On the Inheritance of Handedness

I. Laterality in inbred mice

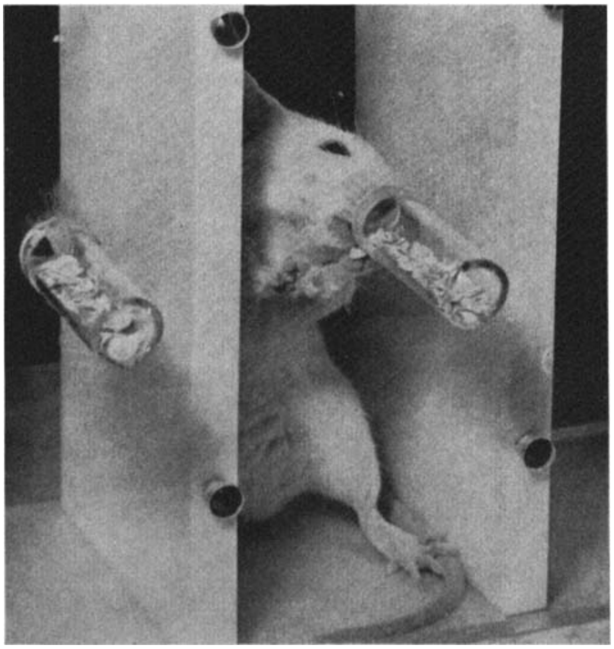
ROBERT L. COLLINS

RIGHT and left handedness are alternative expressions of a fundamental behavioral asymmetry. Although hereditary theories of the origin of human hand preference have occurred periodically in the scientific and popular literature[8], a well-defined relationship between lateral preference and genetic variation has not been successfully established[5, 6]. Part of the difficulty in evaluating the hereditary hypothesis is due to the evident modifiability of hand preference through formal and informal training during early human development and the subsequent entanglement of these environmental effects with the presumed genetic influences[4]. However, it may be possible to evaluate the hereditary hypothesis in detail if lateral preference were demonstrated and studied in laboratory animals whose genetic constitution is controlled, and whose environment presumably does not differentially favor the use of either hand. To this end laterality was studied using highly inbred strains of mice and two hybrid crosses. The results show that an enduring hand preference can be observed in mice and that this preference cannot be attributed to hereditary differences.

Materials and Methods

To define handedness the following criteria were used: a) one hand must be used significantly more frequently than the other in a free-use task; b) this lateral preference should be enduring upon retest; and c) lateral preference should be consistent with lateral superiority measured in a forced-use task. A total of 370 mice from highly inbred strains was tested for paw preference. Specific strain comparisons were made between 114 male C57BL/6J and 101 male DBA/2J mice. The remaining groups included A/J, A/HeJ, C3H/HeJ, C57BL/10J and DBA/1J mice. Hybrid male mice from crosses between C3H/HeJ and DBA/1J and between C57BL/6J and DBA/2J were studied. Mice initially were tested between 2.5 to 3 months of age. Mice were deprived of food for 24 hours prior to testing. Individuals were placed in plastic chambers 11.4 cm high by 6.4 cm deep by 3.8 cm. On the front wall of each cubicle a 9 mm glass feeding tube, equally accessible from the right or left, was attached 5.7 cm from the floor. Maple flavored rolled wheat (Maypo) was placed within the feeding tube so that a subject could withdraw food by using a single paw (Figure 1). Fifty reaches were observed and serially recorded for each mouse. Scores were defined as the number of right paw entries (RPE's) in 50 observations. Low RPE's reflected the predominant use of the left paw, and high RPE's, the right paw. Mice were classified as dextral or sinistral by considering each subject a "coin", which could land either *R* or *L*. A subject who scored above 25 RPE's was designated *R*, and *L* was assigned to one who scored below 25 RPE's. In the infrequent case of a tie score, the "biological coin" was considered to have landed

FIGURE 1—A mouse retrieving a food flake from the feeding tube using the left paw. The photograph was taken near the end of the behavioral sequence. The *feeding tube is equidistant from the two walls of the* chamber.



The author is associate staff scientist, The Jackson Laboratory, Bar Harbor, Maine 04609. Supported by research grants MH 11327 and MH 1775 from the National Institutes of Health. The principles of laboratory animal care as set forth by the National Society for Medical Research are observed in this Laboratory.

on "edge" and was not counted; only four such cases were observed during initial testing.

The consistency of an individual's paw preference over time was determined in three ways. Within the initial testing session, data were divided into two blocks of 25 reaches, and the number of RPE's in each block was compared for 100 randomly chosen mice. To determine short-term consistency, 15 mice were tested 4 times at 4-day intervals. An additional 60 mice were tested 4 times at monthly intervals to evaluate long-term consistency. Pearson product moment correlations were computed for each comparison using RPE scores. Tetrachoric correlations were used to assess the stability of the laterality classifications.

Later in the study an attempt was made to determine whether the preferred paw in food reaching was also the superior paw on a forced-use task. Grip strength was tested in 29 A/J and 32 A/HeJ males. Tape was first placed over one paw. A mouse was then picked up by the tail and allowed to grasp a 6.4 × 6.4 mm mesh wire screen attached to a Grass FT-10 force displacement transducer connected to a Grass polygraph. The mouse was pulled upward until he released the wire screen, and the maximum force displacement was measured in *g* of grip strength. Each mouse was tested 20 to 30 times on the first day. On the second day mice were tested with the opposite paw under the same conditions. Order of testing was balanced with respect to preferred and non-preferred paws, and all procedures were performed double-blind. Data were analyzed by comparing the daily average of the five highest grip strengths for the preferred and non-preferred paws between and within subjects.

Results

If paw preference were a heritable characteristic, laterality differences between strains would provide a convenient basis for further genetic investigation. Figure 2*A* illustrates the frequency distribution of RPE scores for 114 C57BL/6J mice. Modes occurred at 3–5 and 39–44 RPE's. The proportion of right paw reaches was 0.489; left was 0.511. The binomial distribution of expected frequencies according to these proportions is superimposed upon the observed distribution as a dashed line. These frequencies yield the expected distribution of RPE performance under the hypothesis that mice exhibit no lateral preference. The chi-square test between observed and expected frequencies was 2.42×10^3 ($P < 0.0001$, df = 113). Figure 2*B* presents the same information for 101 DBA/2J mice. Modes occurred at 3–5 and 41–44 RPE's. The proportion of right paw entries was 0.494, and left, 0.506; $\chi^2 = 2.17 \times 10^3$ ($P < 0.0001$, df = 100). Individuals in both highly inbred strains used one paw more frequently to retrieve food even though the likelihoods of observing a right or a left reach were approximately equal across subjects. According to the procedure for assigning laterality classifications, the proportions of right paw preferent mice for C57BL/6J and DBA/2J strains were 0.51 and 0.50, respectively. Con-

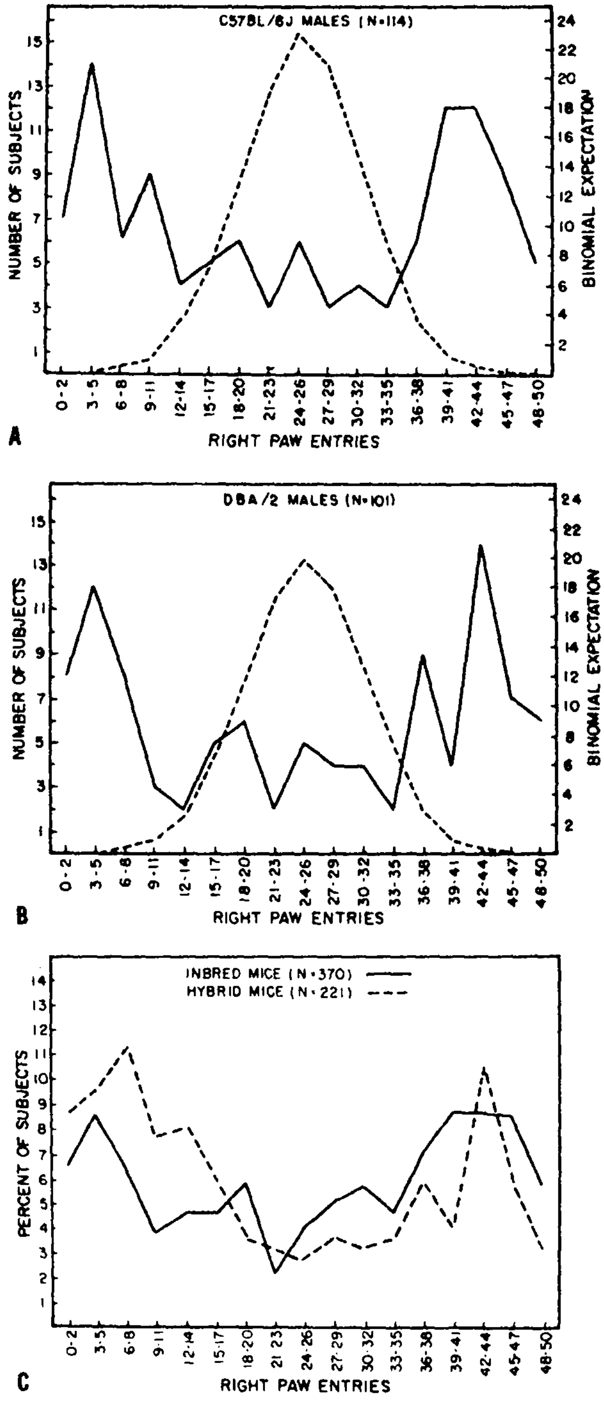


FIGURE 2—*A* **shows the frequency distribution of lateral preference scores for C57BL/6J in mice in terms of the number of right paw entries into the feeding tube in 50 observations. The abscissa is divided into 17 blocks of 3 right-paw entries. The binomial distribution of expected frequencies according to the proportions given in the text is superimposed as a dashed line upon the observed distribution.** *B*—**frequency distribution of lateral preference scores for DBA/2J mice. Expected frequencies are superimposed as a dashed line.** *C*—**mean frequency distributions of lateral preference scores for inbred and hybrid mice.**

sidered in this manner, almost exactly one-half of the mice within both highly inbred strains were dextrals and one half, sinistrals.

Table I summarizes the paw preference data for all experimental groups. A bimodal distribution of paw preference scores was evident for the seven inbred strains and the two hybrid crosses. Since both right and left preferent subjects were observed within each of nine sets of genetically uniform mice, the heritability of paw preference for each group must be close to zero. No association of lateral preferences could be detected in intra-litter comparisons.

Figure 2C illustrates the frequency distributions of RPE's for 370 inbred and 221 hybrid mice. Both distributions are clearly bimodal. Maxima were observed at 3–5 and 42–44 RPE's for inbreds, and at 6–8 and 42–44 RPE's for hybrids. However, the distributions are slightly asymmetrical in opposite directions. Omitting the region of ambilaterality between 21 and 29 RPE's, the percentages of hybrid mice in the left paw preferent region were higher than those for the inbred subjects in 6 of 7 cases. The converse relationship was observed in the right preferent region. Analysis of these distributions indicated that there were statistically different ($\chi^2 = 42.8$, $P < 0.001$, df = 16). For all inbred and hybrid mice, the proportions of dextrals were 0.543 and 0.409, respectively. These values differ statistically ($\chi^2 = 11.4$, $P < 0.001$, df = 1).

Table II summarizes the correlations for the consistency of paw preference scores as well as the stability of the laterality classifications during repeated testing. Within the initial test session, approximately 20 percent of the subjects produced identical RPE scores in the first and second blocks of 25 observations. During the second block, 35 percent of the subjects increased the use of the preferred paw slightly, while 45 percent decreased its use. All 100 mice maintained the same laterality classification in both halves of the initial test. During 4-day retesting there were small variations in the RPE number for some mice. However, the smallest inter-test correlation, observed between tests 1 and 4, was 0.92 and all 16 subjects maintained the same laterality classifications throughout short-term retesting. Thirty C57BL/6J and thirty DBA/2J mice were tested monthly for four months. A small but steady increase in the proportion of mice occupying the two extreme RPE class intervals was observed for C57BL/6J mice; this trend was absent in the DBA/2J subjects. During these retests four C57BL/6J and eight DBA/2J mice exhibited a single fluctuation in their laterality classifications. The classifications for 80 percent of the subjects remained invariant during four months of retesting. All 26 intercorrelations listed in Table II are statistically significant ($P < 0.01$ that ρ or $\rho_t = 0$).

The average grip strength across both paws for A/J mice was 69.1 *g*, and for A/HeJ, 63.0 *g* ($P < 0.05$, $F = 5.17$, df = 1/57). Grip strength for mice tested on the first day with the preferred paw was 68.1 *g*, while that for mice tested with the nonpreferred paw was 64.0 *g*. The 4.1 *g* difference in this between subjects comparison did not reach a conventional level of statistical significance ($P \simeq 0.08$, $F = 3.26$, df = 1/57). There was no interaction of strain and preference. In the within subjects comparison, the average grip strength for 61 mice tested on the preferred side was 64.9 *g*, while the mean for the opposite paw was 61.2 *g*. This 3.7 *g* difference was statistically significant using a two-tailed test ($P < 0.025$, $t = 2.33$, df = 60).

Discussion

Three criteria for the demonstration of functional laterality in mice were supported by these data. Mice used one paw significantly more often than the other to retrieve food; lateral preference was consistent over time; and grip strength was correlated to paw preference within subjects.

Since both dextral and sinistral mice were observed within each of nine genetically uniform populations, it would appear that individual genetic differences are not required to explain laterality differences. In fact, equal representation of the two classes as observed in the two most intensively studied strains produces the maximum possible

Table I. Summary of paw preference data

Strain	Number of subjects	Proportion of right paw entries	Proportion of dextral mice
C57BL/6J	114	0.489	0.51
DBA/2J	101	0.494	0.50
A/J	40	0.562	0.67
A/HeJ	35	0.570	0.51
DBA/1J	42	0.570	0.61
C3H/HeJ	33	0.518	0.58
C57BL/10J	5	0.552	0.60
Total inbred mice	370	0.519	0.543
C3H/HeJ × DBA/1J	64	0.423	0.36
C57BL/6J × DBA/2J	157	0.438	0.43
Total hybrid mice	221	0.434	0.409

Table II. Intercorrelation matrix of paw preference scores and laterality classifications for mice retested at intervals of four days ($n = 16$) and one month ($n = 60$).

	Four day retest			
	1	2	3	4
1	0.92*	0.92†	0.92	0.93
	1.00*	1.00‡	1.00	1.00
2	0.88	—	0.93	0.95
	0.98	—	1.00	1.00
3	0.82	0.93	—	0.97
	0.96	1.00	—	1.00
4	0.75	0.91	0.94	—
	0.85	0.97	0.97	—

Monthly retest

* Within session split-block correlation ($n = 100$).
† Product moment correlation of the number of right paw entries in 50 observations.
‡ Tetrachoric correlation of laterality classifications.

phenotypic variance. This is somewhat surprising considering that the two strains have minimal genetic variance.

The sole experimental result indicating a possible genetic effect upon lateral variation was that the proportion of dextral mice in the two hybrid crosses was smaller than that for the inbred strains. Replication of this finding would suggest that a directional bias in the distribution of lateral asymmetries may be associated with heterozygosity at one or more loci. Accordingly, while these data do not support a conclusion that lateral preference in mice is determined genetically, it cannot be denied that the probability of alternative phenotypic forms may be influenced by genotypic differences. This hypothesis could be tested by studying laterality in additional classical cross generations. For example, using methods of genetic analysis outlined elsewhere[1], the phenotypic distributions of the segregating generations between C57BL/6J and DBA/2J might be congruent to expectations based upon a single or multiple locus difference. However, in view of the ambiguity that would exist between a genotype and either alternative phenotypic form, lateral preference would remain largely unresponsive to selection pressure and the expected realized heritability for each form would be essentially zero. It is of interest to note that in an early study, Peterson reported no change in the frequencies of right and left paw preferent rats through more than seven generations of selection[7]. A similar finding was reported for the heritability of foliar asymmetries in *Cocos nucifera*[3].

In light of the extensive evidence showing genetic effects upon diverse behavioral characters, it is striking that this basic behavioral difference would be so recalcitrant to genetric analysis and manipulation if it were in fact determined by hereditary differences. Indeed, there would seem to be little advantage in having genes determine which hand was preferred. Phenotypic variation arising from environmental sources would insure the continued survival of a species if it were subject to selection pressure for lateral uniformity. In addition, an environmental regulation may confer to each new generation a greater plasticity for adaptation than could be granted genetically. Genetic variation may simply provide the ability to develop functional asymmetries without specifying the alternative phenotypic form, just as genetic variation might favor acquisition of language without specifying the vocabulary and grammar.

On the basis of these experimental results using mice, a mathematical reanalysis of the published data concerning the inheritance of human lateral preference was performed[2]. The results provided no support for a view that human hand preference is determined by individual genetic differences. Thus, *Mus musculus* may not be unique among species in that the lateral preference of its members remains largely unspecified by their genotypes.

Summary

Paw preference was tested experimentally in 591 mice from 7 highly inbred strains and 2 hybrid crosses. Consistency of preference over time and grip strength were assessed. Mice exhibited reliable functional laterality and their individual preferences were enduring. Both right and left paw preferent mice were observed within each of the nine genetically uniform groups. One-half the subjects in C57BL/6J and DBA/2J strains were dextral, and one half, sinistral. This indicates that maximal variation in lateralization may exist in populations possessing minimal genetic variance, and suggests that hand preference is not determined by hereditary factors.

Literature Cited

1. Collins, R. L. A general nonparametric theory of genetic analysis: I. Application to the classical cross. *Genetics* 56:551. 1967.

2. ———. On the inheritance of handedness. II. Reanalysis of human data. (In preparation). 1968.

3. Davis, T. A. The non-inheritance of asymmetry in *Cocos nucifera*. *J. Genetics* 58:42–47. 1962.

4. Falek, A. Handedness: a family study. *Amer. J. Hum. Genet.* 11:56–62. 1959.

5. Fuller, J. L. and W. R. Thompson. Behavior Genetics. John Wiley & Sons, New York. 1960.

6. McKusick, V. A. Mendelian Inheritance in Man. The Johns Hopkins Press, Baltimore. 1966.

7. Peterson, G. M. Mechanisms of handedness in the rat. *Comp. Psychol. Monogr.* No. 46. 1934.

8. Uhrbrock, R. S. Laterality of Function Bibliography (2813 references). American Documentation Inst. Washington, D.C. 1963.