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Hand-preference training in the mouse reveals key elements of its learning and memory process and resolves the phenotypic complexity in the behaviour

Fred G. Biddle and Brenda A. Eales

Abstract: Handedness in the mouse comprises 2 different behaviours. Some strains have a conditional behaviour, in that the mice learn a direction of hand preference in response to reaching for food, whereas other strains have an innate or constitutive behaviour, and prior experience has no measurable effect on their hand preference. However, hybrids from different strains have revealed both recessive and dominant forms of constitutive hand preference. We proposed that kinetic parameters of the learning process would resolve this genetic heterogeneity as well as the phenotypic complexity in the behaviour. We conducted and report here a detailed kinetic analysis of hand-preference training in the C57BL/6J strain. It revealed elements of the fundamental process of learning and long-term memory that underlies the behaviour by documenting consolidation of memory, blocking of this consolidation by an inhibitor of protein synthesis, retention of memory, and speed of learning in response to training reaches. Furthermore, speed of learning is clearly described by 2 parameters that we call "capacity" (or maximum amount of learned preference) and "ability" (or number of training reaches to achieve half the capacity). These 2 kinetic parameters can vary independently among genetically different strains that learn a preference, and we used them to demonstrate that the respective recessive and dominant forms of constitutive hand-preference may be the consequence of a true null or loss of function and a gain of function, possibly a memory regulator, in the learning process. The quantitative measures provide a sensitive and selective method to establish the fundamental learning process underlying mouse hand preference and to demonstrate empirically how genes and contextual environment shape its phenotypic complexity.

Key words: mouse, hand-preference, behavioural genetics, learning, memory, complexity, kinetics, capacity, ability, memory regulator gene.

Résumé : La latéralité manuelle chez la souris présente deux comportements distincts. Certaines lignées affichent un comportement conditionné car ces souris acquièrent une préférence en réponse à un conditionnement alors que d'autres lignées ont un comportement inné ou constitutif et le conditionnement n'a pas d'effet sur leur préférence pour l'une ou l'autre main. Des hybrides entre diverses lignées ont révélé des formes récessives et dominantes de la latéralité manuelle constitutive. Les auteurs proposent que des paramètres cinétiques du processus d'apprentissage pourraient expliquer l'hétérogénéité génétique et la complexité phénotypique du comportement. Les auteurs ont réalisé et rapportent ici une analyse cinétique détaillée du conditionnement à la latéralité chez la lignée C57BL/6J. Ce travail a révélé des éléments du processus fondamental d'apprentissage et de la mémoire à long terme qui sous-tendent le comportement en documentant la consolidation de la mémoire, le blocage de cette consolidation par un inhibiteur de la synthèse protéique, la rétention de la mémoire et la vitesse de l'apprentissage en réponse à une séance d'entraînement. De plus, la vitesse d'apprentissage est clairement déterminée par deux paramètres que les auteurs nomment « capacité » (soit la latéralité maximale pouvant être acquise) et « l'habileté » (soit le nombre de séances d'entraînement nécessaires pour atteindre la moitié de la capacité). Ces deux paramètres cinétiques peuvent varier indépendamment chez des lignées différentes qui acquièrent une préférence et les auteurs les ont utilisés pour démontrer que les formes respectives récessives et dominantes de la latéralité constitutive peuvent résulter d'une mutation nulle, ou perte de fonction, ainsi que d'un gain de fonction, possiblement un régulateur de mémoire, dans le processus d'apprentissage. Les mesures quantitatives fournissent une méthode sensible et sélective pour décrire les processus fondamentaux d'apprentissage qui sous-tendent la latéralité chez la souris et pour démontrer empiriquement comment les gènes et l'environnement façonnent sa complexité phénotypique.

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Mots clés : latéralité manuelle chez la souris, génétique du comportement, apprentissage, mémoire, complexité, cinétique, capacité, habileté, gène régulateur de la mémoire.

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Introduction

Mice use their right and left hands or forepaws to retrieve food from a tube in a small testing chamber, and individual mice show a reliable direction of hand preference. Direction of hand preference is defined as the relative amount of right-paw usage and expressed as a right-paw entry (RPE) score. The testing chamber usually has a food tube equidistant from the right and left sides, and it is described as an unbiased or U-world (Collins 1975). Since the average direction of hand preference from any mouse strain in the U-world is approximately equal hand usage, mouse strains do not appear to differ in the average direction of their hand preference.

Nevertheless, when many individual mice of different strains are assessed, there are conspicuous qualitative differences among the strains in their complex distributions or patterns of RPE scores (e.g., fig. 2 in Biddle et al. 2001). In an attempt to characterize this qualitative variation, hand usage was represented as a degree of lateralization and the direction simply ignored. Degree of lateralization is the absolute value of the difference in relative amount of right-hand and left-hand usage in an individual mouse; it has a minimum numerical value in an individual that uses both hands equally and a maximum value in an individual that uses exclusively its right or left hand (Collins 1968, 1969, 1985; Signore et al. 1991; Roubertoux et al. 2003; Biddle et al. 1993; Biddle and Eales 1996). The fundamental mechanism underlying strain differences in degree of lateralization is unknown, although a quantitative trait locus (QTL) analysis has detected positive association with marker genes (Roubertoux et al. 2003).

Our genetic experiments exposed a greater complexity in mouse hand preference than simply the magnitude of degree of lateralization (Biddle and Eales 1999). It was obvious that, when the numerical values of the original RPE scores are summarized as the mean and variance of a sample or when the RPE scores are transformed to a degree of lateralization and then summarized as the mean and variance of a sample, the summaries do not describe the qualitative differences that are clearly seen in the distributions or patterns of the original data (e.g., patterns of RPE scores in fig. 2 in Biddle et al. 2001). The original measurements are not a continuous distribution, and the measurements from genetically identical individuals are not distributed in a Gaussian manner.

Therefore, we looked in other ways at the patterns of U-world RPE scores among different mouse strains and found that handedness appears to comprise 2 different behaviours (Biddle and Eales 2001). We used an alternative testing chamber, first used by Collins (1975), in which the food tube is flush to the right side (R-world) or left side (L-world). To see the 2 behaviours, we assessed previously untested mice in the R- or L-world and, later, reassessed them in the opposite L- or R-world. In some strains of mice, direction of paw preference changes in the direction of the biased world and it does not return to its expected baseline in the oppositely biased world. The experience of reaching for the food has the effect of conditioning the behaviour of these mice, because they appear to learn a preference in response to reaching, and the context of the testing chamber determines the left or right direction of their paw preference. In other strains, direction of paw preference is not significantly different in a biased world and in the oppositely biased world. These other mice have an innate or constitutive behaviour, because previous experience has no effect on their paw preference.

We proposed that a process of learning and memory determines mouse hand preference. Therefore, naturally occurring, genetic differences among strains influence the learning process, and stochastic or random events in the development of individual mice within a strain as well as in their testing must give rise to the characteristically different, strain patterns of distribution of RPE scores in the U-world. However, there is not a direct relation between the characteristic pattern or shape of the distribution of RPE scores in the U-world and whether a strain has a conditional or a constitutive behaviour. Genetic crosses among strains with conditional and constitutive hand preference have suggested a genetic model (Biddle et al. 2001). Strains with conditional behaviour may differ quantitatively in the amount of preference that they can learn because they are either strong or weak learners, but there is an intriguing genetic heterogeneity among strains with constitutive hand preference. Although mice with a constitutive behaviour cannot learn a direction of preference, their inability to learn is either a recessive or a dominant phenotype in the F_1 hybrid from crosses with apparent strong learners. We suggest that a recessive constitutive behaviour could be the result of a loss of function in the learning process or, possibly, it is a weak learning that we cannot detect. In contrast, dominant constitutive behaviour suggests something novel, because in a genetic background in which it should be possible to learn a preference, it might be the result of a dominantly acting regulator or suppressor of the learning process. Genetic and functional characterization of this dominant regulation may be key to understanding the learning and memory process of hand preference.

Nevertheless, successful analysis of hand preference behaviour depends not only on the ability to distinguish between genetically recessive and dominant forms of constitutive hand preference but also on the ability to distinguish between a true null effect and a very weak learning among strains with a recessive form of constitutive hand preference. We proposed that detailed kinetic analysis of the learning process would provide the objective evidence that a process of learning and memory underlies paw preference in mice, that the learning process represents a valid framework for genetic and functional analysis of this mouse behaviour, and that kinetic parameters of learning can be used to distinguish between a true null effect and a weak learning in mice with a recessive form of constitutive paw-preference behaviour.

We evaluated the kinetic parameters of learning a preference for left-paw usage in C57BL/6J mice, a strain that

appeared to consist of good learners, and we used the definition of learning as "the acquisition of an altered behavioural response due to an environmental stimulus" (Sweatt 2003). By allowing mice to reach in the L-world and by subsequently testing their hand preference in the R-world, we assessed how reaching in the L-world (or L-world training) alters the reaching behaviour in the R-world. Memory or simply the "balance between learning and forgetting" (Abel et al. 1998) is the contextual memory of L-world training that we assess when the L-world trained mice are tested for hand preference in the R-world. We documented the consolidation of memory with time after training, the blocking of this consolidation by an inhibitor of protein synthesis, the retention of the memory, and the rate of learning (or response to number of training reaches). Then, we compared the rate of learning among different mouse strains and their hybrids derived from matings with C57BL/6J. Although we assessed the contextual memory of L-world training, symmetry and preliminary results (Biddle and Eales 1999, 2001; Biddle et al. 2001) predict that training in either the L-world or R-world, followed by testing in the oppositely biased world, will lead to the same inferences about the underlying learning and memory process.

Materials and methods

Mice

C57BL/6JBid, DBA/2JBid, and SWV/Bid are registered inbred strains of the laboratory mouse; their origins in this laboratory were described previously (Biddle et al. 1993; Biddle and Eales 1996). The strains are maintained by continued sister-brother inbreeding. Our "Bid" registered laboratory code (http://dels.nas.edu/ilar_n/ilarhome/index.shtml) is not included in the strain names in the text. F_1 hybrids were produced from C57BL/6J females mated to DBA/2J and SWV males, and their designations are abbreviated as B6 × D2 F_1 and B6 × SWV F_1, respectively. Only female mice were tested from the C57BL/6J strain to simplify the husbandry. C57BL/6J males fought intensely when they were returned to their group cage during the period between tests and would have to be singly caged. Both female and male mice were assessed from SWV and from the B6 × D2 F_1 and B6 × SWV F_1 hybrids. 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 experimental protocols were approved by the Faculty of Medicine Committee on Animal Care of the University of Calgary.

Paw-preference testing

Assessment of paw usage, whether in training reaches or testing reaches, was similar to methods described previously (Biddle and Eales 1999). Mice were at least 10 weeks of age and previously untested. They were fasted and allowed to reach for food in left- or right-biased test chambers with the food tube flush to the left or right side of the chamber, defined as the L-world or the R-world, respectively. The dimensions of the chamber and the food tube were the same as those described by Collins (1975), and our test apparatus accommodated up to 5 mice in individual stations. Flaked or crumbled food was placed in the food tube, and the number of right- and left-paw reaches or entries into the tube to retrieve food was counted. The total number of reaches varied in different experiments. The measure of direction of paw preference is the right-paw entry or RPE score, which is the number of reaches with the right paw in the total number of paw reaches for the specific test.

Elements of learning and memory of hand preference training

Consolidation of memory

Independent groups of previously untested C57BL/6J females were trained in the L-world with 50 paw reaches. They were subsequently tested in the R-world with 50 reaches at 1, 2, 4, or 7 d after training. RPE scores from the R-world tests were compared with the baseline R-world scores of untrained control mice. The difference in mean RPE scores in the R-world between untrained control mice and L-world-trained mice was the measure of learned preference in the left direction.

Blocking consolidation of memory with anisomycin

Anisomycin (Sigma-Aldrich Canada, Oakville, Ont.) was dissolved in 0.9% saline adjusted to pH 7.0–7.4 with 1N HCl according to a published protocol (Abel et al. 1997). The concentration was adjusted to allow an injection volume of 0.01 mL/g body weight. Previously untested C57BL/6J females were trained in the L-world with 50 reaches and immediately (within 1 min) injected subcutaneously with anisomycin by Hamilton syringe at either 150 or 300 mg/kg body weight. One week later, the mice were tested in the R-world with 50 reaches. A saline treatment group was not assessed because the different doses of anisomycin served as treatment control.

Retention of memory

Independent groups of previously untested C57BL/6J females were trained in the L-world with 50 reaches. They were tested in the R-world with 50 reaches at 4, 8, or 16 weeks after training. The RPE scores in the R-world were compared with the 1-week test and control scores. A second test with 50 paw reaches was made in the original L-world, 1 week after the R-world test.

Response to number of training reaches

Independent groups of previously untested C57BL/6J females were trained with different numbers of training reaches in the L-world. After 1 week, they were tested in the R-world with 50 reaches. The RPE scores were compared with control scores. The difference in mean RPE scores in the R-world between untrained (control) and L-world-trained mice was the measure of learned preference in the left direction.

Rate of learning a direction of paw preference in SWV and DBA/2J strains

Compared with C57BL/6J, SWV has a weak conditional paw preference (Biddle et al. 2001). Previously untested SWV and B6 × SWV F_1 mice were trained in the L-world with various numbers of training reaches. One week later, they were tested in the R-world with 50 reaches. RPE scores in the R-world were compared with control scores, and the

rates of learning a left-direction of paw preference in SWV and B6 × SWV F_1 were compared with rates in C57BL/6J.

DBA/2J has a recessive constitutive paw preference because it does not respond to training reaches, but the B6 × D2 F_1 does respond (Biddle et al. 2001). Therefore, previously untested B6 × D2 F_1 mice were trained in the L-world with various numbers of training reaches. One week later, they were tested in the R-world with 50 reaches. RPE scores in the R-world were compared with control scores, and the rate of learning a left-direction of paw preference was compared with the rate in C57BL/6J.

Control mice

C57BL/6J control mice were "chamber control" mice. They were previously untested mice that were fasted and then placed in the L-world chamber without food. They were observed not to reach. They remained in the chamber for approximately 30–45 min, which is the average time required to test a C57BL/6J with 50 reaches. These control mice were fasted again 1 week later, tested in the R-world with food, and were observed for 50 reaches. The SWV, B6 × SWV F_1, and B6 × D2 F_1 control mice were previously untested mice that were tested directly in the R-world with 50 reaches.

Statistical analyses and curve fitting for parameter estimation

Multiple pairwise comparisons were made by ANOVA, followed by Student–Newman–Keuls (SNK) tests (Sokal and Rohlf 1969). The amount of memory was the amount of learned preference, defined as the difference in mean RPE scores in the R-world between trained and control mice. Both the consolidation of the memory of training reaches and the rate of learning a preference appeared to follow a rectangular hyperbolic function. Therefore, a linear transformation, similar to a Hanes-Woolf plot that is used in enzyme kinetics (Dixon and Webb 1964; Segel 1975), was applied to the data. To assess consolidation of the memory of training reaches, the ratio of elapsed time after training to the amount of learned preference was plotted against time. To assess the rate of learning a preference, the ratio of number of training reaches to the amount of learned preference was plotted against number of training reaches. The kinetics of loss of memory of training reaches appeared to be exponential, and a natural logarithmic transformation was applied to the data. Estimation of the parameters of consolidation and retention of memory and of the rate of learning in response to training reaches was done by least-squares regression analysis (Sokal and Rohlf 1969). The alpha level for all tests of statistical significance was set at 0.05.

Results and discussion

Elements of learning and memory of hand preference training in C57BL/6J

Consolidation of memory

Memory of L-world training was consolidated with time after training (Table 1 and Fig. 1). Direction of paw preference in the R-world changed from being right-handed (as in the control mice) to being left-handed in response to the elapsed time after L-world training (Fig. 1A). The difference in mean preference in the R-world between untrained and L-world-trained mice estimates the amount of left direction preference caused by the L-world training (Fig. 1B). The change in magnitude of this difference followed a rectangular hyperbola in response to elapsed time after training and achieved a maximum value. From a linear transformation of the data (Fig. 1C), the inverse of the slope of the regression estimated that a maximum amount of 22.8 RPE units was induced in the left direction by the 50 L-world training reaches, and the *x*-intercept of the regression estimated that 1.4 ± 0.5 (SE) d were required to consolidate half this maximum. Therefore, contextual memory of the 50 training reaches is consolidated over a period of time after training and, in C57BL/6J, it will asymptotically reach the estimated amount of 22.8 RPE units, half of this estimated amount being achieved in 1.4 d. (See later section, Kinetic analysis in C57BL/6J defines capacity and ability to learn, for a fuller presentation of the linear transformation.)

Table 1. Consolidation of contextual memory of 50 L-world training reaches in C57BL/6J mice.

		Test in R-world with 50 reaches	
L-world training Mean RPE±SE	No. of mice	Days after training	Mean RPE±SE*
Control	50	—	32.9±2.8a
13.5±2.2	50	1	22.4±2.4b
12.2±2.1	50	2	19.3±2.4bc
11.9±2.4	50	4	17.6±2.7bc
12.6±2.3	50	7	13.3±2.5c

Note: Consolidation of contextual memory of L-world training is revealed by the change in RPE score in the R-world with elapsed time after training in the L-world.

*Mean RPE scores with the same letter are not significantly different by SNK test ($\alpha = 0.05$).

Blocking consolidation of memory by inhibition of protein synthesis

Anisomycin inhibits cerebral protein synthesis (Davis and Squire 1984) and blocks consolidation of long-term memory (Abel et al. 1997). Treatment of C57BL/6J mice with anisomycin immediately after L-world training blocked the consolidation of contextual memory of this training (Table 2), which is consistent with the hypothesis that a process of learning and long-term memory determines conditional paw preference in C57BL/6J. Since the effect of anisomycin is significant at 300 mg/kg but not 150 mg/kg, there is a dosage effect that could be investigated further.

Retention of memory of L-world training

If memory of the L-world training is being consolidated over time, it must also be decaying. To evaluate this, we tested C57BL/6J mice in the R-world well beyond the 1 week after their L-world training (Table 3). Paw preference of these L-world-trained mice was found to be gradually returning to being right-handed, like the preference of untrained mice in the R-world (Fig. 2A). The difference in mean preference in the R-world between untrained and L-world-trained mice estimates the remaining amount of memory of the 50 L-world training reaches (expressed in RPE

Fig. 1. Consolidation of contextual memory of 50 L-world training reaches in C57BL/6J. (A) Mean paw preference in R-world (mean RPE score ± SE in 50 paw reaches) decreased with the elapsed time after L-world training (data from Table 1). (B) Induced left-preference was estimated by the difference in mean RPE score in the R-world between untrained and trained mice, which increased to a maximum value over time. Curve is from regression analysis in (C). (C) Least-squares regression of the ratio of the number of days after training divided by induced left-preference and plotted against the number of days after training. Maximum amount of learned left-preference was 22.8 RPE units (from the inverse of the slope of the regression of 0.044 ± 0.004 SE); half of the maximum was consolidated in 1.4 ± 0.5 (SE) d (from the *x*-intercept). Regression was significant ($P = 0.01$).

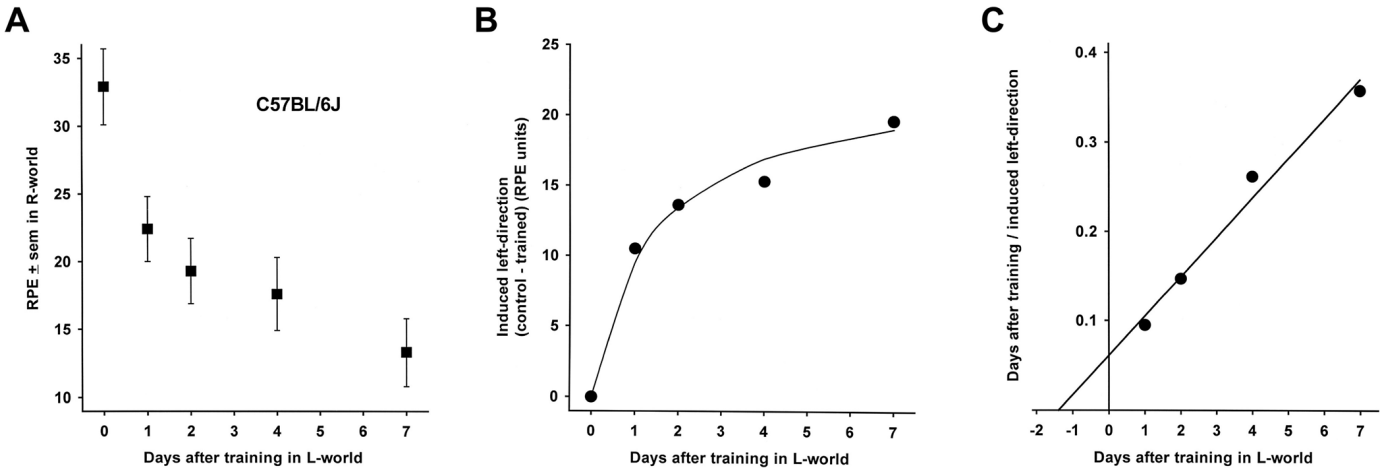


Table 2. Anisomycin blocks the consolidation of contextual memory of 50 L-world training reaches in C57BL/6J mice.

L-world training Mean RPE±SE	No. of mice	Anisomycin (mg/kg)	Test in R-world with 50 reaches Mean RPE±SE*	Inhibition of consolidation (%)
12.6±2.3†	50	0	13.3±2.5a	—
8.9±2.3	24	150	16.8±2.9a	17.9
11.9±2.8	31	300	26.7±2.8b	68.4
Control†	50	0	32.9±2.8b	—

Note: Anisomycin was administered immediately after L-world training, and R-world test was 7 d later. Amount of memory is estimated by the difference in RPE score in the R-world between control and trained mice. Inhibition of consolidation of memory for each dose is estimated by the difference between R-world RPE scores with and without anisomycin relative to the total amount of memory without anisomycin.

*Mean RPE scores with the same letter are not significantly different by SNK test ($\alpha = 0.05$).

†Data repeated from Table 1 to simplify comparisons.

units) (Fig. 2B). From the least-squares regression (Fig. 2C), the exponential decay demonstrates that, at any time after L-world training, the remaining memory of this training is being lost at a constant rate with an estimated half-life of 6.4 weeks. Therefore, approximately 32 weeks (or 5 half-lives) would be required in order for C57BL/6J to lose greater than 95% of the left-hand preference that is induced by the 50 L-world training reaches and to return to within 5% of the paw preference that is expressed by untrained mice in the R-world.

A further test of the L-world-trained mice provided evidence that the learning-and-forgetting process that underlies paw preference is a dynamic process. These data are not rigorously analyzed because they were incidental to the study rather than the result of a separately designed experiment. One week after the R-world test, which assessed the retention of memory of the L-world training, the mice were given another test with 50 reaches in the original L-world (Table 3). The RPE score from the second test in the L-world, compared with the RPE score in the L-world training, demonstrated that L-world-trained mice can be "reconditioned" in the right-direction by their R-world test, but loss of sufficient memory of their original L-world training is required before we can empirically detect it.

Speed of learning in response to training reaches

To determine how quickly C57BL/6J mice can learn a direction of paw preference, we trained groups of mice in the L-world with different numbers of reaches and tested them 1 week later in the R-world with 50 reaches (Table 4, Fig. 3). Direction of paw preference in the R-world changed from being right-handed to being left-handed in response to number of prior L-world training reaches, and it approached a point at which more training reaches had no measurable effect (Fig. 3A). The difference in mean preference in the R-world between untrained and L-world-trained mice estimates

Table 3. Retention of memory of 50 L-world training reaches in C57BL/6J and the effect of the R-world test on a retest in the L-world.

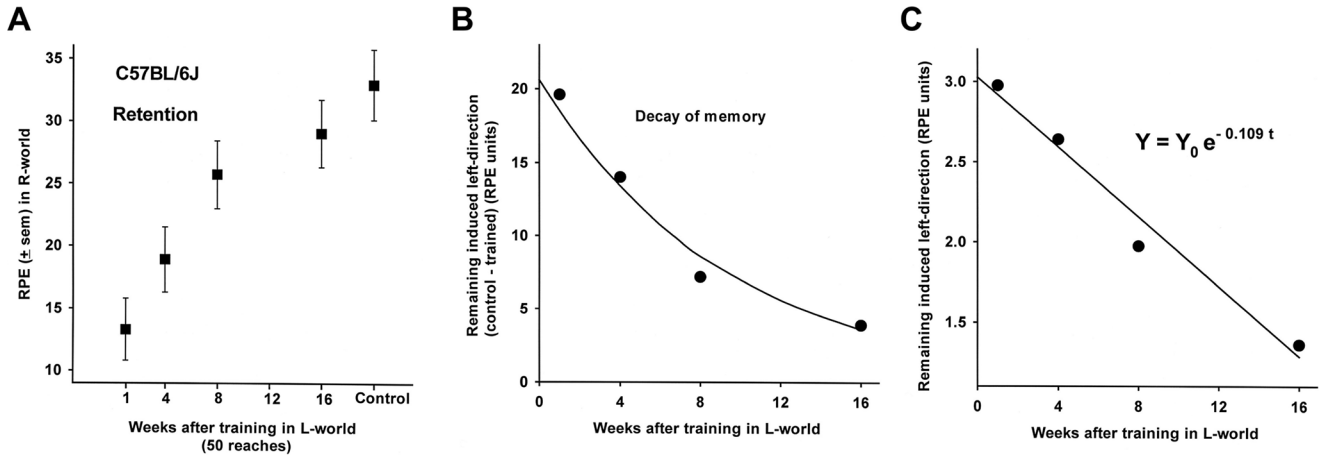
		Test in R-world with 50 reaches		
L-world training Mean RPE±SE	No. of mice	Weeks	Mean RPE±SE*	Second test in L-world with 50 reaches, mean RPE±SE
12.6±2.3[†]	50	1	13.3±2.5a	9.8±2.2
13.8±2.4	50	4	18.9±2.6ab	14.3±2.8
13.9±2.3	50	8	25.7±2.7bc	22.2±3.0
12.3±1.9	50	16	29.0±2.7c	21.5±3.0
Control[†]	50	—	32.9±2.8c	25.9±2.9

Note: Decay of memory is revealed by the change in RPE score in the R-world with elapsed time after training in the L-world. The second test in the L-world, 1 week after the R-world test, demonstrates the effect of the R-world test on direction of hand preference.

*Mean RPE scores with the same letter are not significantly different by SNK test ($\alpha = 0.05$).

[†]Data repeated from Table 1 to simplify comparisons.

Fig. 2. Retention of contextual memory of 50 L-world training reaches in C57BL/6J. (A) Mean paw preference in R-world (mean RPE score ± SE in 50 paw reaches) returned to the preference of untrained control mice with the passage of time (data from Table 3). (B) Induced left-preference was estimated by the difference in mean RPE score in the R-world between untrained and trained mice, which decayed at an exponential rate. Curve is from regression analysis in (C). (C) Least-squares regression of the natural logarithm (ln) of the left-preference remaining at different times after training. Rate constant was $k = -0.109 \pm 0.012$ (SE), and half-life ($t_{1/2}$) was 6.4 weeks (or $ln\ 0.5/k$). Regression was significant ($P < 0.02$).



the amount of left-preference induced by the L-world training (Fig. 3B). The change in magnitude of this difference followed a rectangular hyperbola in response to number of training reaches, and it asymptotically approached a maximum value. From a linear transformation of the data (Fig. 3C), the inverse of the slope of the least-squares regression estimated that 21.5 RPE units was the maximum amount of left-preference that could be induced in C57BL/6J by the L-world training, and the x-intercept of the regression estimated that 10.4 ± 4.0 (SE) training reaches were required to induce half of this maximum.

Kinetic analysis in C57BL/6J defines capacity and ability to learn

The hyperbolic response to increasing number of training reaches (Fig. 3B) is reminiscent of the kinetic features of enzyme-catalyzed or ligand-binding reactions that can be summarized by Henri–Michaelis–Menten–type kinetic equations (Dixon and Webb 1964; Segel 1975). The relation between initial reaction velocity (v) and substrate concentration (s) is often a right rectangular hyperbola of the form

$$(a - y)(b + x) = \text{constant}$$

with limits of a and $-b$. The equation is usually written as

$$(V_{max} - v)(K_m + s) = V_{max}K_m$$

where V_{max} is an estimate of maximum velocity or maximum saturation of enzyme by substrate, and K_m is an estimate of the substrate concentration required for one-half maximum velocity or saturation. K_m is also a measure of the affinity of the enzyme for a substrate. These essential parameters of the kinetic equation have provided useful insight into the biological processes of enzyme-substrate and receptor-ligand interactions.

We applied a Henri–Michaelis–Menten-type equation to the amount of hand preference that was learned from different numbers of training reaches. The relation is written as

$$(D_{max} - d)(K_r + r) = D_{max}K_r$$

and, rearranged to a linear form,

$$r/d = r/D_{max} + K_r/D_{max}$$

Table 4. Response of C57BL/6J to number of L-world training reaches.

L-world training				
No. of reaches	Mean RPE±SE	RPE equivalent to 50 reaches	No. of mice	Test in R-world with 50 reaches, mean RPE±SE*
0†	—	—	50	32.9±2.8a
5	1.4±0.4	14.2	50	27.9±2.7ab
10	2.8±0.5	14.3	50	21.5±2.7bc
20	5.8±1.0	14.6	50	17.8±2.7c
50†	12.6±2.3	12.6	50	13.3±2.5c
100	23.0±4.6	11.5	50	14.0±2.7c

Note: ANOVA of RPE scores in L-world, coded, and standardized to 50 reaches is not significant ($F_S = 0.3087$, degrees of freedom = 4, 245, and $P > 0.75$). Response to number of training reaches is revealed by the change in RPE score in the R-world, 1 week after training.

*Mean RPE scores with the same letter are not significantly different by SNK test ($\alpha = 0.05$).

†Data repeated from Table 1 to simplify comparisons.

(see Fig. 3C) it is similar to a Hanes-Woolf transformation of the Henri–Michaelis–Menten equation. From the measured amount of learned preference d or the difference in mean RPE scores between untrained and trained mice in response to r training reaches, D_{max} is the estimated amount of preference that can be learned and K_r is the estimated number of training reaches that is required for half of this maximum learned preference. In Fig. 3C, the ratio r/d is plotted against r, the asymptote D_{max} is estimated efficiently from the reciprocal of the slope ($1/D_{max}$), and K_r is estimated efficiently by extending the regression to the x-intercept.

We suggest that, for the biological process that determines paw preference in C57BL/6J, D_{max} is the maximum preference that is saturable by training reaches, and K_r is the affinity of the process for training reaches. Therefore, in a functional sense, the parameter D_{max} is a measure of the "capacity" to learn a preference, and the parameter K_r is a measure of the "ability" to learn it. (A similar transformation, based on the Hanes-Woolf transformation of the Henri–Michaelis–Menten equation, was applied to the analysis of consolidation of memory of the 50 L-world training reaches (Fig. 1C). The transformation provided an efficient method to estimate the amount of memory that asymptotically approaches a maximum value with elapsed time after training (Fig. 1B) as well as the amount of time that is required to achieve half of this maximum.)

Genetic factors influence capacity and ability to learn hand preference

We hypothesized that genetic and environmental factors shape paw-preference behaviour by their influence on the underlying learning and memory process and that the effect can be measured by changes in the parameters of capacity (D_{max}) and ability (K_r). To test this hypothesis, we evaluated the rate of learning a paw preference in 2 different mouse strains, SWV and DBA/2J, and their F_1 hybrids from mating with C57BL/6J. Kinetic parameters from this analysis are summarized in Table 5.

Differences in strains with conditional behaviour

SWV mice had conditional paw preference and appeared to learn less preference than C57BL/6J (Biddle and Eales 2001; Biddle et al. 2001). SWV mice responded to training (Table 6 and Figs. 4A and 4B). In comparison with C57BL/6J, SWV mice not only had significantly less capacity for a learned preference (i.e., a smaller D_{max}) but also significantly less ability to learn a preference, because they required significantly more training reaches to achieve half-maximal capacity (i.e., a larger K_r) (summary in Table 5). The B6 × SWV F_1 hybrids also responded to training (Table 7 and Figs. 4A and 4B). B6 × SWV F_1 had capacity and ability parameters that were both intermediate and approximately additive between the C57BL/6J and SWV parental values (summary in Table 5). The kinetic analysis suggests that the elements of the learning and memory processes of both C57BL/6J and SWV were active in the B6 × SWV F_1 hybrid.

Dominant and recessive constitutive paw preference

Relative to conditional paw preference of C57BL/6J, both dominant constitutive and recessive constitutive behaviours are known from strain crosses with C57BL/6J (Biddle et al. 2001). By definition, dominantly acting constitutive paw preference means that neither the specific inbred strain nor its F_1 hybrid from mating with a strong learner, such as C57BL/6J, can learn a preference. Therefore, the conditional behaviour of the C57BL/6J parent would appear to be a recessive phenotype. We suggest that this dominant effect on the behavioural phenotype could be caused by a gain of function that acts as a memory suppressor or regulator of the learning process (Abel and Kandel 1998; Abel et al. 1998; Cardin and Abel 1999). A dominantly acting memory regulator has been suggested to cause the dominant constitutive paw preference in the CDS/LayBid strain (Biddle and Eales 1999).

In contrast, a genetically recessive, constitutive paw-preference behaviour means that a specific inbred strain does not appear to learn a preference, but a conditional behaviour is detectable in the F_1 hybrid from a mating with a strong learner, such as C57BL/6J. Two competing and not trivial hypotheses could explain a recessive genetic effect on paw preference phenotype: either the recessive effect is caused by a true null or loss of function in the learning process, or it is a very weak conditional behaviour that we cannot detect by the testing paradigm. A distinction between the 2 hypotheses is critical for the functional analysis of paw preference behaviour, and we suggested that kinetic analysis might objectively distinguish between the 2 hypotheses.

The DBA/2J strain has genetically recessive, constitutive paw preference. Mice of the DBA/2J strain do not learn a preference in response to reaching, but the B6 × D2 F_1 hybrids from mating with C57BL/6J can learn (Biddle et al. 2001). Without specifying gene number, if a null or loss-of-function mutation causes recessive constitutive preference in DBA/2J, the functional process in the B6 × D2 F_1 heterozygote would be determined only by the active C57BL/6J allele (or alleles), and the gene product of the C57BL/6J allele is expected in only half the amount in the F_1 hybrid. This predicts that the B6 × D2 F_1 mice will have the same ability parameter (K_r) as C57BL/6J to learn a

Fig. 3. Rate of learning a direction of paw preference in C57BL/6J in response to training reaches is described by 2 parameters, referred to as "capacity" and "ability". (A) Mean paw preference in R-world (mean RPE score ± SE in 50 reaches) decreased in response to number of L-world training reaches (data from Table 4). (B) Induced left-preference was estimated by the difference in mean RPE score in the R-world between untrained and trained mice, which increased to a maximum value. Curve is from regression analysis in (C). (C) Least-squares regression of the ratio of the number of L-world training reaches divided by the induced left-preference and plotted against the number of training reaches. Maximum induced left-preference was 21.5 RPE units (from inverse of the slope of the regression of 0.047 ± 0.003 (SE)) and defined the parameter called "capacity". Half of the maximum left-preference was induced by 10.4 ± 4.0 (SE) training reaches (from the *x*-intercept) and defined the parameter called "ability". Regression was significant ($P < 0.001$).

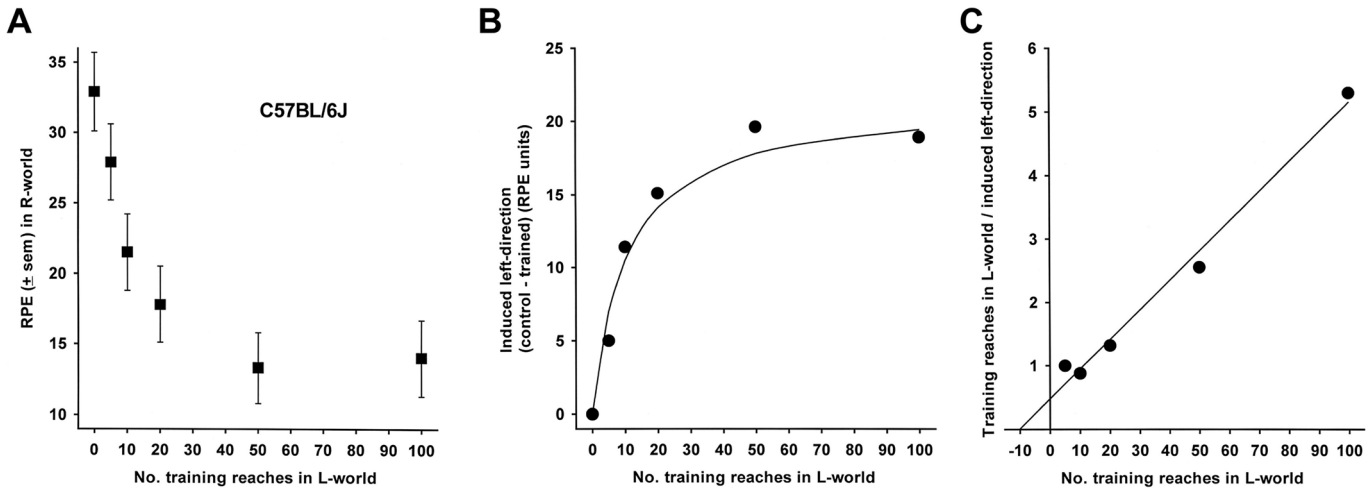


Table 5. Summary of capacity and ability to learn a direction of paw preference in C57BL/6J, SWV, B6 × SWV F_1, and B6 × D2 F_1.

	Capacity (RPE units)		
Genotype	D_{max}	$(1/D_{max})$±SE	Ability (no. of reaches) K_r±SE
C57BL/6J	21.5	(0.047±0.003)	10.4±4.0
SWV	6.8	(0.147±0.016)	63.7±16.9
B6 × SWV F_1	11.4	(0.088±0.001)	26.9±1.7
B6 × D2 F_1	11.8	(0.085±0.005)	13.3±6.6

Note: Capacity (D_{max}) is derived from the inverse of the slope ($1/D_{max}$), and ability (K_r) is derived from the *x*-intercept of the least-squares regression analyses of the learning curves (Figs. 3C, 4B, and 4D).

preference but will have only half the capacity (D_{max}) for a learned preference. Alternatively, DBA/2J might have an undetectable conditional preference. This alternative hypothesis predicts that the B6 × D2 F_1 will have a significantly larger K_r than C57BL/6J, because the ability parameter of the F_1 would be the average of the weak but undetectable ability of DBA/2J and the measurable ability of C57BL/6J. The prediction for this alternative hypothesis is made plausible by the previous kinetic analysis of the weak learning in SWV compared with the strong learning in C57BL/6J (Fig. 4A and 4B).

The B6 × D2 F_1 hybrid responded to training (Table 8 and Figs. 4C and 4D). The B6 × D2 F_1 mice had an ability parameter (K_r) that was similar to that of the C57BL/6J parental strain but only half the capacity (smaller D_{max}) for a learned preference (summary in Table 5). We conclude that only the C57BL/6J alleles determine the learning of paw preference in the B6 × D2 F_1, and we infer that DBA/2J has what appears to be a loss of function in this learning process. Moreover, we believe that this may be the first demonstration of a true null or loss-of-function effect on a behavioural phenotype in the mouse that does not depend on a prior molecular hypothesis for its validation.

Independence of capacity and ability to learn a preference

In addition to the fundamental difference between conditional and constitutive paw-preference behaviours, the essential kinetic parameters of capacity (D_{max}) and ability (K_r) to learn a preference appear to vary independently among genetically defined mice with a conditional behaviour (Table 5). For example, B6 × SWV F_1 and B6 × D2 F_1 mice can learn a similar amount of paw preference because they have a similar capacity (D_{max}), but they have significantly different abilities (K_r) to learn it because they require significantly different numbers of training reaches to achieve the same half-maximal capacity. In contrast, C57BL/6J and B6 × D2 F_1 mice have a similar ability (K_r) to learn a preference but, with this similar ability, they learn significantly different amounts or capacities (D_{max}) of paw preference. Therefore, besides turning on and turning off the underlying process of learning and memory that results in conditional and constitutive paw preference, genetic factors can independently influence the parameters of capacity (D_{max}) and ability (K_r) to learn a direction of preference.

Resolution of phenotypic complexity in paw-preference behaviour

It is clear that a process of learning and memory determines hand preference of mice and that both genes and environment, including the context of the testing chamber, influence direction of hand preference. The U-world testing

Table 6. Response of SWV to number of L-world training reaches.

L-world training				
No. of reaches	Mean RPE±SE	RPE equivalent to 50 reaches	No. of mice	Test in R-world with 50 reaches, mean RPE±SE*
0	—	—	51	29.3±1.9a
50	14.9±1.7	14.9	45	22.0±1.9b
100	35.1±3.5	17.6	58	20.3±2.4b
150	46.1±4.4	15.4	49	19.7±2.3b

Note: Response to number of training reaches is revealed by the change in RPE score in the R-world 1 week after training.

*Mean RPE scores with the same letter are not significantly different by SNK test ($\alpha = 0.05$).

Fig. 4. Capacity and ability to learn a direction of paw preference in SWV and DBA/2J compared with C57BL/6J. (A) Rate of learning a left-preference in SWV (data from Table 6) and B6 × SWV F_1 (data from Table 7) compared with C57BL/6J (from Fig. 3). Curves are from regression analyses in (B). (B) Least-squares regression of the linear-transformed learning curves shows that maximum capacity to learn a left-preference was 6.8 RPE units in SWV (from inverse of the slope of the regression of 0.147 ± 0.016 (SE)) and 11.4 RPE units in B6 × SWV F_1 (from inverse of the slope of 0.088 ± 0.001 (SE)). Ability or number of training reaches for half-maximum capacity was 63.7 ± 16.9 (SE) reaches in SWV and 26.9 ± 1.7 (SE) reaches in B6 × SWV F_1. Least-squares regressions were done, as shown in Fig. 3C, and the significance of the regressions were $P < 0.02$ for SWV and $P < 0.01$ for B6 × SWV F_1. (C) Rate of learning a left-preference in B6 × D2 F_1 (data from Table 8) compared with C57BL/6J (from Fig. 3). Curves are from regression analysis in (D). (D) Least-squares regression of the linear-transformed learning curve shows that maximum capacity of B6 × D2 F_1 to learn a left-preference was 11.8 RPE units (from slope of 0.085 ± 0.005 (SE)), and ability was 13.3 ± 6.6 (SE) reaches. Least-squares regression for B6 × D2 F_1 was done, as shown in Fig. 3C, and it was significant ($P < 0.001$).

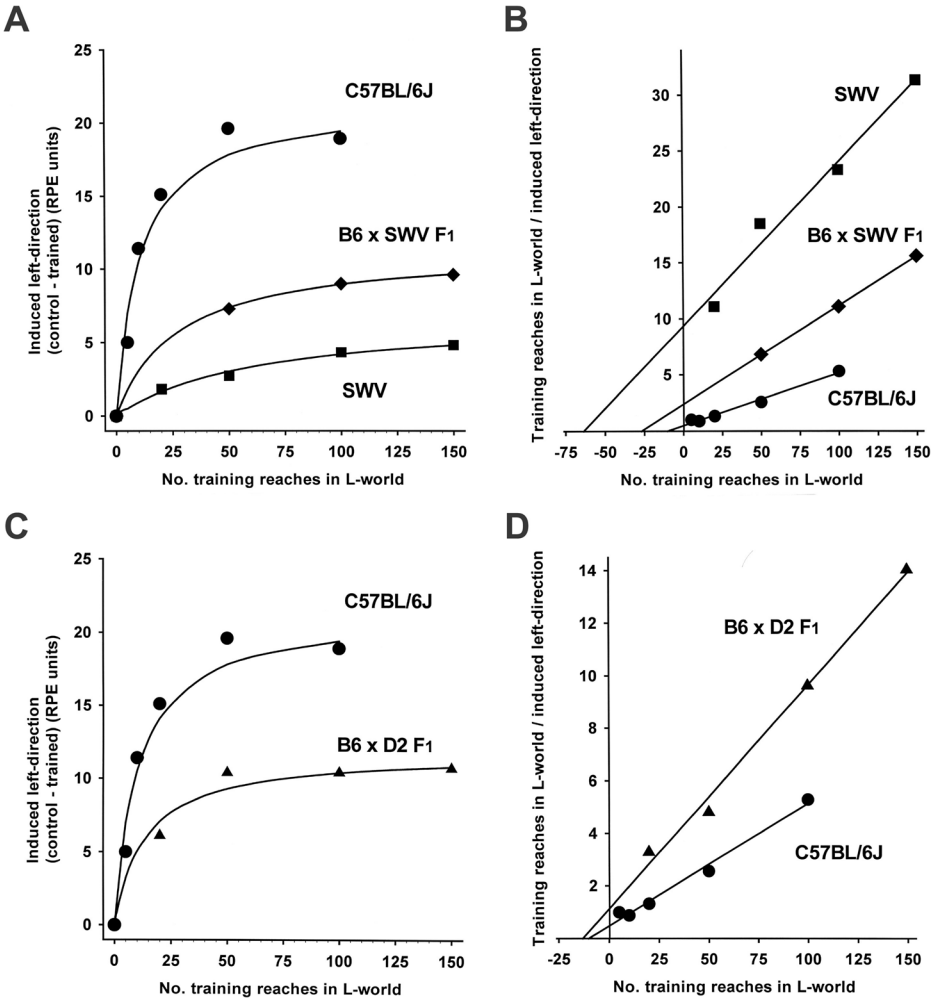


Table 7. Response of B6 × SWV F_1 hybrids to number of L-world training reaches.

L-world training				
No. of reaches	Mean RPE±SE	RPE equivalent to 50 reaches	No. of mice	Test in R-world with 50 reaches, mean RPE±SE*
0	—	—	88	30.7±1.4a
20	6.2±0.7	15.6	70	24.6±1.9b
50	15.2±1.4	15.2	69	20.3±1.9b
100	35.0±3.5	17.5	67	20.3±2.3b
150	48.7±4.5	16.2	67	20.0±2.2b

Note: Response to number of training reaches is revealed by the change in RPE score in the R-world 1 week after training.

*Mean RPE scores with the same letter are not significantly different by SNK test ($\alpha = 0.05$).

Table 8. Response of B6 × D2 F_1 hybrids to number of L-world training reaches.

L-world training				
No. of reaches	Mean RPE±SE	RPE equivalent to 50 reaches	No. of mice	Test in R-world with 50 reaches Mean RPE±SE*
0	—	—	100	31.2±1.1a
20	10.3±0.7	25.8	75	29.4±1.5ab
50	20.8±1.0	20.8	100	28.5±0.9ab
100	39.0±2.2	19.5	100	26.9±1.3b
150	65.8±1.1	21.9	100	26.4±1.2b

Note: Response to number of training reaches is revealed by the change in RPE score in the R-world 1 week after training.

*Mean RPE scores with the sameletter are not significantly different by SNK test ($\alpha = 0.05$).

chamber, which has been used historically to assess hand-preference behaviour, has a centrally placed food tube and does not restrict the ability of the mouse to reach with either hand. Since the U-world cannot reveal the learning and memory process of hand preference, some statements that have been made about hand preference in the U-world are now inappropriate or misleading. They are briefly summarized because they have direct bearing on further genetic analysis.

Hand preference, represented by the RPE score in U-world tests, has been described as being highly reliable in test-retest assessments of individual mice (Collins 1975; Biddle and Eales 1999). Unless hand preference is random every time a mouse reaches, direction of hand preference is expected to be reliable in individual mice in the U-world, regardless of whether it is conditional or constitutive. It is simply reliable for different reasons.

Direction of paw preference in the U-world has appeared to be neutral when the average RPE scores are compared among different mouse strains (e.g., strain survey in Biddle and Eales 1996). Since the U-world does not physically restrict the mouse's ability to reach, average direction of hand usage in the U-world is expected to be neutral, unless direction of preference is the genetically programmed character.

With the current understanding of hand preference in mice, persistence with U-world tests and the "degree of lateralization" measurement only serves to complicate the genetic and functional analysis of the behaviour. Comparisons among individuals in their "degree of lateralization" is equivalent to conducting an analysis of variance when the research question should concern itself with the analysis of cause. Since prior analysis of the learning and memory process was not conducted, it is difficult to interpret the significance of a recent QTL analysis of the degree of lateralization (Roubertoux et al. 2003). Similarly, the functional effect of specific gene substitutions and transgenic inserts on hand preference (e.g., Roubertoux et al. 2005) cannot be assessed without basic knowledge of the background strain behaviour.

Summary

The present analysis has revealed quantitative features of a dynamic learning and memory process that underlies hand-preference behaviour of the mouse. Henri–Michaelis–Menten-type kinetics describes the consolidation of memory in response to elapsed time after training: memory progresses to a quantifiable maximum that is related to the time for half-maximal consolidation. Proteins and gene functions are involved in the process because consolidation is blocked by anisomycin, a protein synthesis inhibitor. The process is dynamic because memory decays at a constant rate, and the mice can be retrained. Henri–Michaelis–Menten-type kinetics also describes the speed of learning a preference in response to number of training reaches: memory progresses to a maximum value, which we call capacity (D_{max}), and this is related to the number of training reaches for half-maximal capacity, which we call the ability constant (K_r). Capacity and ability are genetically independent parameters of the learning and memory process underlying paw-preference behaviour.

Quantitative features of the learning and memory process predict the feasibility of identifying genes and functions that influence the hand-preference behaviour of mice. They provide a clear framework to interpret the phenotypic variation, based on plausible functional models that include not only loss of function but also gain of function, such as dominantly acting regulators or suppressors of the learning process. Similar quantitative features may provide additional methods to characterize genetic and environmental variables that influence other mouse behaviours (Crawley and Paylor 1997; Crawley 2000) and to functionally assess molecular models of learning and memory processes (Milner et al. 1998).

The paw-preference behaviour of mice has some relevance to the interpretation and analysis of human hand preference, because what we see as human hand preference may be a stereotype that needs to be re-evaluated. Family and population studies of human handedness have suggested compelling biallelic, single-gene genetic models (Annett 1995; McManus and Bryden 1992; Klar 1996), and they support the belief that "people are right- or left-handed because of the genes they carry" (McManus 2002). Further, a recent association has been found between the direction of scalp hair-whorl rotation and handedness, suggesting that they may derive from a common genetic mechanism (Klar 2003, 2005). Nevertheless, the genetic models have yet to be confirmed by candidate or marker genes (Van Agtmael et al. 2002, 2003), leaving the cause of human hand preference in a nature vs nurture debate (Provins 1997; Corballis 1997).

The instructive value of the mouse model is that it shows that paw-preference behaviour is clearly influenced by an underlying process of learning and memory, and that genetically determined differences among mice influence this learning and memory process and change the phenotypic expression. Nevertheless, individual mice are like individual people. Individually, they express a reliable hand preference and, after a test, the preference score of the individual does not indicate whether the individual learned the preference or has simply expressed an innate behaviour. In addition, different mice can have the same preference score for genetically different reasons. It was the phenotypic differences in the norms of reaction among replicate samples of genetically defined individuals that revealed the learning and memory process of the mouse behaviour, and this learning and memory process is the functional framework for an analysis of cause of the phenotypic diversity. This is impossible to pursue with people, but insight into human handedness may come from further genetic and functional analysis of mouse behaviour.

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