Lateral asymmetry of paw usage: phenotypic survey of constitutive and experience-conditioned paw-usage behaviours among common strains of the mouse

Fred G. Biddle and Brenda A. Eales

Abstract: Left-right direction of paw usage in the mouse is defined by the right-paw entry (RPE) score, which is the number of reaches with the right paw to retrieve food from a small food tube in a total of 50 right- and left-paw reaches. Two qualitatively different paw-usage behaviours can be identified by the difference in the RPE scores from naive mice in left- or right-biased test chambers and their retest, 1 week later, in the opposite-biased test chamber. In mice with constitutive paw usage, the RPE score may respond to the direction of a biased test chamber, but it returns to the value that is expected for naive mice in the opposite-biased test chamber. In mice with experience-conditioned paw usage, the RPE score responds to the direction of a biased test chamber and does not return to its expected value in the opposite-biased test chamber. In this report, we document the alternate paw usage behaviours in an extended phenotypic survey of different strains that will be useful for its genetic analysis. We also validate an alternate biometrical method to identify constitutive and experience-conditioned paw usage that is based on the mean average RPE score from the biased test and opposite-biased retest of individual mice. This alternate biometrical method demonstrated that, in some strains with experience-conditioned paw usage, there may be asymmetry or an interaction between genotype and the direction of the test sequence. In addition, the strain survey demonstrated that the qualitative difference between constitutive and experience-conditioned paw usage is independent of the well-known quantitative difference in the degree of lateralization of preferred-paw usage.

Key words: mouse, lateral asymmetry of paw usage, left and right handedness, behavioural genetics, constitutive behaviour, experience-conditioned behaviour.

Résumé: La préférence pour l’utilisation de la patte gauche ou droite chez la souris est mesurée à l’aide d’un score dans le test RPE (« right paw entry »). Il s’agit du nombre de fois que la souris utilise la patte droite pour retirer de la nourriture située dans un petit tube lors de 50 tentatives. Deux comportements qualitativement différents peuvent être identifiés sur la base de différences dans le score RPE. Ce dernier est mesuré d’abord chez des souris naïves placées dans des cages ayant le biais. Chez les souris ayant une préférence constitutive, le score RPE peut être influencé par la direction du biais mais le score revient à celui attendu pour des souris naïves lorsque ces souris sont placées dans des cages ayant le biais contraire. Chez les souris ayant une préférence conditionnée par l’expérience, le score RPE répond au biais de la cage mais ne revient pas à la valeur attendue lorsque les souris sont placées dans les cages ayant le biais contraire. Les auteurs rapportent ici la caractérisation phénotypique d’un grand nombre de souches pour ce caractère, une étude utile en vue de l’analyse génétique de ce caractère. Les auteurs ont également validé une méthode biométrique alternative pour identifier le type de préférence au niveau de l’utilisation des pattes (constitutive ou conditionnée par l’expérience). Cette méthode est basée sur la moyenne des scores RPE moyens obtenus lors d’un test biaisé suivi d’un second test avec le biais contraire. Cette seconde méthode a montré que, chez certaines souches à usage conditionné par l’expérience, il peut y avoir asymétrie ou une interaction entre le génotype et la séquence dans laquelle les biais sont utilisés. De plus, l’examen des souches a démontré que la différence qualitative entre usage constitutif et conditionné est indépendante de la différence quantitative (et bien connue) quant au degré de latéralisation de la préférence pour l’utilisation d’une patte.

Mots clés: souris, asymétrie latérale de l’utilisation d’une patte, préférence pour l’utilisation de la patte gauche ou droite, génétique du comportement, comportement constitutif, comportement conditionné par l’expérience.

[Traduit par la Rédaction]
Introduction

There is a common sense understanding of human left-hand and right-hand usage. The bias for right handedness, throughout history and in many different cultures (Coren 1992), has reinforced a belief that there is a genetic component to asymmetry of hand usage and, in turn, an association with cerebral dominance and lateralization of language specialization (McManus and Bryden 1992; Annett 1995). Efforts to evaluate the genetics of hand usage have been fraught with difficulty and no gene has been identified.

The mouse offers a unique opportunity to examine left-right handedness in the absence of both cultural bias and any assumed relationship to other developmental asymmetries. Paw usage is a simple behaviour that can be assessed in mice with a single-paw food-reaching task (Collins 1968, 1969, 1985). Replicated tests with different test paradigms are possible with single genotypes that are defined by the inbred strains, and comparisons of handedness can be made among different strains. Therefore, if allelic forms of genes influence paw usage, it should be possible to identify them.

Paw usage is normally assessed with an unbiased or U-world test, in which a mouse reaches for food from a centrally placed food tube. When mice of different strains are allowed a set number of paw reaches, they express complex patterns or distributions of individual mice with different proportions of left- and right-paw usage. The proportions of left- and right-paw usage by individual mice in a U-world test are reliably expressed on retesting them in a L world or R world (Collins 1975). Flaked or crumbled food was placed in the registered laboratory code “Bid” (Greenhouse 1996). They were assessed previously for the quantitative difference in the degree of lateralization of preferred-paw usage with only a U-world test (Biddle and Eales 1996).

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In an asymmetrical test chamber, the food tube is placed flush to the left or right side of the test chamber and defined, respectively, as a L world or R world (Collins 1975). The phenotypic reaction norm of direction of paw usage by naive mice in test chambers that were asymmetrical and the response of these mice to a retest in test chambers of the opposite world revealed two qualitatively different behaviours, and this observation caused a paradigm shift in the model of paw usage (Biddle and Eales 1999). In some strains, paw usage is a constitutive behaviour and, in others, it is conditioned by the direction of the test chamber and the experience of reaching.

A functional model for the cause of the phenotypic differences in lateral asymmetry of paw usage might be constructed from a genetic analysis of the alternate constitutive and experience-conditioned paw-usage behaviours. Therefore, as a starting point for this analysis, the qualitative difference between constitutive and experience-conditioned paw usage was defined in an extended strain survey. Since replicate assessment of genetically homogeneous samples of mice is required to define the qualitative difference in paw-usage behaviour, an efficient approach to genes that influence the alternate behaviours will be through the analysis of recombinant inbred (RI) strains that have been derived from specific progenitor strain pairs (Taylor 1978; Bailey 1981).

Therefore, in addition to the historical relationship among mouse strains, attention was directed to the progenitors and other closely related strains that were used to produce some of the sets of RI strains. The results are presented in this report.

Materials and methods

Inbred strains of mice

Lateral asymmetry of paw usage was tested in BALB/cByJBid, CLA/Bid, C3H/HeHaBid, C3H/HeSnxBid, DBA/2JBid, FVB/NJ, Line 22c, and WC/ReJBid. The origins of six of these strains were described previously (Biddle and Eales 1996); they have been maintained for more than 20 generations from their sources by continued sister–brother inbreeding and are referenced here with the registered laboratory code “Bid” (Greenhouse 1996). They were assessed previously for the quantitative difference in the degree of lateralization of preferred-paw usage with only a U-world test (Biddle and Eales 1996).

FVB/NJ is an inbred strain that was obtained recently from the Jackson Laboratory (Bar Harbor, Maine), through the courtesy of Dr. R.L. Hawkes (The University of Calgary). FVB/NJ was assessed because it is being used as an inbred rather than a hybrid host genome for random insertional transgenesis (Taketo et al. 1991). With its high fecundity and normal rate of variant marker alleles (Neuhaus et al. 1997), FVB/NJ may be useful for genetic studies of behaviour. It was assessed for paw usage at the +F1−1 generations of continued sister–brother inbreeding from source.

Line 22c has not yet been assigned a registered strain name. It is a completely acallosal inbred strain that is one of a set of RI strains that was developed from the F2 generation from a cross between the 129/ReJ and BALB/cWah1 inbred strains (Wahlsten and Sparkes 1995), both of which have a genetic liability to express the complex trait of deficient–absent corpus callosum (Wahlsten 1982, 1987; Livy and Wahlsten 1991; Wahlsten and Schalomon 1994). Line 22c was obtained from Dr. D. Wahlsten (The University of Alberta), maintained by continued sister–brother inbreeding, and assessed for paw usage at an additional +F15−17 generations in this laboratory.

An important distinction is made between the laboratory mouse and its wild-derived progenitor species of *Mus musculus*. The inbred strains of the laboratory mouse represent a sample of some of the viable genetic recombinants between these different species (e.g., see Discussion in Bonhomme and Guenet 1996; Silver 1996; Beck et al. 2000; Mouse Genome Database (available via http://www.informatics.jax.org)). In this context, CLA is an inbred strain of wild-derived *Mus musculus domesticus* that has not been genetically contaminated during its development by mating with the laboratory mouse.

Paw-usage measurement and summary statistics

Paw usage was assessed as described previously (Biddle and Eales 1999). After a fast of 12–24 h, naive mice were tested in an unbiased or U-world test chamber with a centrally placed food tube or in left- or right-biased test chambers with the food tube placed flush to the left or right sides and defined as a L or R world, respectively (Collins 1975). Flaked or crumbled food was placed in the food tube and the numbers of right- and left-paw entries to retrieve food were counted in a total of 50 paw reaches.

The measures of paw usage are the same as described previously (Biddle and Eales 1999). The primary measure of paw usage is the number of reaches with the right paw in a total of 50 paw reaches (right-paw entry or RPE score); this measure has numerical values of 0 to 50. The RPE score provides a measure of direction of paw usage in response to a specific test chamber; mice with RPE scores of 0–24 are more left-pawed and mice with RPE scores of 26–50.
are more right-pawed. A second measure is the preferred-paw entry (PPE), which is the larger of the number of right- or left-paw reaches in the total of 50 paw reaches. The PPE score provides a measure of the degree or strength of lateralization of the preferred paw, without regard to its left or right direction, and has numerical values of 25 to 50.

U-world measures of paw usage for some strains in this report were calculated from the original data for a previous survey of naive mice (Biddle and Eales 1996). The strains, which had not been tested previously in a U world, were assessed with a sample of naive mice.

The difference between constitutive and experience-conditioned paw-usage behaviours was identified by testing naive mice in a L or R world and, 1 week later, retesting the individual mice in the respective opposite-world test chamber (Biddle and Eales 1999). Figure 1 illustrates the test–retest paradigm and facilitates the description of the contrasts that were made between the RPE scores from the tests to identify the alternate paw-usage behaviours. Within each strain, three critical contrasts were made between the RPE scores from the independent samples of mice that were tested in the opposite and in the same direction. The direction of paw usage of naive mice, measured by the RPE score, moves in response to the direction of the test chamber; in the retest in the opposite direction, 1 week later, the direction of paw usage returns to its expected value in strains with constitutive paw usage, but does not return to its expected value in strains with experience-conditioned paw usage.

Figure 1 also suggested an alternate method for identifying constitutive and experience-conditioned paw-usage behaviours and this alternate method is validated in Results and discussion. The average of the RPE scores in the L- and R-world tests was determined for individual mice and compared between the two test–retest sequences that were done in opposite directions. Strains with constitutive paw usage are expected to have the same mean average of their RPE scores in the two test–retest sequences, and the two mean averages may not differ from the median value of 25 on the 0–50 RPE scale. Strains with experience-conditioned paw usage are expected to differ in the direction of the mean average of their RPE scores, and the direction of the mean average may depend on the left or right direction of the test chamber that was used for their first test as naive mice.

Both females and males were tested in some strains, but only females were tested in other strains. Different sample sizes were tested from the different strains but, within a strain, equal numbers of mice were tested in the two asymmetrical tests of naive mice and their retest in the opposite-biased test worlds. The biometrical tests were taken from Siegel (1956) and Sokal and Rohlf (1969) and are described in Results and discussion. The level of significance for all tests was α = 0.01.

Results and discussion

Direction of paw usage: experience-conditioned and constitutive behaviours

No difference was found in paw-usage behaviour between the sexes in the strains in which both sexes were assessed. Also, there was no difference in paw-usage behaviour between the sexes in the four strains that were described in our previous report (Biddle and Eales 1999). Therefore, all summaries and comparisons are made with the total number of mice. When both sexes were used, their numbers are indicated.

The mean RPE scores from the strains in the present survey are summarized in Table 1 for the asymmetrical tests of naive mice and for their retests, 1 week later, in the opposite direction. The mean RPE scores from U-world tests of naive mice are listed for comparison. It is important to remember that the RPE score, or number of right-paw reaches in the total of 50 paw reaches, provides the measure of direction of paw usage; a RPE score of 0–24 indicates more left-paw usage, and a RPE score of 26–50 indicates more right-paw usage.

The alternate experience-conditioned and constitutive paw-usage phenotypes were identified by statistical inference from three independent contrasts of the mean RPE scores (Biddle and Eales 1999). The contrasts are summarized in Table 2 for each strain, and the statistical inferences are from t tests of the means of the independent samples. To simplify Table 2, the t values and their associated probabilities are not listed, but they are available on request.

Contrast 1 (Table 2) compares the mean RPE scores for the independent samples of naive mice in response to L- and R-world tests. Contrasts 2 and 3 (Table 2) compare the respective mean RPE scores from the retest in the opposite direction with the observed mean RPE score for the independent sample of naive mice that was tested in that direction. Naive mice move their direction of paw usage in response to a biased world, and the difference between mean RPE scores from the biased-world tests of naive mice may or may not be significant (contrast 1 in Table 2). Experience-conditioned paw usage
behaviour is defined by a significant difference in both contrasts 2 and 3; constitutive behaviour is defined by no significant difference in contrasts 2 and 3. In the present survey, two strains have experience-conditioned paw usage and six strains have constitutive paw usage (Table 2).

The association between the RPE scores from the first test of naive mice in a biased world and the retest of the same individuals in the opposite-biased test world is significant for all strains when the associations were tested by Spearman’s rank correlation test in Biddle and Eales (1996) (Table 3). From the strains in this study (Table 3) and in our previous report (Biddle and Eales 1999), a significant association was found, regardless of whether a strain was experience-conditioned or constitutive in its paw-usage behaviour. In hindsight, absence of an association between the RPE scores from an asymmetrical test and the retest in the opposite direction might be expected if paw usage were a completely random behavior.

An alternate method was used to illustrate the association between the RPE score of individual mice to a biased-world test and to a retest in the opposite direction (Figs. 2 and 3; see previous use of the method in Biddle and Eales 1999). Strains with experience-conditioned paw usage are shown in Fig. 2 and strains with constitutive paw usage are shown in Fig. 3.

In Figs. 2 and 3, the average of the two RPE scores for an individual mouse in the L and R worlds (y-axis) is plotted against the numerical difference between the individual’s RPE scores in the L and R worlds (x-axis). The test of naive individuals in a L world and their retest in a R world, 1 week later, is shown in the panels on the left in Figs. 2 and 3; the reverse test sequence is shown in the panels on the right.

It is important to note that the scatter plots in Figs. 2 and 3 are nothing more than a geometric rotation, in the amount of 45° in a counterclockwise direction, of the simple association that would be represented by the two RPE scores from individual mice that are tested in a biased world and retested in the opposite direction. It was the simple association that was assessed by the Spearman’s rank correlation test in Table 3.

The scatter plots in Figs. 2 and 3 appear to be more visually informative because they illustrate the change in RPE score of individual mice when the mice were tested as naive individuals in a biased world and subsequently retested in the opposite biased test world. The summary statistics are the numerical difference between the two RPE scores and the numerical average of the two RPE scores from the biased-world test and the opposite-biased world retest (see Table 4). The inferences that can be drawn from summary statistics of mean values are not always apparent without a visual reference to the population of values that are being summarized (Figs. 2 and 3).

Table 4 summarizes the means of the numerical difference between the two RPE scores and the means of the average of the two RPE scores in a L world and a R world from individual mice for the two test–retest sequences. It is these summary statistics (Table 4) that were explored for an alternate and simpler method of identifying experience-conditioned and constitutive paw-usage behaviours. The four strains (C57BL/6J, CDS/Lay, NOD/Lt, and SWV) that brought the qualitative difference in paw-usage behaviour to our attention are included in Table 4, because the summary statistics were not calculated previously (Biddle and Eales 1999).

The means of the numerical difference between the RPE score in a L world or a R world of individual naive mice and their respective retest in the opposite-biased world show a deviation from a value of 0; see the means of the difference (RPE-L) – (RPE-R) or (RPE-R) – (RPE-L)) ± 95% confi-
dence limits in Table 4. The sign of the deviation is either to the left (–) or to the right (+), depending on the direction of the first test of naive mice, and the deviation is illustrated on the x-axis in Figs. 2 and 3. Most strains express a response to the left direction or the right direction of the test chamber. The only exception to a significant deviation from a value of 0 is BALB/cByJ, which was tested in both test–retest sequences, and Line 22c, which was tested in the left-to-right test–retest sequence (Table 4). The magnitude of the means of the numerical difference in RPE scores is the same for the two test–retest sequences within each strain (Table 4).

As suggested by Fig. 1 and Table 4, it is the mean of the average of the two RPE scores of individual mice in a biased-world test and the opposite-biased world retest that provides an alternate and efficient method of identifying strains with either experience-conditioned or constitutive paw-usage behaviour; see $\frac{1}{2}[(RPE-L) + (RPE-R)]$ and $\frac{1}{2}[(RPE-R) + (RPE-L)] \pm 95\%$ confidence limits in Table 4. Inspection of the mean of the average RPE scores (±95% confidence limits) from the two independent test–retest sequences for each strain demonstrates that there is a significant difference between the two means only for the four strains that have experience-conditioned paw usage. The mean values (±95% confidence limits) from these four strains are presented in bold in Table 4 and the difference between the two means of the average RPE scores is significant for each of the four strains when they are compared by t tests ($P < 0.001$).

Strains with experience-conditioned paw-usage behaviour express a net deviation from a value of 25 for the distribution of the average of their L-world and R-world RPE scores from the two test and retest sequences for individual mice (Table 4). This is illustrated by the values plotted on the y-axis in Fig. 2. The direction of the deviation from the median value of 25 on the 0–50 RPE scale depends on the direction of the first test of naive mice. Strains with a constitutive paw-usage behaviour (Fig. 3) do not show a net deviation from the median value of 25 for the average of their RPE scores (Table 4).

In addition, the deviation from the median value of 25 for the means of the average of the L-world and R-world RPE scores of individual mice might be expected to be symmetrical for the two test–retest sequences. Inspection of the means from the two test–retest sequences of the four experience-conditioned strains (presented in bold in Table 4) suggests that the deviation may not be symmetrical for all strains. There may be an asymmetry or a greater deviation to the “left” for the C57BL/6J and FVB/NJ strains, but not for the C3H/HeHa and SWV strains. This suggests an unexpected interaction between genotype and the direction of the test sequence; the “inherent” distribution of the proportions of left- and right-paw usage by some strains may not be symmetrically distributed around the median value of 25 on the 0–50 RPE scale. This could be explored further with the F₁ hybrid generations from different experience-conditioned strains. There was some evidence for this potential interaction from the unbiased U-world tests of some strains and the deviation of their mean RPE scores from a median value of 25 (Biddle and Eales 1996).

Without knowing the potential for this interaction between the genotype and the direction of the test sequence among
different experience-conditioned strains, it may not be possible to test and retest mice in only one direction to develop a test statistic for individual mice that will clearly distinguish between the alternate experience-conditioned and constitutive paw-usage behaviours. Such a test might use an expected null value of 25 to test for the significance of difference in the average direction of paw usage. It would be required, for example, to assess the alternate qualitative difference in experience-conditioned and constitutive paw-usage behaviours among the genetically unique individuals in segregating generations of standard genetic crosses.

Figures 2 and 3 and Table 4 complement the conclusions that are drawn from the critical contrasts in Table 2. They illustrate the behaviour that is observed with individual mice.

Table 3. Summary of Spearman’s rank correlation ($r_s$) tests for association between the right-paw entry (RPE) scores of naive mice tested in a L world or a R world and retested 1 week later in the respective opposite-biased test world.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sample size for each test–retest sequence</th>
<th>Test–retest sequence $r_s$</th>
<th>Left to right</th>
<th>Right to left</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/cByJ</td>
<td>40</td>
<td></td>
<td>0.813</td>
<td>0.576</td>
</tr>
<tr>
<td>C3H/HeHa</td>
<td>75</td>
<td></td>
<td>0.792</td>
<td>0.790</td>
</tr>
<tr>
<td>C3H/HeSnJ</td>
<td>26</td>
<td></td>
<td>0.787</td>
<td>0.773</td>
</tr>
<tr>
<td>CLA</td>
<td>55</td>
<td></td>
<td>0.705</td>
<td>0.783</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>101</td>
<td></td>
<td>0.835</td>
<td>0.841</td>
</tr>
<tr>
<td>FVB/NJ</td>
<td>83</td>
<td></td>
<td>0.783</td>
<td>0.800</td>
</tr>
<tr>
<td>Line 22c</td>
<td>60</td>
<td></td>
<td>0.631</td>
<td>0.763</td>
</tr>
<tr>
<td>WC/ReJ</td>
<td>75</td>
<td></td>
<td>0.870</td>
<td>0.847</td>
</tr>
</tbody>
</table>

Note: Equal sample sizes were assessed in the two test–retest sequences of each strain, but different sample sizes were assessed from the different strains. Each correlation is highly significant ($P < 0.001$) when tested against the null hypothesis of no association.

Fig. 2. Response of strains with experience-conditioned paw usage to a biased-world test as naive mice and to a retest 1 week later in the opposite-biased test world. The average of the two right-paw entry (RPE) scores ($y$-axis) is plotted against the numerical difference between the two RPE scores ($x$-axis). Naive mice that were tested in a L world and retested 1 week later in a R world are shown in the panels on the left; mice that were tested in the reverse sequence are shown in the panels on the right.
of genetically different and inbred strains and provide a simple, alternate, biometrical method for identifying experience-conditioned and constitutive paw-usage behaviours. Experience-conditioned paw-usage behaviour is clearly distinguished from constitutive paw-usage behaviour among different strains by scanning the means of the average RPE scores from individual mice in the two test-retest sequences (Table 4).

Degree of lateralization of preferred-paw usage among experience-conditioned and constitutive strains

The degree of lateralization of preferred-paw usage (mean PPE score), without regard to its left or right direction, is summarized in Table 5 for the biased-world test sequences. The mean PPE scores from a U-world test are listed for comparison. As found previously (Biddle and Eales 1999), there is little or no change in the mean PPE score from the different test worlds and test sequences among the strains.

Figure 4 compares the degree of lateralization of preferred-paw usage in the 12 strains that have been defined with the alternate experience-conditioned and constitutive paw-usage behaviours. It includes the four strains from the original report that demonstrated that paw usage is experience-conditioned in C57BL/6J and SWV and constitutive in CDS/Lay and NOD/Lt (Biddle and Eales 1999). The strains are numerically rank-ordered by the degree of lateralization of their preferred-paw usage (mean PPE score) from a U-world test of naive mice. Among the 12 strains, experience-conditioned and constitutive paw-usage behaviours do not appear to be logically associated with a specific mean PPE score, although no experience-conditioned strain has yet been identified that is as weakly lateralized as the constitutive CDS/Lay strain (Fig. 4). The qualitative difference between experience-conditioned and constitutive behaviour may be independent of the quantitative difference in the degree of lateralization of preferred-paw usage.

Genetics of lateral asymmetry of paw usage

The qualitative difference between experience-conditioned and constitutive paw-usage behaviours was identified in a previous report (Biddle and Eales 1999). It was brought to our attention by an apparent epistatic effect when the genetic difference in degree of lateralization of preferred-paw usage was assessed between the very weakly lateralized CDS/Lay and the highly lateralized C57BL/6J strains. Constitutive paw usage of CDS/Lay is dominant to experience-conditioned paw usage of C57BL/6J and, if the same direction of dominance is found among other strain pairs with the alternate behaviours, recessive experience-conditioned paw usage may be caused by a loss of function. Nevertheless, until the F1 hybrids from different strains with the alternate behaviours are themselves phenotypically assessed for the alternate behaviours, it is equally possible that dominant constitutive paw usage of CDS/Lay may be caused by a gain of function relative to experience-conditioned C57BL/6J.

The qualitative difference in paw-usage behaviour was also suggested to be independent of the quantitative differ-
The present survey confirms that the alternate experience-conditioned and constitutive paw-usage behaviours are found among common strains of the laboratory mouse that differ widely in their degree of lateralization of preferred-paw usage. Therefore, which behaviour is the wild type or “normal” behaviour cannot be defined. In this regard, the constitutive paw-usage behaviour of the CLA inbred strain of wild-derived *M. m. domesticus* (Tables 2 or 4) might suggest a tentative answer. Nevertheless, other inbred strains of the wild-derived progenitor species of the laboratory mouse need to be assessed, to put the behaviour of CLA into the

<table>
<thead>
<tr>
<th>Sample size(^a)</th>
<th>Left to right ((\text{RPE-L}) – (\text{RPE-R}))</th>
<th>Right to left ((\text{RPE-R}) – (\text{RPE-L}))</th>
<th>(\frac{1}{2}(\text{RPE-L}) + (\text{RPE-R}))</th>
<th>(\frac{1}{2}(\text{RPE-R}) + (\text{RPE-L}))</th>
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</thead>
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<tr>
<td>BALB/cByJ 40</td>
<td>–1.0±1.8</td>
<td>2.6±2.8</td>
<td>25.9±3.0</td>
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<tr>
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<td>–6.7±2.3</td>
<td>7.5±2.5</td>
<td>20.5±3.4</td>
<td>30.3±3.3</td>
</tr>
<tr>
<td>C3H/HeSnJ 26</td>
<td>–6.0±3.6</td>
<td>5.2±3.9</td>
<td>25.4±5.5</td>
<td>24.0±6.5</td>
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<tr>
<td>CLA 55</td>
<td>–3.4±2.5</td>
<td>3.0±2.3</td>
<td>22.6±3.2</td>
<td>24.1±3.2</td>
</tr>
<tr>
<td>DBA/2J 101</td>
<td>–5.2±1.8</td>
<td>6.9±1.7</td>
<td>25.0±2.8</td>
<td>23.1±2.9</td>
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<tr>
<td>FVB/NJ 83</td>
<td>–4.1±2.1</td>
<td>3.6±2.0</td>
<td>19.6±3.0</td>
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<tr>
<td>Line 22c 60</td>
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<td>WC/ReJ 75</td>
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</tbody>
</table>

**Table 4.** Comparison of the numerical difference and the numerical average of the right-paw entry (RPE) scores of naive mice that were tested in a L world or R world and retested 1 week later in the respective opposite-biased test world.

- **Note:** Equal sample sizes were assessed in the two test–retest sequences of each strain, but different sample sizes were assessed from the different strains.
- **Table 5.** Mean preferred-paw entry (PPE) scores ± 95% confidence limits from the test of naive mice in a L world or a R world and their retest in the opposite-biased test world 1 week later; the mean PPE scores from a U-world test of naive mice are shown for comparison.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Test and direction</th>
<th>Naive L</th>
<th>R after L</th>
<th>Naive U</th>
<th>Naive R</th>
<th>L after R</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/cByJ</td>
<td>32.4±1.8</td>
<td>33.3±2.0</td>
<td>38.5±2.0</td>
<td>32.4±0.8</td>
<td>31.5±1.8</td>
<td></td>
</tr>
<tr>
<td>C3H/HeHa</td>
<td>38.8±1.8</td>
<td>39.9±1.9</td>
<td>42.0±2.2</td>
<td>39.0±1.8</td>
<td>40.9±1.7</td>
<td></td>
</tr>
<tr>
<td>C3H/HeSnJ</td>
<td>36.8±3.0</td>
<td>38.5±2.8</td>
<td>40.4±1.7</td>
<td>39.3±3.2</td>
<td>41.3±2.5</td>
<td></td>
</tr>
<tr>
<td>CLA</td>
<td>36.2±1.9</td>
<td>34.8±2.0</td>
<td>36.3±2.1</td>
<td>34.5±1.8</td>
<td>36.4±1.8</td>
<td></td>
</tr>
<tr>
<td>DBA/2J</td>
<td>37.2±1.4</td>
<td>39.7±1.4</td>
<td>37.0±1.2</td>
<td>37.8±1.5</td>
<td>39.7±1.5</td>
<td></td>
</tr>
<tr>
<td>FVB/NJ</td>
<td>37.2±1.7</td>
<td>39.6±1.7</td>
<td>35.3±1.5</td>
<td>37.3±1.5</td>
<td>38.6±1.6</td>
<td></td>
</tr>
<tr>
<td>Line 22c</td>
<td>33.1±1.5</td>
<td>35.2±1.9</td>
<td>33.2±1.8</td>
<td>33.9±1.7</td>
<td>35.6±2.0</td>
<td></td>
</tr>
<tr>
<td>WC/ReJ</td>
<td>36.7±1.4</td>
<td>38.8±1.5</td>
<td>41.4±1.9</td>
<td>37.4±1.5</td>
<td>38.5±1.6</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Values in parentheses are number of females and males \(4\), \(\sigma\) in each test of naive mice and their respective retest.

\(^a\)PPE score was calculated from original data in Biddle and Eales (1996).
context of the two behaviours that are polymorphic among strains of the laboratory mouse.

Genetic analysis of the qualitative and quantitative differences in paw-usage behaviour cannot be done with the random individuals in the usual segregating generations of the F₂ or the first backcross (BC₁). Definition of the qualitative and quantitative differences requires replicate assessment and paired comparisons between genetically homogeneous individuals. In addition, there may be asymmetry in the direction of paw usage among some experience-conditioned strains, as suggested by the apparent interaction between genotype and the direction of the test sequence (Table 4). Therefore, an efficient entry to the genetics of asymmetry of paw usage will be through the analysis of a set of RI strains that has already been derived from a progenitor-strain pair with the alternate experience-conditioned and constitutive paw-usage behaviours. Each RI strain in the set would allow the replicate assessment of genetically homogeneous individuals. Segregation of the qualitative difference in behaviour among the individual RI strains might be caused by segregation and homozygosity of alternate and allelic forms of genes that are identical by descent from the respective experience-conditioned and constitutive progenitor strains. Evidence for segregation of the quantitative difference in degree of lateralization of preferred-paw usage and for association between the quantitative and qualitative traits could also be assessed among the RI strains with alternate experience-conditioned and constitutive behaviours.

BALB/cByJ, C3H/HeJ, and DBA/2J were each used as the alternate progenitor strain to construct a set of RI strains from an initial cross to C57BL/6J. The sets of RI strains were developed by inbreeding from random pairs from the respective F₂ generations and are designated CXB, BXH, and BXD, respectively (e.g., Bailey 1981; Silver 1996; Taylor 1996). Since both BALB/cByJ and DBA/2J are constitutive in their paw-usage behaviour compared with experience-conditioned C57BL/6J, both the CXB and BXD RI strains might be used. The BXD set of RI strains has the largest number of individual strains, with 35 strains extant, and is the most extensively documented for mapped marker genes (Taylor 1978; Taylor et al. 1999). Since a minimum number of 13 RI strains will detect the chromosomal location of a major gene locus by association with a reasonable density of mapped marker genes (Bailey 1981), a systematic assessment of paw usage among the BXD RI strains may provide the best entry to the genetics of asymmetry of paw usage. Also, the larger number of independent BXD RI strains will permit an assessment of potential complexity in the behaviour beyond single-locus genetic models.

Alternate paw-usage behaviour in two closely related substrains of C3H (Tables 2 or 4) suggests that the qualitative difference might be caused by allelic substitution at a major gene locus; however, the potential for ancestral genetic contamination during development of these two substrains cannot be excluded. Nevertheless, the BXH RI strains are precluded from analysis until the specific C3H/HeJ strain, which was used to construct the BXH RI strains, has been assessed for paw-usage behaviour.

**Corpus callosum and asymmetry of paw usage**

Directional asymmetry of human handedness has been tied inexorably to cerebral dominance (specialization) and lateralization of speech and language (Bishop 1990; Coren 1990; Corballis 1997; Provins 1997). Interest in the asymmetry of hand usage is based on the hypothesis that it might provide clues to asymmetries of cerebral dominance. If there are allelic forms of genes that influence the asymmetry of hand usage, they should be discoverable, but no gene has been discovered.

Genetically determined deficiencies and (or) absence of the corpus callosum have been characterized in the mouse and studied for their potential role in behavioural dysfunction (Lipp and Wahlsten 1992). Previously, we demonstrated that there is no direct cause and effect relationship between presence–absence of the corpus callosum and the degree of lateralization of paw usage (Biddle and Eales 1996). In the present report, the CDS strain has an anatomically normal corpus callosum (Biddle and Eales 1996) and the Line 22c strain has complete absence of the corpus callosum (Wahlsten and Sparks 1995). Both CDS and Line 22c express a constitutive and weakly lateralized paw usage (Table 4; Fig. 4). Therefore, there can be no direct and obvious cause and effect relationship between the presence and absence of the major interhemispheric commisure of the corpus callosum and the behaviour difference of constitutive and experience-conditioned paw usage.

**Summary and conclusions**

The present analysis of the lateral asymmetry of paw usage forms a starting point for a genetic analysis that may move this mouse behaviour from an interesting phenomenon to an informative model. The qualitative difference in lateral asymmetry of paw usage is not a dichotomy between left-
paw versus right-paw usage. Instead, it is a qualitative difference in a behaviour that is context dependent; it is a difference between a direction of paw usage that is either experience conditioned or constitutive. This qualitative difference is shown to be simple to identify among common strains of the laboratory mouse, which differ widely and, apparently, independently in their degree of lateralization of preferred-paw usage. The progenitor strains of two different sets of RI strains are identified and characterized with alternate paw-usage behaviours. These RI strains may provide an entrance to the genetic cause of the qualitative difference in paw-usage behaviour and the identification of genes that influence the asymmetry of paw usage in the mouse.

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References


