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Dynamic Agent-Based Model of Hand-Preference Behavior Patterns in the Mouse

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Using a new agent-based model that mimics the learning process in hand-reaching behavior of individual mice, we show that mouse hand preference is probabilistic, dependent on the environment and prior learning. We quantify the learning capabilities of three inbred strains and show that population distributions of hand preference emerge from the properties of individual mice. The model informs our understanding of gene–environment interactions because it accommodates genotypic differences in learning and memory abilities, and environmental biases. We tuned each strain’s model to match their experimental hand-preference distributions in unbiased worlds and, by comparing simulations and experiments, identified and quantified a constitutive left-bias in hand preference of one strain. The models, tuned for unbiased worlds, match experimental measures in left- and right-biased worlds and in biased worlds after previous training. New measures quantitatively assess this matching, revealing that two strains, previously considered non-learners of hand preference, actually have significant learning ability and we confirm this with new experiments. Model mice match the kinetics of hand-preference learning of one strain and predict the limits of learning. We conclude that genetically evolved hand-preference behavior in mice is inherently probabilistic to provide robustness and allow constant adaptability to ever-changing environments.

Keywords adaptation · behavior · hand-preference · population · probabilistic

1 Introduction

Recent measurements of gene expression and other biochemical processes show that the dynamics of biological systems is stochastic (Yu, Xiao, Ren, Lao, & Xie, 2006) and vital processes, such as cell differentiation, can have a probabilistic nature (Samoilov, Price, & Arkin, 2006; Suel, Garcia-Ojalvo, Liberman, & Elowitz, 2006). Noise in gene expression generates pheno-

typic variability among genetically identical individuals (Arkin, Ross, & McAdams, 1998), but the diversity of complex traits, such as behavior, also arises from differences in genotype and the probabilistic nature of interactions between individuals and their environment (Den Nijs & Sterk, 1980; van Baarlen, van Dijk, Hoekstra, & de Jong, 2000).

Hand preference in the mouse is one example of a behavioral trait with a probabilistic nature, dependent

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on genotype and prior experiences. Genetically identical mice exhibit diverse behaviors when subject to identical experimental tests (Biddle, Coffaro, Zeihr, & Eales, 1993; Biddle & Eales, 1996; Collins, 1968, 1969, 1975). Regardless of this diversity at the individual level, populations of different strains have characteristic distributions of behavioral responses (e.g., see Biddle & Eales, 2006; Takeda & Endo, 1993). We model and assess how these distributions emerge at the population level from the properties of individual mice.

Paw usage is a relatively simple behavior to observe when laboratory mice reach for food. Individual mice express a reliable ratio of right and left paw reaches. Replicated tests are possible for single genotypes in the form of genetically defined inbred strains and comparison of handedness can be made between different strains. Laboratory mice may provide a useful model of hand preference in the absence of cultural biases and other developmental asymmetries that have confounded the analysis of human handedness.

Paw usage has been assessed in unbiased (U-world) test chambers (Figure 1), in which a mouse reaches for food from a centrally placed food tube (Biddle et al., 1993; Biddle & Eales, 1996; Collins, 1968, 1969). The right-paw entry or RPE score quantifies the direction of paw usage and is the number of reaches made with the right paw in a specified number of reaches (Collins, 1968). Since most inbred strains have approximately equal numbers of right- and left-pawed mice in the U-world, direction of paw preference, in the sense of right versus left, appeared to be genetically neutral. The reliable and characterized different shapes of the distributions of RPE scores among inbred strains led to the initial conclusion that genetic differences in the mouse trait reside in the degree of laterality variation with learning of the preferred paw, rather than in its direction.

Subsequently, asymmetrical test chambers (biased worlds) demonstrated the learning and memory property in paw usage and identified genetic differences (Biddle & Eales, 1999). The food tube is placed to the left or right side in these asymmetrical test chambers, defined as L-world or R-world respectively (Collins, 1975). Response of previously untested or naïve mice to reaching in a biased world and to retesting in an oppositely biased world illustrated how paw usage depends on prior experience, current test environment and mouse genotype (Biddle & Eales, 1999). In some strains, direction of paw usage is determined by the bias of the test chamber and it is conditioned by the experience of reaching; in others, direction is mostly a constitutive behavior (Biddle & Eales, 2001).

Experiments with biased test chambers showed even more phenotypic complexity than U-world experiments. Distributions of RPE scores of a population are not continuous, and, even for populations of genetically identical individuals, they are usually not Gaussian-like. Thus, mean and variance values of RPE scores of samples of mice are not sufficient to capture, and they often conceal, the qualitative differences in hand-preference behavior between mouse strains (Biddle & Eales, 2006; Biddle, Jones, & Eales, 2001). Furthermore, genetically identical mice are rarely phenotypically identical, as shown by the individual-to-individual variation in RPE measurements within an inbred strain.

We hypothesize that critical information about regulatory mechanisms is contained in the patterns of phenotypic diversity of hand-preference behavior of genetically identical individuals and in the differences between these patterns among genetically distinct individuals. Success in the functional analysis of behavior depends on analysis of distributions of behavioral phenotypes because the distributions provide insight not visible in mean values or descriptors of distribution shape. Thus, an analytical framework that models this individual-to-individual variation in a predictive way is necessary to relate noisy behavioral phenotypes to their deterministic genotypes. Since RPE scores of individual mice of any strain are not deterministic, the major problem in analyzing hand preference is the need for large samples of mice, making them costly and, thus, rarely done. Therefore, a simulator that reproduces hand-preference experiments could play an important role in the functional analysis of this behavior.

We propose a stochastic agent-based model that reproduces hand-preference behavior of mice at the individual and population levels. In the model, phenotypic expression of genotype and the interactions between individuals and the environment are probabilistic. Only the properties of an individual mouse of each strain are pre-established and they have a probabilistic, rather than deterministic, character. These properties were inferred from the RPE distributions of each strain in a U-world and then tested in various other situations and compared with experimental data. The
results show how the different RPE distributions of mouse strains emerge from the differences between genotypes and the probabilistic nature of phenotypic expression of individual mice.

In the model, mice of the same strain are identical, and mice of different strains differ only in the amount of learning from each successful reach, which has been shown to be a genetically determined trait (Biddle & Eales, 2006). By tuning the models of individual mice of different strains from RPE measurements of naïve mice in a U-world, we found that the population distributions of RPE scores emerge when many, initially identical mice are sampled. Importantly, without further tuning, the model mice matched the results of various other experiments, such as in biased worlds or determining the kinetics of mice learning response to training reaches, indicating that the model has the capability of predicting behavior because the underlying regulatory mechanism in real mice is likely to be identical in all situations. Additionally, comparisons between model and experiments allowed identifying previously unknown features of mouse hand-preference behavior.

2 Experiments, Model, and Simulations

2.1 Mouse Strains and Hand-Reaching Experiments

C57BL/6JBid, CDS/LayBid, and DBA/2JBid are registered inbred strains of the laboratory mouse and their origins to our laboratory were described or extensively referenced in Biddle et al. (1993) and Biddle and Eales (1996). The strains are maintained with continued sister–brother inbreeding. Our “Bid” laboratory code is not included in the strain names in the text. 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 the experimental protocols. Paw usage was experimentally assessed as in Biddle and Eales (1999). After a fast of 12–24 h, mice were placed in a test chamber and they reached for food from a food tube placed equidistant from the left and right sides of the chamber (U-world; see Figure 1) or near the left or right sides of the chamber (L- or R-world). The food tube in biased worlds is placed such as to allow reaching for food with either paw. Rolled oats or crumbled laboratory diet was placed in the food tube and the number of R- and L-paw entries to retrieve food was counted, usually in a total of 50 reaches.

The experimental data, used in the present analysis, were collected over several years (1999 through 2006). On their own, these experiments would be expensive because of the need to test large numbers of mice of each strain. The validation of the results of the present study demands an amount of biological data that requires collection over several years. Importantly, the models of each strain did not require any adaptation to this fact, which allows us to conclude that the population behavior of each strain has not changed over the years.

2.2 Agent-Based Model

The agent-based model simulates the paw-reaching behavior of single mice, by subjecting model mice to paw-reaching events at consecutive time intervals (one time unit apart in the simulations). At each reach, the relative position between mouse and food determines which paw the mouse ought to use to reach for the food. The world bias defines the probability by which the food ought to be reached with each paw. For example, in a left-biased world, it is more likely
that the food ought to be reached with the left paw than with the right paw. When describing biased worlds we use the following convention: “p:q bias”, such that: p + q = 100. For example, in a world with 40:60 bias, at each reach, there is a 60% probability that the food should be reached with the left paw.

The mouse memory of previous events determines the probability of using the right or left paw at the next reach. In practice, we model memory by providing all mice with the same fixed amount of memory units, here set to 1000. The fraction of right memory units (RMU) at a given moment defines the probability that the mouse uses the right paw at the next reach, while the rest are left memory units (LMU), defining the probability of using the left paw.

Naïve unbiased model-mice have an equal number of RMU and LMU. The probability of using either paw changes during a simulation by two processes. Each time the mouse uses the correct paw to reach for the food, it records it by increasing the probability of using the same paw in the next reach. The amount by which such probability is varied corresponds to how much a mouse learns from a successful reach and is the feature that is unique to each strain. The amount learned when reaching with the left paw can be set to differ from the amount learned when reaching with the right paw (the learning process would, in that case, be biased).

These paw-usage probabilities are continuously affected by memory decay (Biddle & Eales, 2006), implemented as first order stochastic chemical reactions by the stochastic simulation algorithm (Gillespie, 1977). One reaction converts LMU into RMU and the other does the opposite. These two decay reactions have equal rates, so they will continuously equalize the probabilities of using left and right paws.

In order to compare the paw-preference behaviors of populations of model mice with populations of real mice, we numerically assess the behavior of populations of model mice. That is, to obtain the RPE distribution of a population of model mice, we simulate the dynamics and measure the RPE of many individual model mice, thus numerically assessing the behavior of the population of model mice, rather than using some mean field model of the population’s average behavior. When referring to “numerical results,” these are the observations of the behavior measured from simulations of many independent model mice.

### 2.3 Measures for Assessing Hand-Preference Behavior and Learning

The primary measure, the RPE score of a mouse, is the number of reaches with the right paw by an individual in a total of 50 reaches (Collins, 1968).

The average adaptation ratio (AR) assesses the matching at the population level when mice are tested in two worlds of opposite bias. AR is the fraction of mice with RPE scores of the same bias in both test worlds. If the first world is an L-world followed by an R-world, the AR(L1 → R2) of an individual mouse (mi) is given by Equation 1 (where x1 and x2 are given by conditions 2 and 3, respectively):

\[
\text{AR}(L_1 \rightarrow R_2) = \frac{x_1 + x_2}{2}
\]

If \([50 - \text{RPE}(m_i(L_1))] > 25 + 3 \Rightarrow x_1 = 1,\]

else \(x_1 = 0 \) (2)

If \(\text{RPE}(m_i(R_2)) > \text{RPE}(m_i(L_1)) \Rightarrow x_2 = 1,\]

else \(x_2 = 0 \). (3)

The population’s AR is the average of the AR of all individual mice. From condition 2, a mouse is only considered adapted to the first world (L-world) if its RPE > 28, rather than 25, that is, half the number of reaches. This threshold prunes out noise-related fluctuations. A population’s average AR ranges from 0 to 1. AR is 0 if all mice have an RPE opposite to the world (i.e., test chamber) bias in both worlds. The expected AR for random paw preference is 0.48 (because of the threshold); correct paw preferences in both test chambers gives an AR of 1.

Finally, the population’s improvement ratio (IR) is the fraction of mice whose RPE value in the second U-world is more biased than in the first U-world, as long as this bias is in the same direction in the two worlds. Individual mice have an IR of 0 or 1. For example, if an individual’s RPE score in the first 50 reaches is 25 or less and, if the RPE in the second 50 reaches is equal to or smaller than the RPE in the first set of 50 reaches, IR is 1 since its bias to the left is the same or higher; otherwise, IR is 0. By convention, mice with an RPE of 25 in both worlds have an IR of 0, and mice
with RPE scores of 0 or 50 in both worlds have an IR of 1 since no further improvement is possible. The expected IR of mice with random paw usage or no learning is 0.25 rather than null; this is the expected fraction of times a mouse with random paw usage chooses the correct paw in the first U-world and then in the second U-world.

Comparing the values of these measures of hand-preference behavior in experiments with real mice and simulated model mice allows a test of how well model mice reproduce the experimentally observed behaviors at the population and the individual levels. Additionally, to determine if the behavior of model mice fits real mice, as we will explain later, we do visual comparisons of the RPE distributions and the two-sample Kolmogorov-Smirnov (K-S) tests.

3 Results and Discussion

In this section, we compare the simulations with experimental measurements of hand preference. When measuring RPE values, both for experimental mice and for simulated data, the RPE value of each mouse was computed from 50 paw reaches, unless stated otherwise. First, we tune the model to match the experimental measurements of RPE distributions of populations of naïve mice of various strains in U-worlds (Figure 2a to d). Tuning consists in setting the values of left and right training rates (LTR and RTR) for each strain that best fit the experimental measurements.

The decay rate of learning is constant during the lifetime of the model mouse and equal valued in all strains (set to 0.01 t⁻¹, where t is 1 time unit). What ultimately determines the shape of RPE distributions is the ratio between learning rate and decay so these two quantities could, in principle, have multiple possible values. This value of decay was the one that we heuristically found to best fit both the learning curve of C57BL/6J mice (Figure 8) and the RPE distribution of C57BL/6J in a U-world. Once set for C57BL/6J, we assume equal decay rates for the other strains for simplicity.

The RPE distribution of model mice with no ability to learn is shown in Figure 5 for comparison. Usually, RPE ratios of individual mice are binned (i.e., sorted into a series of ranges of numerical values). For comparison purposes, we normalize the RPE value of 50 reaches of each mouse from 0 to 1 (dividing it by the total number of reaches), and set bins with a range of values of 0.1. All mice that had a normalized RPE value between 0 and 0.1 are placed in bin 1, inclusively; mice with a RPE between 0.1 (exclusively) and 0.2 (inclusively) are placed in bin 2. The procedure is continued across the RPE distribution. The bias to the left introduced by the binning process is minimal; namely, if one randomly generates a number N of RPE values from a normal distribution (with mean of 25) and then bins these values as described, approximately 0.5098% will be placed in bins corresponding to left-biased RPE and 0.4902% will be in bins corresponding to a right-biased RPE (Figure 5). Note that there are 0 to 25, that is, 26 left-binned RPE values, versus 25 to 50, that is, 25 right-binned RPE values, out of 51 possible values.

Afterwards (Figure 6), the tuned models are assessed in left- and right-biased worlds and in biased worlds after previous training and their RPE distributions are compared with those from experimental mice. For these experiments that measure the RPE distributions of populations of naïve mice of various strains in U-worlds and in biased worlds, the assessment of matching between numerical and experimental results is done by visual comparison of the RPE distributions, and by two-sample Kolmogorov-Smirnov (K-S) tests to assess their goodness of fit (implemented in Matlab 2007a). The sample size is 150 for each experimental data set, except for 101 DBA/2J mice (Figure 6b1 and b2), and 1,000 mice for the numerical data sets. In all cases, setting α to 0.01, the K-S test confirmed the goodness of fit (p ≥ α) of numerical and experimental distributions, except for the results in Figure 6a2. Additionally, the adaptation ratio (AR) is computed in the latter experiment to assess the matching at the population level and is the fraction of mice with individual RPE scores with the same bias as each of the worlds.

Next, we show numerical and experimental results of individual mice, placed in two sessions in a U-world in RPE scatter plots (Figure 7), to illustrate the association and changes in the RPE score of individual mice subjected to two temporally separated tests in a U-world. The RPE score of individual mice in the first U-world (x-axis) is plotted against its RPE score in the second U-world (y-axis) (Biddle & Eales, 1999). From these data, we computed the improvement ratio (IR), that is, the fraction of mice whose RPE value in the second U-world is more biased than in the first, if this bias is in the same direction in the two U-worlds.
Finally, we compare the kinetics of the learning response of model and experimental mice with the number of training reaches. The learning curve (Figure 8) shows the average RPE score in 50 reaches in an R-world of a mouse population, for various numbers of previous training reaches in an L-world (Biddle & Eales, 2006).

3.1 Tuning the Model in an Unbiased U-world

The only variable of the model that is tuned is the learning rate of individual mice per successful reach for CDS/Lay, DBA/2J, and C57BL/6J strains, so that the RPE distributions of model naïve mice of each strain match the experimentally measured distributions of naïve mice in the U-world. The notation used to describe the training rate of a mouse strain is: “strain (LTR,RTR).” Paw-reaching events are separated by 1 time unit (t) interval and the stochastic decay rate of training is 0.01 t⁻¹. Since it is a U-world, at each reach there is a 50% chance that left and right paw ought to be used.

The initial tuned values of LTR and RTR are CDS/Lay(25,25), DBA/2J(60,60) and C57BL/6J(95,95) and distributions of RPE scores from simulating 1000 mice of each strain are compared with experimental data (Figure 2). Numerical and experimental data fit well in CDS/Lay and DBA/2J (Figure 2a and b), but not as well in C57BL/6J (Figure 2c). More experimental C57BL/6J mice are left biased than right biased. This constitutive bias was incorporated into the C57BL/6J model by setting a higher LTR and lower RTR, that is, C57BL/6J(105,85), improving the fit with the experiments (Figure 2d). Regarding the model’s sensitivity to the values of LTR and RTR, we note that varying the values, found to give the best fit, by five or more units results in RPE distributions that do not fit the experimental data satisfactorily.

It can be seen in Figure 2d that the model of C57BL/6J mouse does not fully capture all features involved in the learning process of real C57BL/6J mice. Real mice have, in comparison to model mice, an excess of mice in bin 1 and a deficit in bin 2. Also, bins 9 and 10 have a deficit while bins 6 and 7 have an

![Figure 2](http://example.com/f2.png)

**Figure 2** RPE distributions of 50 hand reaches in a U-world of numerically simulated and experimental mice. (a) Naïve CDS/Lay (experimental data) and model of naïve CDS/Lay with training rate of (25,25). (b) Naïve DBA/2J (experimental data) and model of naïve DBA/2J with training rate of (60,60). (c) Naïve C57BL/6J (experimental data) and model of naïve C57BL/6J with training rate (95,95). (d) Naïve C57BL/6J (experimental data) and model of naïve C57BL/6J with biased training rate of (105,85).
excess. The fact that there are differences in both left- and right-biased real and model mice shows that such differences are not caused by the binning procedure, and that not all features of the learning process in this strain are entirely captured by the present model.

We ran simulations of 50 reaches in 10 sets of 1,000 mice with the tuned C57BL/6J model mice to estimate the variability in the RPE distributions between each set. The average standard deviation of the normalized heights of the bins in these 10 sets was 0.0083% and the maximum difference between a bin height and its mean height was 2.5%. Thus, samples of 1,000 mice are sufficient to obtain consistent results between runs. Simulations with 100 mice are still consistent, but simulations with populations smaller than 100 mice vary significantly, because of an insufficient sample size (importantly, the same appears to be true in real mice).

From the simulations we also computed the correlation between scores obtained for the first 25 and the last 25 reaches for all model mice. The fraction of model mice whose paw preference was biased to the same side in both sets of reaches was 50% for non-learners, 62% for model CDS/Lay, 84% for model DBA/2J, and 92% for model C57BL/6J. Note that all model mice are initially naïve and have no preexisting lateralization (except C57BL/6J), which allows us to conclude that a high correlation between RPE values in the two U-worlds does not imply preexistence of lateralization.

To illustrate how the difference between RTR and LTR affects the RPE distribution, Figure 3 shows the RPE distributions of 1,000 model mice without bias in learning (95,95), with a weak bias in left-paw learning (105,85), and with a stronger bias (115,75). A small bias to the left (in bin 1 compared to bin 10) is visible in the unbiased population (95,95) as a result of the binning procedure.

We tested the effect of number of reaches on the observed RPE distributions. Historically (Collins, 1968, 1969), the RPE is obtained from sets of 50 reaches. We tested how the number of reaches alters the final RPE distribution in a model mouse with a learning rate of (60,60) and show the RPE distributions when populations of these mice are allowed to make 50, 100, and 250 consecutive reaches (Figure 4). When allowed 1,000 consecutive reaches, most mice are equally distributed in bins 1 and 10, and a small percentage (<5%) is in the neighboring bins (because of decay of learning).

From Figure 4 one can observe that 50 reaches are not sufficient to measure the steady state behavior of these model mice. Interestingly, this number of reaches is sufficient for C57BL/6J (Figure 8), but because of the weaker learning rate of these model mice, approximately 150 reaches are needed to reach the limits of learning (defined by the ratio between learning and decay).

Lastly, a numerical simulation of 50 reaches in a U-world was done with 1,000 model mice with null
LTR and RTR, that is, with a learning rate of (0,0) (Figure 5). Comparison of the RPE distributions from the three strains of experimental mice (Figure 2a to d) with the “null-model” mice shows that these strains, and perhaps all strains of experimental mice, have at least some learning and memory ability and it differs between strains.

As seen in Figure 5, the binning procedure introduces a small bias in hand preference of approximately 0.1% for a population of 1,000 mice (for 100 mice this bias is approximately 1%). That is, 50.1% of the mice are in bins 1 to 5, and the rest in bins 6 to 10 for reasons previously described. Note that, in the RPE distribution of C57BL/6J (Figure 2d) 57% of the mice are left biased while 43% are right biased (in a population of 100 mice). The “spurious” bias, resulting from the binning procedure, is much smaller than the ~7% bias in the RPE distribution of C57BL/6J (Figure 2d) showing that the bias in paw preference in the C57BL6J strain is biologically significant.

The relation between learning rate and decay of memory shapes the RPE distributions in Figure 2. As the learning per successful reach is increased, the resulting distributions go from Gaussian-like, to flat, to U-shaped. The fact that a set of genetically identical naïve mice gives rise to RPE distributions rather than a single sharp peak implies that phenotypes are distinct because of the stochastic nature of the response of individual mice and the interactions between mouse and environment.

A critical issue in the analysis of mouse hand preference has been the predictive value of a hand-preference score among genetically different mice. The RPE scores of individual mice do not uniquely predict the strain phenotype and, hence, the genotype. Conversely, the identity of the strain (genotype) does not predict the RPE score of individual mice. The same problem occurs regarding the detection of directional biases in hand preference. Simulations provide a way out of these biometrical conundrums. For example, with limited prior knowledge of the U-world RPE scores, the simulator estimated the LTR and RTR of individual mice of each strain. From there, the models predicted both the phenotypic distributions of RPE scores and the differences in these distributions between CDS/Lay, DBA/2J and C57BL/6J, including the constitutive bias in left-hand preference of C57BL/6J in the U-world. Thus, it might be possible to use the model to explore the underlying functional biology by assessing its ability to predict the response of a strain to different environmental conditions. To that end, we assessed the models in different environments, but from this point onward no changes were made in the parameter values of the model of each strain that was tuned in the U-world.

### 3.2 Training Naïve Mice in an L-World and Retesting in an R-World

Comparing the experimental and numerical RPE distributions of naïve mice in an L-world is the first test of the ability and accuracy of the models to predict phenotypic distributions in conditions different from the one where they were tuned. Then, after a 1-week interval to allow consolidation of learning (Biddle & Eales, 2006), the mice were retested in an R-world. A 1-week interval, during which only decay of learning occurs, is modeled by a waiting period of 75 time units, found empirically to best fit the data. Matching between model and experimental mice is assessed by comparing RPE distributions and average AR values, and by the K-S two-sample test.

Model C57BL/6J mice match the experiments (Figure 6c1 and c2). Model DBA/2J mice (Figure 6b1 and b2) also match. However, model CDS/Lay mice (Figure 6a1 and a2) match in the first set of reaches (L-world), but the matching was not successful in the retest in the R-world. The K-S test confirmed these results.
Figure 6  RPE distributions of 50 hand reaches in biased worlds. (a1) Naïve CDS/Lay (experimental data) and model of naïve CDS/Lay in an L-world (40:60 bias). (a2) CDS/Lay (experimental data) and model of CDS/Lay, after being in the L-world and 1-week interval, now in an R-world (60:40 bias). (b1) Naïve DBA/2J (experimental data) and model of naïve DBA/2J in an L-world (40:60 bias). (b2) DBA/2J (experimental data) and model of DBA/2J, after being in the L-world and 1-week interval, now in an R-world (60:40 bias). (c1) Naïve C57BL/6J (experimental data) and model of naïve C57BL/6J in an L-world (40:60 bias). (c2) C57BL/6J (experimental data) and model of C57BL/6J, after being in the L-world and 1-week interval, now in an R-world (60:40 bias).

Figure 6a2 shows differences between observed and simulated RPE distributions. Several factors might explain the inability of the model to match CDS/Lay in the R-world. The observed/experimental results seem leptokurtic (excess at peaks and tails), which might arise because the population of CDS/Lay mice expresses two phenotypes in biased worlds, that is, two distinct (LTR,RTR) values. Another possibility is that the weaker learning ability of CDS/Lay mice might be compensated by unknown mechanisms in some environmental conditions. This will be explored in future studies.

Finally, the numerical and experimental average adaptation ratios, AR(LW to RW), for CDS/Lay, DBA/2J, and C57BL/6J were, respectively, 0.59 (num.) and 0.59 (exp.), 0.65 (num.) and 0.64 (exp.), and 0.69 (num.) and 0.71 (exp.) and confirms that there is good matching between numerical and experimental data at
the population level. AR captures the differences between strains (consistently ~10%) and it is sensitive enough to estimate the degree of matching between numerical and experimental data (consistently ~2% difference). Similar simulations, starting in an R-world followed by an L-world retest a week later, equally matched the experiments (Biddle & Eales, 1999, 2001; data not shown). Again, there was a deviation of the numerically simulated CDS/Lay mice from their observed experimental results, supporting our contention that the weak learning ability in CDS/Lay needs to be further explored.

3.3 Replicate Tests in U-World with 1-Week Interval Between Tests

We determined whether the model mice match the ability to learn a direction of hand preference in the U-world by simulating an experiment where mice are tested twice in a U-world, separated by a 1-week interval (Figure 7). This test assessed whether there are unaccounted factors in the model in a re-learning situation.

In the previous experiment (Figure 6), we noted that a change in the direction of hand preference as a result of training is not easily detectable in the DBA/2J strain. The same conclusion was reached in a previous study (Biddle & Eales, 2001), where the distribution of RPE scores in DBA/2J mice did not deviate from an equal number of individuals in each of the binned values across the RPE interval scale. However, a significant training ability was detected in DBA/2J by computing the average IR, which measures the effect of training at the individual level.

These experiments were previously done for C57BL/6J and CDS/Lay strains in two consecutive U-worlds only to show the correlation between directions of hand preference in the two tests (Biddle & Eales, 1999). Here we use these tests and do an identical experiment on DBA/2J mice and with the simulator to detect learning and memory in hand preference and show that any strain can learn if it has an IR above 0.25 (the expected IR for model mice with null learning). Causes for differences in IR among different mouse strains will be assessed by further genetic studies.

DBA/2J training ability becomes visible by testing its tuned model with 50 reaches in the U-world, followed by a 1-week interval and another 50 reaches in the U-world (Figure 7). The IR values confirmed the match between model and experiments. The average IR values from numerically simulated (num.) and experimental (exp.) mice are (0.42 num., 0.44 exp.) for CDS/Lay (Figure 7a) and (0.74 num., 0.72 exp.) for C57BL/6J (Figure 7c) (experimental data from Biddle & Eales, 2001). When we calculated the IR of DBA/2J model mice, its value (0.74) was surprisingly similar to the IR of both model and experimental C57BL/6J mice. We tested this prediction by collecting new data from 100 DBA/2J naïve mice (Figure 6b1) and measured an average IR of 0.70, confirming the IR predicted by the model.

While this matching shows the model’s ability to predict results in different experimental contexts, we note that the IR is binary and only accounts for whether the L- or R-biased direction of hand preference in the first U-world is maintained at the same value or increases in the same direction in the second U-world. The IR does not account for how much the directional bias in hand reaching changed between each set of 50 reaches and, obviously, the degree of change differs in DBA/2J and C57BL/6J.

Importantly, the ability to predict the mouse behavior in a re-learning situation shows that the re-learning dynamics of real mice is identical to the dynamics of the first-time learning and the tuned models are able to predict hand preference behavior in replicated sets of hand-reaching events on the individual mouse. Also, decay of training appears to affect hand preference in a simple way because there is no detectable unexpected effect of residual memory on the re-learning dynamics.

3.4 Kinetics of Learning Response to Number of Training Reaches

We tested the model’s ability to predict the kinetic response to number of prior training reaches. Experimental data were reported for the quantitative effect of prior biased-world training in C57BL/6J on its direction of hand preference in an oppositely biased world (Biddle & Eales, 2006). Groups of naïve mice were trained in an L-world with respectively 0, 5, 10, 20, 50, and 100 reaches, and after a 1-week interval each of these groups was given 50 reaches in an R-world. Direction of paw preference in the R-world changed from right to left, in response to the increasing number of prior L-world training reaches. Interestingly, the
change to left-hand preference reached a saturation point where more training reaches in the L-world had no further measurable effect on direction of hand preference in the R-world.

The ideal test of the model is whether it can predict and detect such a saturation point or the limits of training/learning. Here, the rate of decay of training plays an important role. The 1-week interval, between L-world training and the R-world test, was again set to 75 t, found empirically to fit the previous experiments.

We simulated 100 independent populations of C57BL/6J mice. The averaged results of C57BL/6J model-mice and experimental data are shown in Figure 6 and the matching is virtually perfect. We verify that the results are within the expected variation by computing the standard deviation of the mean values.

Figure 7 Comparison of RPE scores of a first test of 50 hand reaches of naïve mice in a U-world and then the same mice moved, with a 1-week interval, to another U-world for 50 more reaches. Each data point value in the x-axis is the RPE value of a mouse in the first U-world and, in the y-axis, is its RPE value in the second U-world. For each strain, the left panel shows the experimental data and the right panel shows the data from the numerical simulations: (a1) CDS/Lay (experimental data); (a2) model of CDS/Lay; (b1) DBA/2J (experimental data); (b2) model of DBA/2J; (c1) C57BL/6J (experimental data); (c2) model of C57BL/6J. The computed average improvement ratio (IR) is noted in each graph.
measured for 100 simulated mouse populations. The amplitude of error bars in Figure 8 is ±1.5 (the standard deviation of each data point is ~1.5). This shows that the experimental measurements are within the range of values predicted by the model, thus, the model matches exactly the limits of training/learning of C57BL/6J.

The matching (Figure 8) demonstrates that the model captures biologically realistic features in the hand-reaching behavior. First, it shows that the model mice retain information from their training, which has consequences on subsequent hand-reaching events, identical to real mice. Second, it shows that the time between consecutive reaches and between sets of consecutive reaches is well tuned, given the other parameter values.

In previous reports, the learning abilities of the CDS/Lay and DBA/2J strains appeared to be below the level at which they could be detected in a reasonable number of reaches (Biddle & Eales, 1999, 2001, 2006). The simulator provides a framework to guide these studies by estimating the required sample sizes to detect both learning and the limits of learning in hand-preference behavior.

In retrospect, the matching between model and experiments suggests that the nature of paw choice in real mice is probabilistic and the functional differences between strains are in how much the probabilities of using each paw can vary with training. Also, we show that the model can be used to identify inherent genetic biases in the direction of paw preference, such as the bias of C57BL/6J to left-paw usage. This was done by providing the model with a functional mechanism to accommodate directional biases, which was simply to allow individuals to learn more from a successful left-paw reach than from a successful right-paw reach.

4 Conclusions

A surprisingly simple stochastic agent-based model of paw-preference behavior can be produced for mice to match observations of paw reaching not only from individual mice but also from populations of various genotypes. Importantly, the characteristically different distributions of right- and left-paw usage emerge from the genetic differences between individual mice of the different genotypes.

In the model, phenotypic expression of genotype is deterministic in the sense that mice with identical genotype have the same training rate per successful reach. Also, the amount of training per successful reach is deterministic. However, the distributions of RPE scores are generated by successful training events that are probabilistic. The key finding is that simulated paw-reaching experiences in identical mice generate distributions of RPE scores, rather than a single RPE value for all mice, because of the different reaching experiences of individual mice throughout their training sessions that result from the stochastic nature of paw choice.

Assuming deterministic phenotypic expression of genotype and constant training rate are most likely approximations of reality in paw preference. Real mice might acquire more training in early successful reaches than in later ones, and it is not likely that all mice in a population have exactly the same training rate (as assumed here). Nevertheless, the success of the model suggests that the approximations are not far from the truth. First, reaching experiments are standardized for real mice under defined laboratory conditions; thus, the acquired training likely varies little between successful reaches. Second, the success of matching is strong evidence that training rates of mice
of the same strain with identical genotype are, in fact, very similar and the genetic trait of the mouse is its training rate, as the model assumes, rather than its individual RPE value.

Other models could be built in different frameworks. For example, it is possible to match the RPE distributions of each strain with Beta distributions. However, such a model would not be able to generate the population distributions from the behavior of individual mice, that is, it would not capture the learning process and memory acquisition of individual mice. Additionally, we showed that noise and the probabilistic nature of the events cannot be ignored in mouse paw choice. In our model, noise arises from the probabilistic nature of individual choices, which is what occurs in reality. Alternatively, in a stochastic ODE model for example, noise would have to be introduced at the population level and, thus, it would not reflect the true nature of the source of stochasticity that lies in the individual mouse choices.

We suggest that the model provides the beginning of a unique insight to the nature and function of the regulatory mechanisms underlying hand-preference behavior in mice. The models of all strains assume a degree of learning through hand-reaching experience and only differ in how much the probability of right-paw reaching varies as the result of a successful right-or left-paw reach. The fit with the experiments suggests that paw choice in real mice might also be genetically regulated as a probabilistic choice, rather than being a fixed trait. If so, not only would the mouse be able to adapt to any world bias, but also it would maintain the ability to detect and respond to changes in bias, regardless of the amount of previous training. In other words, maintaining a probabilistic choice of paw usage may allow the mouse to constantly probe better ways of interacting with the world and to adapt to changes that would not be possible with a deterministic mechanism. This might be an important example of an evolved genetic mechanism whose robustness and adaptability results from its probabilistic nature. It is now of importance to determine what gene network regulates this mechanism of learning and memory in mouse paw-preference behavior.

The maintenance of a probabilistic choice in paw usage in the model results from the combination of training and memory decay, leading to a plateau of paw preference with non-null probability of using either paw. Experiments on the limits of training in real mice support this conclusion (Biddle & Eales, 2006) and, for that reason, the model matched the kinetics of learning of hand preference in C57BL/6J.

Memory consolidation was not explicitly included in our model and requires further studies. A period of time is needed to consolidate memory of paw-preference training in mice and this consolidation can be blocked in a dose–response manner by inhibitors of protein synthesis (Biddle & Eales, 2006). Here, we assumed that mice have instantaneous consolidation of training because the interval between the sets of reaching events was sufficient to accommodate the consolidation process.

Many intriguing associations have been identified between different developmental traits and different measures of hand-preference behavior of individual mice, either within an inbred strain and between genetically different strains. For example, morphological variation in size of the inter-cerebral hemispheric callosal fiber tract, including its complete absence, was positively associated with different measures of hand preference in some but not other strains (Biddle & Eales, 1996; Bulman-Fleming, Wainwright, & Collins, 1992; Gruber, Waanders, Collins, Wolfer, & Lipp, 1991; Ward, Tremblay, & Lassonde, 1987). Variation in different measures of immune reactivity has been associated with hand preference in some but not all strains and, in some cases, observable in one, but not both sexes (Denenberg, Mobraaten, et al., 1991; Denenberg, Sherman, Schrott, Rosen, & Galaburda, 1991; Neveu, Barneoud, Vitiello, Betancur, & Le Moal, 1988; Neveu, Betancur, Vitiello, & Le Moal, 1991) and attributable to a genetic difference in maternal uterine effect during gestation (Denenberg et al., 1992). We believe that we cannot account for these additional complexities at the present stage of the research presented here because they are beyond the scope of the assumptions of the present model. It is likely that a new layer of complexity, regarding brain activity, might have to be introduced in a more developed model as we begin to model hand preference in specific individuals within genetically identified and different mice strains.

We note that the results of the present study do not allow determining the exact underlying difference between the strains that causes the different behavioral patterns. For example, we cannot determine if the differences are at the level of gene sequences or they...
are to the result of potential epigenetic processes. However, it is possible to rule out interaction with maternal environmental factors as a possible cause because a population that is generated, for example, from two mice that are highly lateralized to the left, has an RPE distribution identical to what would be expected if we had initially picked a pair of weakly lateralized mice of the same genotype. At the same time, the learning and memory property of hand-preference behavior of mice will require attention to standardized hand-reaching protocol because some early studies counted different numbers of hand reaches in different mice, and normalized the observations to 50 reaches. These results cannot be reproduced by our model unless the total number of reaches of each mouse is provided (e.g., Denenberg, Mobraaten, et al., 1991; Denenberg, Sherman, et al., 1991; Denenberg et al., 1992). Finally, in some cases, the experimental sample size limits the possibility of confronting model and experiments.

Our results have established hand preference of the mouse as a complex adaptive behavior in which individuals show a directed response to a changing environment and future performance depends on past experience. Moreover, the mouse shows us that genetics enters the regulation of its hand-preference behavior at two functionally different levels. The first level is that different strains (different genotypes) learn at different rates in response to a successful hand reach and, therefore, the past experience in different genotypes has different and genotype-specific effects on future performance. The second level is that, despite a directed response to a changing environment in asymmetrical test worlds, some genotypes show yet another genetic difference by their constitutive ability to learn more from a left- or a right-hand reach than they learn with their opposite hand.

Finally, hand-preference behavior in mice has relevance to our understanding of human handedness. A genetically determined and tractable model of a learning and memory process is emerging for the mouse. Without over-interpreting our results, we anticipate that this mouse behavior will inform our understanding of other biological functions that involve learning and memory as well as human hand-preference behavior. Nevertheless, while it is strongly heritable (Warren, 1980), human hand preference also depends on cultural environment and, thus, it will pose far more challenges.

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