning can be assessed in the UW setting, it would have the advantage of allowing the detection of correlations in temporal series of paw choices that would indicate that there is learning within a training session.

Mouse Strains

CDS/LayBid, DBA/2JBid, and C57BL/6JBid are registered inbred strains of the laboratory mouse. Their origins are described in (Biddle and Eales 1996) and they are maintained as genetic reference strains with continued sister-brother inbreeding. For brevity, our ILAR registered laboratory code (“Bid”) is not included in the strain names here (http://dels.nas.ed.global/ilar.Lab-Codes). We assessed paw preference of C57BL/6J, DBA/2J and CDS/Lay mice as each strain has a documented ability to learn a bias in paw preference with training in biased test chambers, but differ in learning abilities in that context (Ribeiro et al. 2010). We used 300 mice in this study (100 mice of each strain). This amount was shown to be required for the distributions of paw preference of the strains to be strongly consistent between measurements due to the stochastic nature of the paw reaching behavior (Ribeiro et al, 2010).
The mice were cared for in accordance with the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care (www.ccac.ca) and the Animal Care Committee of the University of Calgary approved all the experimental procedures as standard operating procedures or as modified standard procedures. After weaning from the breeding colony, mice for paw preference testing were housed in groups of up to 5 mice per cage; males (1 or 2) were usually caged with females to prevent fighting. Cages were clear polycarbonate (29.5 cm x 19.0 cm and 13 cm high) with stainless steel wire grid lids that held feed and water bottle; bedding material was aspen wood chip and virgin unbleached “krinkle cut” paper for nesting. Food (rodent formula 5020, PMI Nutrition International, www.labdiet.com) and water were available ad libitum. The mouse rooms were maintained under controlled conditions (20 ± 1 °C, relative humidity 40%) and 14:10 h light cycle (lights on at 0800 hours). Paw preference testing was conducted in the second half of the light period in the same colony room. Mice were raised specifically for this experiment and were tested usually at 10 – 12 weeks of age. At the conclusion of the testing period, the mice were euthanized by cervical dislocation (according to our SOP E1, General Euthanasia, Animal Health Unit, University of Calgary).

Paw-reaching Measurements
Paw usage was assessed as described in (Biddle & Eales 1999). The mice were fasted for 24 h or less, using an approved modified SOP H20 on Overnight Fasting of Rodents (Animal Health Unit). When the mice were placed in the testing chamber, their paw reaches to retrieve food from the food tube were neither frenetic nor reluctant. Shorter fasting periods, of 1-2 h, of the mouse strains used in this report did not provide test mice that appeared to be motivated to reach into the food tube and retrieve food.

Dimensions of the testing chambers are the same as described in (Collins 1975): “The test apparatus was fabricated of Plexiglas and consisted of five in-line testing cubicles whose inside dimensions were 3.8 cm wide by 5.5 cm deep by 11.5 cm high. A 9-mm glass feeding tube was attached to the front wall of each cubicle 5.75 cm from the floor.” The only difference is that we use a Plexiglas feeding tube, instead of glass. In UW testing chambers, the feeding tube is placed equidistant from the left and right side of the cubicle. Food was placed into the feeding tube with a small spatula and, depending on the individual’s enthusiasm to reach for food and eat, we used the commercial mouse diet or human-grade, natural rolled-oat cereal (unflavored) that was ground into a coarse powder. Sufficient food was placed in the tube so that the mouse reached carefully without digging frenetically.

In the present study, 100 mice of each strain were allowed 50 paw reaches in each of two testing sessions and the number of right- and left-paw entries to retrieve food was counted. At the end of the first session, the mice were returned to their home cage. One week later, the mice were refasted and retested as in the first testing session, as this interval appears to be optimal for memory consolidation (Biddle & Eales 2006).
In 50 mice from each strain, the sequence of paw reaches was recorded. Previously untested mice are described as “naïve”. The measure of paw preference is the right paw entry or RPE score and it is the number of reaches the mouse made with its right paw to retrieve food from the feeding tube in 50 reaches. Population measurements of RPE scores are presented in binned distributions (bin size 3).

Model of Unbiased Non-learner Mouse in an Unbiased World

We use ‘null model’ mice with zero learning (Ribeiro et al. 2010) to compare deviations from actual behaviour (Anderson et al. 2007). The model null-learner mouse is memoryless and each choice of paw is random (unbiased). The behavior of a population of null-learner mice provides an objective ‘metric of learning and memory ability’ and aids in identifying if differences in behaviour among strains are significant by providing a magnitude of learning ability, which previously was defined only as the net differences in average test scores among mice that appear to learn a preference (Biddle & Eales 2006).

In probabilistic unbiased non-learner model mice the probabilities of reaching with the left \( P_L \) and right paw \( P_R \) are identical \((0.5)\) at each reach and the number of reaches using either paw follows a binomial distribution. \( P_R(k) \), the probability that a model mouse uses its right paw \( k \) times in \( n \) reaches, is given by (1), where \( p \) is 0.5:

\[
P_R(k) = \binom{n}{k} p^k (1 - p)^{n-k} = \binom{n}{k} (0.5)^n, \quad \sum_{k=0}^{n} P_R(k) = 1
\]

The RPE distribution of a population of unbiased non-learner mice given \( n \) reaches for each mouse can be deduced from (1), or it can be numerically sampled.
Measures of Learning Ability Between Training Sessions (IR and WIR)

To quantify the degree of learning between training sessions we use the ‘improvement ratio’ (IR). Learning implies using prior knowledge to determine future actions. In our setting, a mouse that acquires a bias in the first UW is expected, if due to learning, to maintain or further increase that trend in the second UW. Note that becoming more lateralized in a UW is not seen as an “improvement of efficiency in reaching” per se. The term “improvement” is used in the sense of further biasing of an acquired bias.

The population’s IR is the fraction of mice with a RPE in the second UW equal to or more biased than in the first UW, if this bias is in the same direction in the two UWs. In a total of 50 paw reaches, IR is 1 if a mouse's RPE in the first UW is 25 or less and if the RPE in the second UW is equal to or less than the RPE in the first UW; otherwise, IR is 0. Similarly, IR is 1 if a mouse’s RPE in the first UW is 25 or greater and if the RPE in the second UW is equal to or greater than in the first set of reaches; otherwise, IR is 0. By convention, mice with RPE of 25 in both worlds have IR of 0.

Learning ability of a mouse strain can be determined by comparing its IR with the IR of unbiased non-learner mice. The IR for a population of 1 million non-learner mice with random unbiased paw-usage was numerically determined to be 0.33, i.e., 33% of mice with no ability to learn will be ‘spurious’ learners by chance; this result can also be obtained analytically from equation (1). The maximum IR possible for a population is 1.

IR is a binary measure of the fraction of mice that improves; thus, it does not capture the extent to which a mouse RPE varies due to learning. For this we use the ‘weighted
IR’ (WIR). For individual mice, we define that WIR = 0 if IR = 0; else, the WIR equals the sum of two components. The first, WIR_1, associated with the first UW, computes how much the RPE differs from the expected RPE given random paw choice: WIR_1 = |25 – RPE(UW1)|. The second, WIR_2, equals how much the RPE in the UW_2 differs from the RPE in UW_1: WIR_2 = |RPE(UW_2) – RPE(UW_1)|. For a mouse i:

\[
WIR(\text{mouse i}) = IR_i \times \left( |25 - RPE_i(UW_{i1})| + |RPE_i(UW_{i2}) - RPE_i(UW_{i1})| \right) \tag{2}
\]

A population’s WIR is the average WIR of the mice (including those with WIR = 0). The numerically estimated WIR of a population of non-learners is 1.23 and the maximum possible WIR is 25 (if the test is based on 50 reaches). From the WIR alone, one cannot determine whether a population’s learning ability is high because most mice changed their RPE score by some amount or because a few mice changed their RPE by a large amount; observing WIR and IR jointly makes this distinction.

Measure of Learning Ability Within a Training Session

Sequences of paw choices in 50 reaches were recorded in two UW tests separated by a one-week interval in 50 previously untested mice of each of the C57BL/6J, DBA/2J, and CDS/Lay strains. We also generated a time series of 50 reaches for unbiased model non-learner mice. If mice learn within training sessions, their sequences of consecutive paw choices ought to be positively correlated. We use the autocorrelation of the series of
paw choices of a mouse as a measure of its degree of learning within a training session (Box et al. 2008; Chatfield 2004; Orfanidis 1988).

If a mouse’s paw-preference behavior changes within a training session due to learning, a high positive autocorrelation between consecutive paw choices is expected and a decrease of autocorrelation with the “lag” between choices is also expected, where “lag” is defined as the number of reaches separating two reaches in a sequence of reaches. If the behavior is purely constitutive, the autocorrelation should be invariant with the lag. Finally, if paw choices are purely random, we expect very weak autocorrelation, similar to that of the null-model mice.

We computed the unbiased autocorrelation of the time series of paw choices of each mouse using the ‘xcorr’ (cross-correlation) function in Matlab 7.4 (version 2007a) and averaged the results for all mice of each strain. For each mouse, the autocorrelation is computed between all reaches, i.e., for all time lags. A mouse with 50 reaches has 49 lags of size 1, i.e., between the first and second reach, between the second and the third, etc., but only 1 lag of size 49 and that is between the first and the last reaches.

To avoid biases in the autocorrelation calculation, we assign values of +1 and -1, respectively, to right-paw and left-paw choices, in each time series of reaches.

Tests of Statistical Significance

Biometrical inferences are based on standard methods (Sokal & Rohlf 1995; Whitlock & Schluter 2009) and alpha is set to 0.05 for tests of statistical significance.
RESULTS

Figure 2 illustrates the strain differences in variation in paw preference by comparing the distributions of RPE scores after 50 paw reaches in the first UW training session of the CDS/Lay, DBA/2J and C57BL/6J strains and the non-learner model mice. The shapes of the population distributions of RPE scores are consistent with previous assessments of these strains in a UW (e.g., Biddle & Eales 1996; Ribeiro et al. 2010).

FIGURE 2

Table 1 summarizes the 50-reach RPE scores in UW1 and UW2 training sessions separated by the 1-week interval. Also shown are the mean RPE of mice of each strain and of model null-learner mice. The mice are described as left-pawed (0 - 23 RPE) or right-pawed (27 - 50 RPE), without regard to the degree of lateralization; individuals with 24 - 26 RPE are considered to be ambilateral (neither left- nor right-pawed). In this sample of mice, there appears to be a small deviation to more left-paw usage in DBA/2J and C57BL/6J, similar in both UW1 and UW2 training sessions for each strain. The populations mean RPE scores are strikingly similar for each strain in the two training sessions. By themselves, these mean RPE scores from the 100 mice of each strain do not reveal that there is a change in paw-preference behavior due to learning.

TABLE 1
The numerical measures of improvement ratio (IR) and weighted improvement ratio (WIR) are summarized in Fig. 3. There is a quantitative increase in the bias of paw preference due to learning between the two training sessions in the UW and there appears to be a functional relationship between the IR and WIR scores. Deviations of these measures from the IR and WIR values of the null learner model mice provide a numerical context to assess the magnitude of these measures from each mouse strain. Clearly, the three strains increase their bias in paw preference between the sessions, and learn different amounts from the training sessions. The results confirm observations previously reported for the three mouse strains (Ribeiro et al. 2010).

Upon inspection of Fig. 3, IR is similar in DBA/2J and C57BL/6J, but the higher WIR of C57BL/6J confirms their stronger response to training than DBA/2J (i.e., a higher degree of change in bias occurs in C57BL/6J than in DBA/2J). CDS/Lay is the weakest learner, but it has some degree of learning since its IR and WIR are significantly higher than in model null learners. The measures of IR and WIR show that, provided there is time for memory consolidation, training causes a behaviour modification due to learning and it is expressed as an increase in the bias of paw preference. This suggests that paw-preference behaviour is not strongly “constitutive”, despite the fact that some strains appear to express a small population bias in direction of paw preference (Table 1).
We further confronted, in each strain, each mouse’s paw choice in the last reach in the first UW with the first choice in the second UW. For null learners, the probability of the two choices being identical is 50%. Values above 50% indicate long-term stability of the learning process. We found that in C57BL/6J, DBA/2J and CDS/Lay, this ratio is 82%, 75%, and 53%, respectively, confirming the long-term effects of previous learning.

Paw Preference Changes Within a UW Training Session

We recorded the paw choices in 50 consecutive reaches in the two UW training sessions for 50 mice of each strain to determine if and how the behaviour of each mouse is modified during a training session and if such changes are in agreement with the learning ability as quantified by IR and WIR. For comparison, we generated a random series of paw choices for unbiased, non-learner model mice. From these data, we compute the unbiased autocorrelation of the time series of paw choices of each mouse and the mean autocorrelation for each strain. This quantity is computed for all 49 possible time lags, given reaches 1 to 50. Figures 4a and 4b show the values of autocorrelation for each time lag for each strain and model non-learner mice for UW1 and UW2 sessions. A linear trend line and its equation are added to estimate the change in autocorrelation as a function of the lag. Note that a more appropriate approximation for the autocorrelation values as a function of lag would be of the form: $\alpha + \beta e^{\gamma \tau}$. However, since the values are well fit by the first order Taylor expansions of this expression in all strains, we opted for showing these in Figures 4a and 4b. The first order approximation is
of the form \((\alpha + \beta\gamma)x\), where \(\beta\gamma\) (the inclination) is a measure of the decrease of correlation between choices with lag.

FIGURE 4

To determine whether the differences in autocorrelation values between the strains are significant, we assessed the distributions of autocorrelations from the strains and the null model for every time lag with a logical set of pair-wise Kolmogorov-Smirnov tests, \(i.e.,\) C57BL/6J vs. DBA/2J, DBA/2/J vs. CDS/Lay, and CDS/Lay vs. null model mice. An increase of the \(p\)-value with the lag is expected since, among other reasons, the number of samples decreases with increase in lag. Results are shown in Figure 5 for the first UW and the differences are significant. Identical \(p\)-values were obtained for the measurements in the second UW (data not shown).

FIGURE 5

The autocorrelation analyses in both UW training sessions (Fig. 4a,b) address our questions in the context of a null learner. The mean autocorrelation for all lags fluctuates around 0 in null learners, due to their random unbiased choices. Learners show weaker fluctuations in autocorrelation as their future paw choices are based on past choices.

Future paw choice in all mice is positively correlated with current and past choices in both training sessions in the UW. Therefore, memory is used to decide paw choice which implies an underlying learning process. C57BL/6J has the highest autocorrelation between successive choices for all lags, followed by DBA/2J, and then CDS/Lay. These
results are in agreement with the inferences from the IR and WIR measures and indicate that paw-preference behavior in the second UW is regulated by what was learned in the previous session. This behavior modification due to learning from past paw choices occurs in the apparent absence of reward-punishment stimulus.

The degree to which paw choice depends on constitutive behaviour or on learning ability is not yet established. However, it is clear that learning plays a definite role, given the comparisons of behavior between the two UW tests, summarized by IR and WIR. The slopes of the trend lines on the positive autocorrelations in Fig. 4a,b suggest that constitutive behavior plays a very weak, if any, role since the mice of all strains modified their behavior to different extents within and between the training sessions. The stronger the learning ability, the weaker is the relative correlation for reaches with increasing lag and the biggest negative slope is for C57BL/6J, which is the strongest learner in the long term between training sessions, followed by DBA/2J, and then CDS/Lay.

The results indicate that paw-preference behavior is highly moldable, with relatively little training, in that the number of reaches in the test is very small compared to the number of reaches a mouse does in the day-to-day life prior to the test. Such adaptability indicates that constitutive behaviours have little, if any, influence on paw preference.

Note that strong correlation between consecutive paw choices inevitably causes high correlation between choices separated by big lags even if only the previous reach is used to make the next decision. Thus, in the short term of a training session, a relatively faster decrease in association with past reaches indicates faster modification of the paw-reaching behaviour within the test. Interestingly, adaptability is likely to require decline of short term memory and, at best, very weak dependence on constitutive behaviours.
The similarity of the patterns of autocorrelation between consecutive UW sessions suggests that the mechanism regulating the learning of paw preference in the three inbred strains is genetically determined. The only apparent difference is that, in all strains, the slopes of the trend lines are smaller in the second UW. This is explained by the fact that, as mice accumulate training, they are expected to change their behaviour more slowly as they reach the limits in bias of paw preference (Biddle & Eales 2006; Ribeiro et al. 2010).

The increases with lag in the magnitude of the fluctuations of the mean autocorrelation are expected (Fig. 4) as the number of samples assessed necessarily decreases with the lag. Relevantly, the fluctuations are stronger for all lags for the model null-learners, followed by CDS/Lay, then DBA/2J, and finally C57BL/6J (Fig. 4). This is further proof that the stronger is the learning ability of a mouse strain, the less random is the paw-preference behavior and the more predictable is the effect of training.

Given these evidences of learning within training sessions, we attempted to observe the changes in bias directly, e.g., by observing how the variance of the biases of each mouse changes within a session. The results are not conclusive in that they neither prove nor disprove the conclusions drawn from the autocorrelation. The only measures we found able to detect behavior modification within training sessions are measures such as the autocorrelation (e.g. mutual information). Direct measures of bias modification within training sessions are not sensitive enough, given the number of mice and reaches in each sample. For this sample size, direct measures of bias modification appear to be only able to detect bias modifications between training sessions.

DISCUSSION
We used unbiased test chambers (UW) to study the dynamics of right- and left-paw choices in three inbred strains of mice. We analyzed and compared the sequences of consecutive paw reaches of previously untested mice in two training sessions separated by a 1-week interval. Mice from the C57BL/6J, DBA/2J and CDS/Lay strains learn a paw preference when trained in a UW since they gain a bias in paw preference in the first UW and further increase that bias in the second UW, virtually always to the same direction as in the first UW, confirming previous observations (Ribeiro et al. 2010).

The three strains differ in the degree of change of lateralization in paw preference with training, indicating that this learning ability is genetically determined to some extent. Since our results are reproducible, regardless of the degree of lateralization of the pair of mice chosen within an inbred strain to produce a new population (Collins 1969, 1991), the handedness measure of “degree of lateralization of the preferred paw” in an individual mouse cannot be the genetic trait in mice. In other words, the degree of paw preference expressed by an individual mouse is contextual and dependent on experience and interaction with the environment. Therefore, without a prior knowledge of that context, the measure of degree of lateralization does not reveal the genotype of a mouse.

Our results suggest that, prior to the mice being tested, any biases in their environment are unlikely to have affected the measurements. If there were significant environmental biases, the mean RPE score of all strains in UW would not be virtually unbiased since training in biased worlds is known to bias the mean RPE of the population in the direction of the biased world (Biddle & Eales 2006).

Analysis of the sequence of consecutive paw choices shows that future choices by individual mice are positively correlated with previous choices when mice are tested in
the first UW as well as in the subsequent UW training session. C57BL/6J mice have the highest positive autocorrelation between successive paw choices for all lags in the sequences of reaches, followed by DBA/2J, and then CDS/Lay, while for model non-learners the mean autocorrelation for all lags is null. Learning or constitutive biases are the two possible causes of positive autocorrelation between successive paw choices. However, constitutive biases cannot explain the variation of the autocorrelation value with the lag between paw choices, since such variation implies a gradual modification of bias within a training session, which implies learning. If paw preference was mostly a constitutive behavior, the autocorrelation would be invariant with the lag.

Relevantly, it is not necessary to have strong constitutive biases in order to exhibit strongly biased behaviours. Strong biases can result from constant reinforcement of initially weak biases that are caused by random unbiased paw choices in the first reaches.

A difference in the rate of memory decay between strains is another explanation that needs to be ruled out as the direct cause of the different autocorrelation values and the variation of autocorrelation with lag between the strains. Note that C57BL/6J has the highest autocorrelation (Fig. 4) and it is also the one with highest IR and WIR (Fig. 3); similarly, CDS/Lay has the lowest autocorrelation and it is the one with lowest IR and WIR. This would not occur if differences in memory decay, rather than learning ability, were the cause of the strain differences in behavior. Stronger memory decay would cause higher variation in autocorrelation with lag, but would also cause lower IR and WIR.

It is of interest to observe that although the C57BL/6J strain is the strongest learner, i.e., has the highest positive autocorrelation between consecutive paw reaches, it also has the fastest decrease in autocorrelation with increasing lag between reaches (Fig. 4). Note
that although the autocorrelation is expected to tend to zero with increasing lag, having higher autocorrelation for lag 1 does not necessarily imply that it decreases faster with lag, unless one assumes, e.g., that all strains use the same number of previous choices to make a decision and have the exactly same rate of memory decay. If paw preference is an adaptive behaviour in mice, an ability to form and retain new associations may give better adaptation in searching and reaching for food. We speculate that a better strategy for adaptation might be to retain information from more immediate past reaches than from distant past reaches. These observations are reminiscent of the reciprocal and antagonistic relationships in the dynamics of short- and long-term memory acquisition and retention found in association with natural variation in the foraging (for) gene and the “rover” and “sitter” phenotypes in D. melanogaster (Kaun et al. 2007; Mery et al. 2007).

In summary, learning of paw preference in the mouse is gradual and can occur in the apparent absence of reward-punishment stimulus. Learning ability is the genetically determined difference between strains and it explains the differences in paw-preference behavior in the UW at the individual as well as at the population level. Biases at the individual level likely result from weak biases that appear by chance early in training and that are enhanced with training, as in a positive feedback mechanism, due to learning. Studies suggest that interactions between individuals may play a role on human hand preference (Ghirlanda et al. 2009). It might be that our mouse populations have equal amounts of left and right-pawed individuals because such interactions do not occur in the laboratory conditions. It is plausible that stochastic fluctuations in paw preference in each initially unbiased mouse, combined with interactions between mice when in the natural environment, would cause lateralization to the left or right at the population level (as a
“Baldwin-like” effect). The present study does not allow confirming or ruling out the possible effects of interactions between mice on individual paw preference. Also, from our results it is premature to speculate about laterality in other species as this requires expanding our sampling to genetically different laboratory mouse strains and other species. We suggest that the autocorrelation analysis done here could be considered in replicated assessment of laterality of paw usage in other species.

Nevertheless, our conclusions are surprisingly in agreement with those from a study of human hand preference (McManus et al. 1988) that concluded that the degree of lateralization is not due to the direction of brain lateralization, but instead it reflects a positive feedback mechanism whereby hand usage itself makes that hand more preferred for future actions. Such a mechanism is also most likely present in mice where small initial fluctuations in paw preference become strong biases due to learning and, most importantly, it explains how individual mice in an inbred strain can exhibit strong biases in paw preference and almost no bias at the population level.

Our study cannot explain the functional causes of the different learning rates of paw preference between strains, but it may lay a foundation for it. For example, it is unknown whether the differences are genetically regulated processes or epigenetic, but we can, for example, rule out interactions with maternal environmental factors since a population generated from two highly lateralized mice (to the same side) has the same paw preference distribution as when it is generated from a pair of weakly lateralized mice of the same genotype (Collins 1991). A question in future studies of hand preference is why there is heritable variation in this adaptive behaviour. One possibility is that the regulating genes have other functions (Buchanan et al. 2009) and their effect on paw
preference is, to some extent, coincidental. Another possibility, which does not exclude the first, is that “optimality” in learning rate depends on the rate of environmental change (Ribeiro 2008) and differences in regulatory factors are evolutionary historical antecedents.

One final question deserves attention. Why do mice learn a direction of paw preference in a UW when use of either paw is equally rewarded and involves the same effort? We observed that mice learn within training sessions and the correlation between consecutive paw choices decreases with increasing lag between the reaches. Perhaps mice integrate previous successes/punishments to make subsequent decisions. Given the absence of punishment in our experimental setting, they may bias paw preference based only on having “more successes” using one paw than the other, due to randomly using that paw more often and all reaches being successful. Such a mechanism of biasing paw preference would be useful in adapting to environments where one paw would be more efficient in reaching than the other.

Another possible contributing factor, which does not exclude the first, is that using one paw more often than the other requires less effort as the mouse specializes. However, this can only explain increasing bias with training and not the appearance of an initial bias, since in initially unbiased mice, the use of either paw involves the same effort. Additionally, we did not detect any difference in motor skills or strength in the mice of the three strains studied; thus, different memory abilities are still required to explain their different degrees of biasing with training. Specialization and consequent decrease of effort, may, nevertheless, enhance the biasing in paw preference. This appears to be the case in Gombe’s chimpanzees (McGrew & Marchant 1999), where more lateralized
individuals are more efficient in fishing for termites. In the future, this may be tested by confronting degrees of biasing in cages where the reaching for food involves different physical effort. The findings in (McGrew & Marchant 1999) further support the hypothesis that laterality emerges from learning rather than being constitutive as well as that it is a stochastic process. Namely, while laterality was found to be beneficial, not all animals developed it, implying that its development may be, as in mice, based on stochastic accumulation of deviations from ambilaterality.

Acknowledgments

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Table 1

Mean RPE and percentage of left- and right-pawed mice from the 50-paw reach tests of 100 CDS/Lay, DBA/2J and C57BL/6J in the two U-worlds (UW1 and UW2). Mean RPE of 1000 null learners model mice is also shown. Mice with RPE scores of 24 to 26 are considered ambilateral.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>UW1 (RPE)</th>
<th>UW2 (RPE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean RPE</td>
<td>0 – 23</td>
</tr>
<tr>
<td>CDS/Lay</td>
<td>25.5</td>
<td>45 %</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>21.9</td>
<td>59 %</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>23.6</td>
<td>52 %</td>
</tr>
<tr>
<td>Null learner</td>
<td>25</td>
<td>34.5 %</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. Mouse using its right paw to reach for food in unbiased UW test chamber. The food tube is equidistant from the left and right sides of the UW. Reproduced with permission (Biddle et al. 1993).

Figure 2. RPE distributions from 50 paw reaches in UW of previously untested (A) model null-learner mice (N = 1000) and the (B) CDS/Lay (N = 100), (C) DBA/2J (N = 100), and (D) C57BL/6J (N = 100) strains.

Figure 3. Relationship between weighted improvement ratio (WIR) and improvement ratio (IR) for the model null learner mice, CDS/Lay, DBA/2J and C57BL/6J strains.

Figure 4. Mean autocorrelation values of the series of paw choices as a function of the lag between choices for each strain and model null learner mice when they are (A) naive and (B) after a 1-week interval. Linear approximations are shown in the figure.

Figure 5. Significance of the difference in autocorrelation at each lag from the inbred strains and model null learner mice. The distributions of autocorrelations for every time lag were compared with a logical set of pair-wise Kolmogorov-Smirnov tests between C57BL/6J and DBA/2J, between DBA/2J and CDS/Lay, and between CDS/Lay and model null learner mice. Y-axis is in log scale.
Learning of paw preference in mice is strain-dependent, gradual and based on short-term memory of previous reaches

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ABSTRACT

We study the dynamics of mouse paw-preference learning in unbiased symmetrical test chambers where mice can freely choose which paw to reach. When compared to non-learner model mice, three strains exhibit different degrees of learning within and between two training sessions separated by a one-week interval. While paw preference is probabilistic, positive autocorrelation between paw choices made by individual mice in a training session shows that bias in paw preference changes gradually with successive paw reaches and it is concordant between sessions. Within a session, the degree of positive autocorrelation between consecutive paw choices differs among strains and it decreases faster with increasing lag between reaches in stronger than in weak learners, suggesting that the degree of learning is the genetic variable in mice. We propose that strong biases in individual mice result from weak biases that appear by chance early in training and increase by a positive feedback mechanism due to learning from training. This explains how individual mice of each strain are strongly biased when there is almost no bias at the population level. Decrease of autocorrelation with lag shows that constitutive behaviours play a minor role and, hence, there is adaptability in paw preference to environmental changes. We conclude that paw-preference learning in mice is genetically dependent on past experience, changes gradually within training sessions, and is based on short-term memory; it also has long-term effects, provided time for memory consolidation.

Key words: paw preference, mouse, autocorrelation, null model, learning, probabilistic, adaptation, short and long term memory
INTRODUCTION

The probabilistic nature of the interaction between individuals and their environment is a source of diversity in complex traits (Den Nijs & Sterk 1980; van Baarlen et al. 2000). Phenotypic heterogeneity in morphology and behaviour is a key factor in adaptability and survival (Hamilton et al. 1990; Maynard Smith 1978; Mayr 1997, 2004). What determines the extent of phenotypic diversity within populations still requires much study (Mayr 1997, 2004), especially in behavioural traits such as human hand preference (McManus 2002; Tommasi 2009; Rodriguez et al. 2010). Genes responsible for influencing such behavioural traits are also largely unknown (Corballis 2009).

Paw preference of laboratory mice provides a practical model without the cultural biases and developmental asymmetries that affect human handedness (Medland et al. 2009). When mice reach for food, their paw preference is a quantifiable behavioural trait and the inbred strains allow replication and comparison of the trait among different genotypes under various experimental conditions (Biddle & Eales 2006). Simplicity of mouse pawedness should facilitate characterization of its underlying learning process.

FIGURE 1

Mouse paw usage was first assessed in unbiased or ‘U-World’ (UW) test chambers (Fig. 1) with a central food tube that does not hamper reaching with either forepaw (Collins 1968). Direction of paw preference is the right-paw entry (RPE) or the number of reaches with the right paw in a specified number of reaches. Populations of genetically
identical individuals in the form of inbred strains show wide phenotypic diversity in RPE values (Collins 1968; Signore et al. 1991; Takeda & Endo 1993; Biddle & Eales 1996).

In the UW, paw preference appears to be genetically neutral because most strains have an equal number of right and left-pawed mice (Collins 1968; Biddle & Eales 1996). Selection of parents with more biased paw usage in the C57BL/6J strain does not produce more left- or right-pawed offspring (Collins 1969). The reproducible and distinct shape of the RPE distributions of the inbred strains implies that genetic differences in this mouse trait are in the degree of lateralization, not in its direction (Collins 1985). In agreement, bidirectional selective breeding of genetically heterogeneous mice produced divergent lines of highly lateralized and weakly lateralized mice in a few generations and these lines can be maintained by random breeding without losing the selected behaviour (Collins 1985, 1991). Quantitative trait locus analysis detected a positive, but yet to be validated, association between variation in degree of lateralization and marker genes on mouse chromosome 4 (Roubertoux et al. 2003). Paw preference in mice has also been studied in other contexts, such as its dependence on the corpus callosum (Ward et al. 1987; Bulman-Fleming et al. 1992; Gruber et al. 1991; Biddle & Eales 1996), hippocampal “mossy fibre projections” (Lipp et al. 1996) and immune reactivity (Neveu et al. 1988; Neveu et al. 1991; Denenberg et al. 1991a, 1991b).

Paw preference and motor lateralization has been studied in other animals, such as cats (Wells & Millsopp 2009), horses (McGreevy & Thomson 2006) and dogs (Wells 2003; Poyser et al. 2006; Batt et al. 2008). One result, relevant to the present study, is that distributions of handedness scores from different tests of individual dogs (Poyser et al. 2006) show large variation in lateralization of the left- and right-paw preference, whereas
there is no bias at the population level (e.g., Wells 2003; Batt et al. 2008). This is also observed in several mouse strains (Biddle and Eales 1996, 2006).

Large sample size in these studies appears to be necessary (Ribeiro et al. 2010). For example, it is still unclear whether nonhuman primates exhibit significant bias in hand preference at the population level or simply express a sampling error that is inflated when individuals with small and unequal sample sizes make a disproportionate contribution to population-level measures (Palmer 2002, 2003). Resolution of these questions will aid in better understanding the evolutionary origin of human hand preference (Rogers 2009).

Here we focus on the role of learning in the paw preference behaviour of various mouse strains and ask what determines the degree of lateralization in an unbiased, symmetrical UW where mice freely choose the paw to reach. As in other animals (Magat & Brown 2009), a process of learning and memory regulates mouse paw preference in biased test chambers with the food tube placed to the right or left side (Biddle & Eales 1999). Kinetic analysis established that paw preference in biased worlds is an adaptive behaviour since mice show a directed response to changing environment and their future performance is genotypically dependent on past experience (Biddle & Eales 2006). Therefore, mice learn paw preference when training in biased worlds and different strains differ in how much the training affects the behaviour (Ribeiro et al. 2010).

Although the distributions of RPE scores of the inbred strains are reproducible, the RPE score of a mouse does not predict its strain of origin (Collins 1969). Paw preference in the UW was suggested, but not formally demonstrated to have a stochastic component (Collins et al. 1993). Recently, it was proposed that learning rate per successful reach of
individual mice is the genetically deterministic trait that “shapes” the different RPE
distribution of each strain (Ribeiro et al. 2010).

We address three questions about paw-preference behaviour in the UW. Do mice
learn a direction of paw preference in a UW and, if so, why, when use of either paw
provides equal reward and involves the same effort? How does paw preference change
with training in a UW: Is it a gradual change during a training session or is it detectable
only when comparing tests after a period of memory consolidation? Finally, do mice have
constitutive biases in the direction of paw preference and what is the relative importance
of constitutive behaviour versus training in mice of different inbred strains? To address
these questions, we examine the process of reaching in individual mice on a reach-to-
reach basis during training sessions, compare results between training sessions, and
compare the measurements with model null-learner mice with random paw choices.

METHODS

When referring to “memory”, we mean “implicit memory” or an unconscious form of
memory. In tests of implicit memory, there is no conscious effort to retrieve recent
experiences to perform the tests, which we believe to be the case here (Roediger 1990;
Schacter 1987). When referring to “learning”, we imply “procedural learning”, i.e. a
behavioural change by acquisition of implicit memory (Nissen & Bullemer 1987).

Measurements of paw preference were made in unbiased test chambers, described as
U-world or UW test chambers. We assume that reaching with the right or left paw in a
UW involves identical effort and that both possible choices for the mouse are equally
rewarded. This is necessary if one is to assess what aspect of the behaviour is constitutive and what aspect is due to learning because, in a UW setting, the mice ought to use a constitutive behaviour “unbiased” by environmental factors, such as would occur in right- or left-biased test chambers. A previous study suggested that mice change paw preference in the absence of a reward versus punishment stimulus in a UW (Ribeiro et al. 2010). Therefore, if learning can be assessed in the UW setting, it would have the advantage of allowing the detection of correlations in temporal series of paw choices that would indicate that there is learning within a training session.

Mouse Strains

CDS/LayBid, DBA/2JBid, and C57BL/6JBid are registered inbred strains of the laboratory mouse. Their origins are described in (Biddle and Eales 1996) and they are maintained as genetic reference strains with continued sister-brother inbreeding. For brevity, our ILAR registered laboratory code (“Bid”) is not included in the strain names here (http://dels.nas.ed.global/ilar.Lab-Codes). We assessed paw preference of C57BL/6J, DBA/2J and CDS/Lay mice as each strain has a documented ability to learn a bias in paw preference with training in biased test chambers, but differ in learning abilities in that context (Ribeiro et al. 2010). We used 300 mice in this study (100 mice of each strain). This amount was shown to be required for the distributions of paw preference of the strains to be strongly consistent between measurements due to the stochastic nature of the paw reaching behavior (Ribeiro et al, 2010).
The mice were cared for in accordance with the *Guide to the Care and Use of Experimental Animals* of the Canadian Council on Animal Care (www.ccac.ca) and the Animal Care Committee of the University of Calgary approved all the experimental procedures as standard operating procedures or as modified standard procedures. After weaning from the breeding colony, mice for paw preference testing were housed in groups of up to 5 mice per cage; males (1 or 2) were usually caged with females to prevent fighting. Cages were clear polycarbonate (29.5 cm x 19.0 cm and 13 cm high) with stainless steel wire grid lids that held feed and water bottle; bedding material was aspen wood chip and virgin unbleached “krinkle cut” paper for nesting. Food (rodent formula 5020, PMI Nutrition International, www.labdiet.com) and water were available ad libitum. The mouse rooms were maintained under controlled conditions (20 ± 1 °C, relative humidity 40%) and 14:10 h light cycle (lights on at 0800 hours). Paw preference testing was conducted in the second half of the light period in the same colony room. Mice were raised specifically for this experiment and were tested usually at 10 – 12 weeks of age. At the conclusion of the testing period, the mice were euthanized by cervical dislocation (according to our SOP E1, General Euthanasia, Animal Health Unit, University of Calgary).

**Paw-reaching Measurements**
Paw usage was assessed as described in (Biddle & Eales 1999). The mice were fasted for 24 h or less, using an approved modified SOP H20 on Overnight Fasting of Rodents (Animal Health Unit). When the mice were placed in the testing chamber, their paw reaches to retrieve food from the food tube were neither frenetic nor reluctant. Shorter fasting periods, of 1-2 h, of the mouse strains used in this report did not provide test mice that appeared to be motivated to reach into the food tube and retrieve food.

Dimensions of the testing chambers are the same as described in (Collins 1975): "The test apparatus was fabricated of Plexiglas and consisted of five in-line testing cubicles whose inside dimensions were 3.8 cm wide by 5.5 cm deep by 11.5 cm high. A 9-mm glass feeding tube was attached to the front wall of each cubicle 5.75 cm from the floor.” The only difference is that we use a Plexiglas feeding tube, instead of glass. In UW testing chambers, the feeding tube is placed equidistant from the left and right side of the cubicle. Food was placed into the feeding tube with a small spatula and, depending on the individual’s enthusiasm to reach for food and eat, we used the commercial mouse diet or human-grade, natural rolled-oat cereal (unflavored) that was ground into a coarse powder. Sufficient food was placed in the tube so that the mouse reached carefully without digging frenetically.

In the present study, 100 mice of each strain were allowed 50 paw reaches in each of two testing sessions and the number of right- and left-paw entries to retrieve food was counted. At the end of the first session, the mice were returned to their home cage. One week later, the mice were refasted and retested as in the first testing session, as this interval appears to be optimal for memory consolidation (Biddle & Eales 2006).
In 50 mice from each strain, the sequence of paw reaches was recorded. Previously untested mice are described as “naïve”. The measure of paw preference is the right paw entry or RPE score and it is the number of reaches the mouse made with its right paw to retrieve food from the feeding tube in 50 reaches. Population measurements of RPE scores are presented in binned distributions (bin size 3).

Model of Unbiased Non-learner Mouse in an Unbiased World

We use ‘null model’ mice with zero learning (Ribeiro et al. 2010) to compare deviations from actual behaviour (Anderson et al. 2007). The model null-learner mouse is memoryless and each choice of paw is random (unbiased). The behavior of a population of null-learner mice provides an objective ‘metric of learning and memory ability’ and aids in identifying if differences in behaviour among strains are significant by providing a magnitude of learning ability, which previously was defined only as the net differences in average test scores among mice that appear to learn a preference (Biddle & Eales 2006).

In probabilistic unbiased non-learner model mice the probabilities of reaching with the left ($P_L$) and right paw ($P_R$) are identical (0.5) at each reach and the number of reaches using either paw follows a binomial distribution. $P_R(k)$, the probability that a model mouse uses its right paw $k$ times in $n$ reaches, is given by (1), where $p$ is 0.5:

$$P_R(k) = \binom{n}{k} p^k (1 - p)^{n-k} = \binom{n}{k} (0.5)^n , \quad \sum_{k=0}^{n} P_R(k) = 1 \quad (1)$$

The RPE distribution of a population of unbiased non-learner mice given $n$ reaches for each mouse can be deduced from (1), or it can be numerically sampled.
Measures of Learning Ability Between Training Sessions (IR and WIR)

To quantify the degree of learning between training sessions we use the ‘improvement ratio’ (IR). Learning implies using prior knowledge to determine future actions. In our setting, a mouse that acquires a bias in the first UW is expected, if due to learning, to maintain or further increase that trend in the second UW. Note that becoming more lateralized in a UW is not seen as an “improvement of efficiency in reaching” per se. The term “improvement” is used in the sense of further biasing of an acquired bias.

The population’s IR is the fraction of mice with a RPE in the second UW equal to or more biased than in the first UW, if this bias is in the same direction in the two UWs. In a total of 50 paw reaches, IR is 1 if a mouse's RPE in the first UW is 25 or less and if the RPE in the second UW is equal to or less than the RPE in the first UW; otherwise, IR is 0. Similarly, IR is 1 if a mouse’s RPE in the first UW is 25 or greater and if the RPE in the second UW is equal to or greater than in the first set of reaches; otherwise, IR is 0. By convention, mice with RPE of 25 in both worlds have IR of 0.

Learning ability of a mouse strain can be determined by comparing its IR with the IR of unbiased non-learner mice. The IR for a population of 1 million non-learner mice with random unbiased paw-usage was numerically determined to be 0.33, i.e., 33% of mice with no ability to learn will be ‘spurious’ learners by chance; this result can also be obtained analytically from equation (1). The maximum IR possible for a population is 1.

IR is a binary measure of the fraction of mice that improves; thus, it does not capture the extent to which a mouse RPE varies due to learning. For this we use the ‘weighted
IR’ (WIR). For individual mice, we define that WIR = 0 if IR = 0; else, the WIR equals the sum of two components. The first, WIR₁, associated with the first UW, computes how much the RPE differs from the expected RPE given random paw choice: WIR₁ = |25 – RPE(UW₁)|. The second, WIR₂, equals how much the RPE in the UW₂ differs from the RPE in UW₁: WIR₂ = |RPE(UW₂) – RPE(UW₁)|. For a mouse i:

\[
\text{WIR(mouse } i ) = IR_i \times (|25 - RPE_i(UW_1)| + |RPE_i(UW_2) - RPE_i(UW_1)|)
\]

A population’s WIR is the average WIR of the mice (including those with WIR = 0). The numerically estimated WIR of a population of non-learners is 1.23 and the maximum possible WIR is 25 (if the test is based on 50 reaches). From the WIR alone, one cannot determine whether a population’s learning ability is high because most mice changed their RPE score by some amount or because a few mice changed their RPE by a large amount; observing WIR and IR jointly makes this distinction.

Measure of Learning Ability Within a Training Session

Sequences of paw choices in 50 reaches were recorded in two UW tests separated by a one-week interval in 50 previously untested mice of each of the C57BL/6J, DBA/2J, and CDS/Lay strains. We also generated a time series of 50 reaches for unbiased model non-learner mice. If mice learn within training sessions, their sequences of consecutive paw choices ought to be positively correlated. We use the autocorrelation of the series of
paw choices of a mouse as a measure of its degree of learning within a training session (Box et al. 2008; Chatfield 2004; Orfanidis 1988).

If a mouse’s paw-preference behavior changes within a training session due to learning, a high positive autocorrelation between consecutive paw choices is expected and a decrease of autocorrelation with the “lag” between choices is also expected, where “lag” is defined as the number of reaches separating two reaches in a sequence of reaches. If the behavior is purely constitutive, the autocorrelation should be invariant with the lag. Finally, if paw choices are purely random, we expect very weak autocorrelation, similar to that of the null-model mice.

We computed the unbiased autocorrelation of the time series of paw choices of each mouse using the ‘xcorr’ (cross-correlation) function in Matlab 7.4 (version 2007a) and averaged the results for all mice of each strain. For each mouse, the autocorrelation is computed between all reaches, i.e., for all time lags. A mouse with 50 reaches has 49 lags of size 1, i.e., between the first and second reach, between the second and the third, etc., but only 1 lag of size 49 and that is between the first and the last reaches.

To avoid biases in the autocorrelation calculation, we assign values of +1 and -1, respectively, to right-paw and left-paw choices, in each time series of reaches.

Tests of Statistical Significance

Biometrical inferences are based on standard methods (Sokal & Rohlf 1995; Whitlock & Schluter 2009) and alpha is set to 0.05 for tests of statistical significance.
RESULTS

Figure 2 illustrates the strain differences in variation in paw preference by comparing the distributions of RPE scores after 50 paw reaches in the first UW training session of the CDS/Lay, DBA/2J and C57BL/6J strains and the non-learner model mice. The shapes of the population distributions of RPE scores are consistent with previous assessments of these strains in a UW (e.g., Biddle & Eales 1996; Ribeiro et al. 2010).

FIGURE 2

Table 1 summarizes the 50-reach RPE scores in UW1 and UW2 training sessions separated by the 1-week interval. Also shown are the mean RPE of mice of each strain and of model null-learner mice. The mice are described as left-pawed (0 - 23 RPE) or right-pawed (27 - 50 RPE), without regard to the degree of lateralization; individuals with 24 - 26 RPE are considered to be ambilateral (neither left- nor right-pawed). In this sample of mice, there appears to be a small deviation to more left-paw usage in DBA/2J and C57BL/6J, similar in both UW1 and UW2 training sessions for each strain. The populations mean RPE scores are strikingly similar for each strain in the two training sessions. By themselves, these mean RPE scores from the 100 mice of each strain do not reveal that there is a change in paw-preference behavior due to learning.

TABLE 1
The numerical measures of improvement ratio (IR) and weighted improvement ratio (WIR) are summarized in Fig. 3. There is a quantitative increase in the bias of paw preference due to learning between the two training sessions in the UW and there appears to be a functional relationship between the IR and WIR scores. Deviations of these measures from the IR and WIR values of the null learner model mice provide a numerical context to assess the magnitude of these measures from each mouse strain. Clearly, the three strains increase their bias in paw preference between the sessions, and learn different amounts from the training sessions. The results confirm observations previously reported for the three mouse strains (Ribeiro et al. 2010).

FIGURE 3

Upon inspection of Fig. 3, IR is similar in DBA/2J and C57BL/6J, but the higher WIR of C57BL/6J confirms their stronger response to training than DBA/2J (i.e., a higher degree of change in bias occurs in C57BL/6J than in DBA/2J). CDS/Lay is the weakest learner, but it has some degree of learning since its IR and WIR are significantly higher than in model null learners. The measures of IR and WIR show that, provided there is time for memory consolidation, training causes a behaviour modification due to learning and it is expressed as an increase in the bias of paw preference. This suggests that paw-preference behaviour is not strongly “constitutive”, despite the fact that some strains appear to express a small population bias in direction of paw preference (Table 1).
We further confronted, in each strain, each mouse’s paw choice in the last reach in the first UW with the first choice in the second UW. For null learners, the probability of the two choices being identical is 50%. Values above 50% indicate long-term stability of the learning process. We found that in C57BL/6J, DBA/2J and CDS/Lay, this ratio is 82%, 75%, and 53%, respectively, confirming the long-term effects of previous learning.

Paw Preference Changes Within a UW Training Session

We recorded the paw choices in 50 consecutive reaches in the two UW training sessions for 50 mice of each strain to determine if and how the behaviour of each mouse is modified during a training session and if such changes are in agreement with the learning ability as quantified by IR and WIR. For comparison, we generated a random series of paw choices for unbiased, non-learner model mice. From these data, we compute the unbiased autocorrelation of the time series of paw choices of each mouse and the mean autocorrelation for each strain. This quantity is computed for all 49 possible time lags, given reaches 1 to 50. Figures 4a and 4b show the values of autocorrelation for each time lag for each strain and model non-learner mice for UW1 and UW2 sessions.

A linear trend line and its equation are added to estimate the change in autocorrelation as a function of the lag. Note that a more appropriate approximation for the autocorrelation values as a function of lag would be of the form: $\alpha + \beta e^{\gamma x}$. However, since the values are well fit by the first order Taylor expansions of this expression in all strains, we opted for showing these in Figures 4a and 4b. The first order approximation is
of the form \((\alpha + \beta) + \beta \gamma x\), where \(\beta \gamma\) (the inclination) is a measure of the decrease of correlation between choices with lag.

To determine whether the differences in autocorrelation values between the strains are significant, we assessed the distributions of autocorrelations from the strains and the null model for every time lag with a logical set of pair-wise Kolmogorov-Smirnov tests, \(i.e.,\) C57BL/6J vs. DBA/2J, DBA/2/J vs. CDS/Lay, and CDS/Lay vs. null model mice. An increase of the \(p\)-value with the lag is expected since, among other reasons, the number of samples decreases with increase in lag. Results are shown in Figure 5 for the first UW and the differences are significant. Identical \(p\)-values were obtained for the measurements in the second UW (data not shown).

The autocorrelation analyses in both UW training sessions (Fig. 4a,b) address our questions in the context of a null learner. The mean autocorrelation for all lags fluctuates around 0 in null learners, due to their random unbiased choices. Learners show weaker fluctuations in autocorrelation as their future paw choices are based on past choices.

Future paw choice in all mice is positively correlated with current and past choices in both training sessions in the UW. Therefore, memory is used to decide paw choice which implies an underlying learning process. C57BL/6J has the highest autocorrelation between successive choices for all lags, followed by DBA/2J, and then CDS/Lay. These
results are in agreement with the inferences from the IR and WIR measures and indicate that paw-preference behavior in the second UW is regulated by what was learned in the previous session. This behavior modification due to learning from past paw choices occurs in the apparent absence of reward-punishment stimulus.

The degree to which paw choice depends on constitutive behaviour or on learning ability is not yet established. However, it is clear that learning plays a definite role, given the comparisons of behavior between the two UW tests, summarized by IR and WIR. The slopes of the trend lines on the positive autocorrelations in Fig. 4a,b suggest that constitutive behavior plays a very weak, if any, role since the mice of all strains modified their behavior to different extents within and between the training sessions. The stronger the learning ability, the weaker is the relative correlation for reaches with increasing lag and the biggest negative slope is for C57BL/6J, which is the strongest learner in the long term between training sessions, followed by DBA/2J, and then CDS/Lay.

The results indicate that paw-preference behavior is highly moldable, with relatively little training, in that the number of reaches in the test is very small compared to the number of reaches a mouse does in the day-to-day life prior to the test. Such adaptability indicates that constitutive behaviours have little, if any, influence on paw preference.

Note that strong correlation between consecutive paw choices inevitably causes high correlation between choices separated by big lags even if only the previous reach is used to make the next decision. Thus, in the short term of a training session, a relatively faster decrease in association with past reaches indicates faster modification of the paw-reaching behaviour within the test. Interestingly, adaptability is likely to require decline of short term memory and, at best, very weak dependence on constitutive behaviours.
The similarity of the patterns of autocorrelation between consecutive UW sessions suggests that the mechanism regulating the learning of paw preference in the three inbred strains is genetically determined. The only apparent difference is that, in all strains, the slopes of the trend lines are smaller in the second UW. This is explained by the fact that, as mice accumulate training, they are expected to change their behaviour more slowly as they reach the limits in bias of paw preference (Biddle & Eales 2006; Ribeiro et al. 2010). The increases with lag in the magnitude of the fluctuations of the mean autocorrelation are expected (Fig. 4) as the number of samples assessed necessarily decreases with the lag. Relevantly, the fluctuations are stronger for all lags for the model null-learners, followed by CDS/Lay, then DBA/2J, and finally C57BL/6J (Fig. 4). This is further proof that the stronger is the learning ability of a mouse strain, the less random is the paw-preference behavior and the more predictable is the effect of training.

Given these evidences of learning within training sessions, we attempted to observe the changes in bias directly, e.g., by observing how the variance of the biases of each mouse changes within a session. The results are not conclusive in that they neither prove nor disprove the conclusions drawn from the autocorrelation. The only measures we found able to detect behavior modification within training sessions are measures such as the autocorrelation (e.g. mutual information). Direct measures of bias modification within training sessions are not sensitive enough, given the number of mice and reaches in each sample. For this sample size, direct measures of bias modification appear to be only able to detect bias modifications between training sessions.

**DISCUSSION**
We used unbiased test chambers (UW) to study the dynamics of right- and left-paw choices in three inbred strains of mice. We analyzed and compared the sequences of consecutive paw reaches of previously untested mice in two training sessions separated by a 1-week interval. Mice from the C57BL/6J, DBA/2J and CDS/Lay strains learn a paw preference when trained in a UW since they gain a bias in paw preference in the first UW and further increase that bias in the second UW, virtually always to the same direction as in the first UW, confirming previous observations (Ribeiro et al. 2010).

The three strains differ in the degree of change of lateralization in paw preference with training, indicating that this learning ability is genetically determined to some extent. Since our results are reproducible, regardless of the degree of lateralization of the pair of mice chosen within an inbred strain to produce a new population (Collins 1969, 1991), the handedness measure of “degree of lateralization of the preferred paw” in an individual mouse cannot be the genetic trait in mice. In other words, the degree of paw preference expressed by an individual mouse is contextual and dependent on experience and interaction with the environment. Therefore, without a prior knowledge of that context, the measure of degree of lateralization does not reveal the genotype of a mouse.

Our results suggest that, prior to the mice being tested, any biases in their environment are unlikely to have affected the measurements. If there were significant environmental biases, the mean RPE score of all strains in UW would not be virtually unbiased since training in biased worlds is known to bias the mean RPE of the population in the direction of the biased world (Biddle & Eales 2006).

Analysis of the sequence of consecutive paw choices shows that future choices by individual mice are positively correlated with previous choices when mice are tested in
the first UW as well as in the subsequent UW training session. C57BL/6J mice have the
highest positive autocorrelation between successive paw choices for all lags in the
sequences of reaches, followed by DBA/2J, and then CDS/Lay, while for model non-
learners the mean autocorrelation for all lags is null. Learning or constitutive biases are
the two possible causes of positive autocorrelation between successive paw choices.
However, constitutive biases cannot explain the variation of the autocorrelation value
with the lag between paw choices, since such variation implies a gradual modification of
bias within a training session, which implies learning. If paw preference was mostly a
constitutive behavior, the autocorrelation would be invariant with the lag.

Relevantly, it is not necessary to have strong constitutive biases in order to exhibit
strongly biased behaviours. Strong biases can result from constant reinforcement of
initially weak biases that are caused by random unbiased paw choices in the first reaches.

A difference in the rate of memory decay between strains is another explanation that
needs to be ruled out as the direct cause of the different autocorrelation values and the
variation of autocorrelation with lag between the strains. Note that C57BL/6J has the
highest autocorrelation (Fig. 4) and it is also the one with highest IR and WIR (Fig. 3);
similarly, CDS/Lay has the lowest autocorrelation and it is the one with lowest IR and
WIR. This would not occur if differences in memory decay, rather than learning ability,
were the cause of the strain differences in behavior. Stronger memory decay would cause
higher variation in autocorrelation with lag, but would also cause lower IR and WIR.

It is of interest to observe that although the C57BL/6J strain is the strongest learner,
i.e., has the highest positive autocorrelation between consecutive paw reaches, it also has
the fastest decrease in autocorrelation with increasing lag between reaches (Fig. 4). Note
that although the autocorrelation is expected to tend to zero with increasing lag, having higher autocorrelation for lag 1 does not necessarily imply that it decreases faster with lag, unless one assumes, e.g., that all strains use the same number of previous choices to make a decision and have the exactly same rate of memory decay. If paw preference is an adaptive behaviour in mice, an ability to form and retain new associations may give better adaptation in searching and reaching for food. We speculate that a better strategy for adaptation might be to retain information from more immediate past reaches than from distant past reaches. These observations are reminiscent of the reciprocal and antagonistic relationships in the dynamics of short- and long-term memory acquisition and retention found in association with natural variation in the foraging (for) gene and the “rover” and “sitter” phenotypes in *D. melanogaster* (Kaun et al. 2007; Mery et al. 2007).

In summary, learning of paw preference in the mouse is gradual and can occur in the apparent absence of reward-punishment stimulus. Learning ability is the genetically determined difference between strains and it explains the differences in paw-preference behavior in the UW at the individual as well as at the population level. Biases at the individual level likely result from weak biases that appear by chance early in training and that are enhanced with training, as in a positive feedback mechanism, due to learning.

Studies suggest that interactions between individuals may play a role on human hand preference (Ghirlanda et al. 2009). It might be that our mouse populations have equal amounts of left and right-pawed individuals because such interactions do not occur in the laboratory conditions. It is plausible that stochastic fluctuations in paw preference in each initially unbiased mouse, combined with interactions between mice when in the natural environment, would cause lateralization to the left or right at the population level (as a
“Baldwin-like” effect). The present study does not allow confirming or ruling out the possible effects of interactions between mice on individual paw preference. Also, from our results it is premature to speculate about laterality in other species as this requires expanding our sampling to genetically different laboratory mouse strains and other species. We suggest that the autocorrelation analysis done here could be considered in replicated assessment of laterality of paw usage in other species.

Nevertheless, our conclusions are surprisingly in agreement with those from a study of human hand preference (McManus et al. 1988) that concluded that the degree of lateralization is not due to the direction of brain lateralization, but instead it reflects a positive feedback mechanism whereby hand usage itself makes that hand more preferred for future actions. Such a mechanism is also most likely present in mice where small initial fluctuations in paw preference become strong biases due to learning and, most importantly, it explains how individual mice in an inbred strain can exhibit strong biases in paw preference and almost no bias at the population level.

Our study cannot explain the functional causes of the different learning rates of paw preference between strains, but it may lay a foundation for it. For example, it is unknown whether the differences are genetically regulated processes or epigenetic, but we can, for example, rule out interactions with maternal environmental factors since a population generated from two highly lateralized mice (to the same side) has the same paw preference distribution as when it is generated from a pair of weakly lateralized mice of the same genotype (Collins 1991). A question in future studies of hand preference is why there is heritable variation in this adaptive behaviour. One possibility is that the regulating genes have other functions (Buchanan et al. 2009) and their effect on paw
preference is, to some extent, coincidental. Another possibility, which does not exclude the first, is that “optimality” in learning rate depends on the rate of environmental change (Ribeiro 2008) and differences in regulatory factors are evolutionary historical antecedents.

One final question deserves attention. Why do mice learn a direction of paw preference in a UW when use of either paw is equally rewarded and involves the same effort? We observed that mice learn within training sessions and the correlation between consecutive paw choices decreases with increasing lag between the reaches. Perhaps mice integrate previous successes/punishments to make subsequent decisions. Given the absence of punishment in our experimental setting, they may bias paw preference based only on having “more successes” using one paw than the other, due to randomly using that paw more often and all reaches being successful. Such a mechanism of biasing paw preference would be useful in adapting to environments where one paw would be more efficient in reaching than the other.

Another possible contributing factor, which does not exclude the first, is that using one paw more often than the other requires less effort as the mouse specializes. However, this can only explain increasing bias with training and not the appearance of an initial bias, since in initially unbiased mice, the use of either paw involves the same effort. Additionally, we did not detect any difference in motor skills or strength in the mice of the three strains studied; thus, different memory abilities are still required to explain their different degrees of biasing with training. Specialization and consequent decrease of effort, may, nevertheless, enhance the biasing in paw preference. This appears to be the case in Gombe’s chimpanzees (McGrew & Marchant 1999), where more lateralized
individuals are more efficient in fishing for termites. In the future, this may be tested by confronting degrees of biasing in cages where the reaching for food involves different physical effort. The findings in (McGrew & Marchant 1999) further support the hypothesis that laterality emerges from learning rather than being constitutive as well as that it is a stochastic process. Namely, while laterality was found to be beneficial, not all animals developed it, implying that its development may be, as in mice, based on stochastic accumulation of deviations from ambilaterality.

Acknowledgments

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References


Biddle, F. G. & Eales, B. A. 1996. The degree of lateralization of paw usage (pawedness) in the mouse is defined by three major phenotypes. *Behavior Genetics, 126*, 391-406.


Table 1

Mean RPE and percentage of left- and right-pawed mice from the 50-paw reach tests of 100 CDS/Lay, DBA/2J and C57BL/6J in the two U-worlds (UW1 and UW2). Mean RPE of 1000 null learners model mice is also shown. Mice with RPE scores of 24 to 26 are considered ambilateral.

<table>
<thead>
<tr>
<th>Mouse</th>
<th>UW1 (RPE)</th>
<th>UW2 (RPE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean RPE</td>
<td>0 – 23</td>
</tr>
<tr>
<td>CDS/Lay</td>
<td>25.5</td>
<td>45 %</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>21.9</td>
<td>59 %</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>23.6</td>
<td>52 %</td>
</tr>
<tr>
<td>Null learner</td>
<td>25</td>
<td>34.5 %</td>
</tr>
</tbody>
</table>
Figure 1. Mouse using its right paw to reach for food in unbiased UW test chamber. The food tube is equidistant from the left and right sides of the UW. Reproduced with permission (Biddle et al. 1993).

Figure 2. RPE distributions from 50 paw reaches in UW of previously untested (A) model null-learner mice (N = 1000) and the (B) CDS/Lay (N = 100), (C) DBA/2J (N = 100), and (D) C57BL/6J (N = 100) strains.

Figure 3. Relationship between weighted improvement ratio (WIR) and improvement ratio (IR) for the model null learner mice, CDS/Lay, DBA/2J and C57BL/6J strains.

Figure 4. Mean autocorrelation values of the series of paw choices as a function of the lag between choices for each strain and model null learner mice when they are (A) naive and (B) after a 1-week interval. Linear approximations are shown in the figure.

Figure 5. Significance of the difference in autocorrelation at each lag from the inbred strains and model null learner mice. The distributions of autocorrelations for every time lag were compared with a logical set of pair-wise Kolmogorov-Smirnov tests between C57BL/6J and DBA/2J, between DBA/2J and CDS/Lay, and between CDS/Lay and model null learner mice. Y-axis is in log scale.
Acknowledgments

A.S.R. thanks the support of the Academy of Finland and Tekes. F.G.B. thanks the Alberta Children's Hospital Research Foundation for salary support and the Life and Environmental Science Animal Resource Center and Department of Biological Sciences for accommodating this work within the Faculty of Science of the University of Calgary. We thank Sharif Chowdhury and Antii Hakkinen for useful advice.
Figure 2
Figure 3

A scatter plot showing the relationship between WIR and IR for different strains:

- C57BL/6J
- DBA/2J
- CDS/Lay
- Null

The data points are plotted on a linear scale, with WIR on the y-axis and IR on the x-axis.