The basis for the heterogeneous morphological response (hollow circular and flattened circular forms) to the cyclic AMP analogs and PGE_1 is not clear. It is possible that these Schwannoma clones are not genetically stable and recloning may provide lines with differing morphological effects. Another potential basis for the heterogeneity is that the open circular shape may be derived from the flat circular form. An invagination or membrane retraction may occur in the flattened circular, "fried-egg–like" form resulting in the open circular form. Formation of the myelin sheath in vivo is accomplished by movement of the nucleated portion of the Schwann cell around the nerve axon (*15*). In culture, the movement of the nucleus in the flattened circular form away from its initial central position could lead to retraction of the membrane, resulting in the hollow circular from (Fig. 1C may illustrate such an event). It should be emphasized that all of these various morphological forms exist in the untreated and control cultures but in much lower percentages than in the cyclic AMP analog–treated cultures. Furthermore, the effect is not a result of the enrichment of a given cell population through differential lethality. Cells exclude trypan blue after exposure to cyclic AMP analogs and cell number does not decrease. Actual numbers of circular cells, as well as percentages, increase as a result of the cyclic AMP analog treatment. Thus it appears that the major difference between control and cyclic AMP–treated cultures is quantitative, not qualitative.

Whether biochemical characteristics that distinguish Schwannoma cells in culture (*16*) are also affected by cyclic AMP must await further investigation. It is not yet clear whether cyclic AMP directly promotes the morphological changes accompanying cellular differentiation (*17, 18*) or effects the change by altering adhesiveness of the cells to substrate (*19*). Nevertheless, the cyclic AMP effect on these cultured Schwannoma cells may provide a model system useful in studying Schwann cell morphology, membrane synthesis, and myelination.

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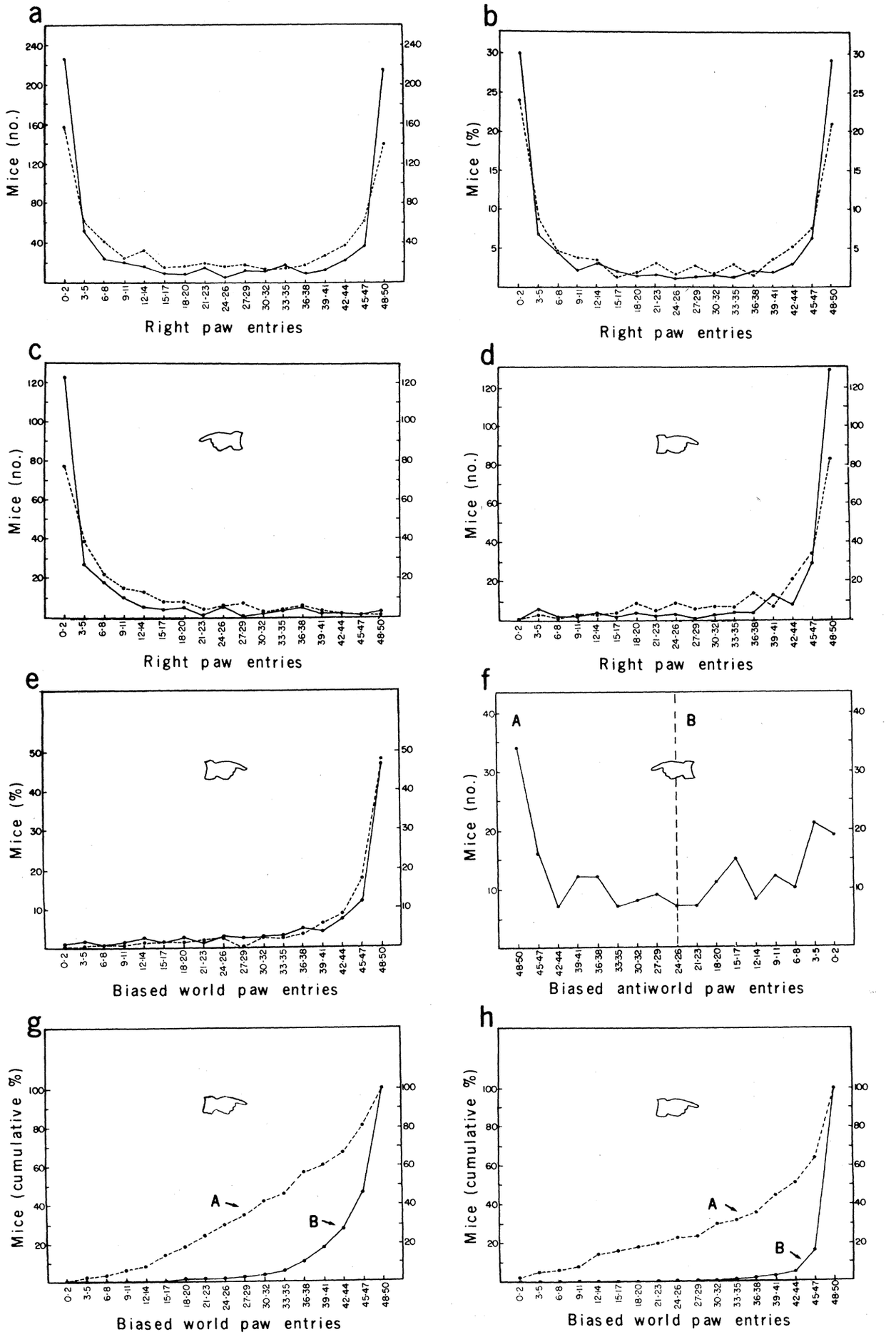
When Left-Handed Mice Live in Right-Handed Worlds

Abstract. *Many C57BL/6J inbred mice were tested for paw preference. In unbiased worlds, half were left-handed, and females were more strongly lateralized. In biased worlds, approximately 10 percent exhibited lateral preferences inconsistent with the world bias, and males were more strongly lateralized. Influences of world bias appear to be superimposed upon an already laterally dichotomized population. Initial left-right sense, it is posited, arises as an outcome of a seemingly random process.*

Right- and left-handedness are not unique to humans. Mice also show a consistent hand preference. Experimental studies of laterality in inbred mice (*1*) and retrospective studies of handedness in man (*2*) have led me to conclude that lateral sense is not specified in any unambiguous manner by the individual's genome. If right- and left-handedness arise not from heritable genetic instructions, then how does lateral sense develop, and what influences affect its expression? The following studies of laterality in mice tested in unbiased and biased worlds may help in a solution to this question.

Mice of the highly inbred C57BL/6J strain (*3*), 709 animals 8 to 12 weeks of age, were first tested for paw preference in an unbiased world. Mice were deprived of food for 24 hours before testing, and were then placed into a cubicle in which sweetened rolled wheat (Maypo) was available in a feeding tube that was attached to the front wall equidistant from the right and left sides (*4*). This constitutes my definition of an unbiased or U world. Fifty reaches for food were observed for every mouse, and the number of right paw entries (RPE's) was used as one index of laterality. Each mouse was retested 1 week later. Figure 1a illustrates the RPE scores for 709 mice tested in the U world. Data were cast into 17 class intervals. Most mice were either strongly right-handed or strongly left-handed, whereas only a few were ambilateral. The distributions are strikingly "U"-shaped. In test 1, 41.8 percent of mice scored in the two most extreme class intervals, 0 to 2 and 48 to 50 RPE's; in test 2, 62.2 percent were in these intervals. If mice that scored exactly 25 RPE's are excluded, 334 of 702 or 47.6 percent of mice could be designated as dextral in test 1; and 337 of 707 or 47.7 percent, in test 2. The strength of lateralization was increased in the second testing. This difference is statistically significant (*5*).

Figure 1b illustrates the RPE scores for the same 341 female and 368 male mice. Individual test 1 and test 2 scores were averaged (*6*). Female mice were more strongly lateralized than males in the U world. Whereas 59.5 percent of female mice scored in the two extreme intervals, only 45.1 percent of male mice scored therein ($\chi^2 = 14.75$, $P < .001$). This result presents a clear example of a genetic effect on laterality.


a
Mice (no.)
Right paw entries
b
Mice (%)
Right paw entries
c
Mice (no.)
Right paw entries
d
Mice (no.)
Right paw entries
e
Mice (%)
Biased world paw entries
f
A
B
Mice (no.)
Biased antiworld paw entries
g
Mice (cumulative %)
A
B
Biased world paw entries
h
Mice (cumulative %)
A
B
Biased world paw entries

This effect is on the strength of expressed laterality, and not on its sense.

An additional 320 C57BL/6J mice, 6 to 8 weeks of age, were tested for paw preference. However, in these tests the feeding tube was located flush with the right side of the wall, as faced by the mouse (Fig. 2). This constitutes my definition of a right-biased or R world. To obtain the left-biased or L world, the apparatus was inverted. Each mouse was assigned randomly to either the R or L world (162 to R, 158 to L). In a replicate experiment with 115 C57BL/6J mice, 55 were assigned to the R world and 60 to the L world. Data for replicate experiments were statistically indistinguishable and were therefore pooled.

Figure 1, c and d, presents the RPE scores for mice tested in the L and R worlds, respectively. The distributions of paw preference scores are markedly "J"-shaped (*7*). Although most mice exhibited RPE scores that were consistent with the world bias, 8.7 to 14.7 percent exhibited RPE scores opposite to the bias (*8*). These fractions are not unlike the proportions of left-handers in human societies (*9*). In R and L worlds, as in the U world, there was a marked increase in the proportions of mice occupying the extreme classes on retesting (*10*).

Figure 1e illustrates the biased world paw entries (BWPE's), averaged across tests, for 215 female and 220 male mice. The BWPE scores were obtained by a mirror-image superposition of the L world distribution onto the R world distribution. For example, RPE scores of 10 in an L world and of 40 in an R world are both equivalent to a BWPE score of 40. Male mice were more strongly lateralized in biased worlds than female mice. This was more pronounced on retesting. Consider BWPE intervals 39 to 50, the region of moderately to strongly expressed laterality consistent with the world bias. In test 1, 72.3 percent of males and 64.7 percent of females scored in this region ($\chi^2 = 2.93$, $.05 < P < .10$). In test 2, 88.6 percent of males and 75.4 percent of females scored therein ($\chi^2 = 13.05$, $P < .001$). This sex difference may be interpreted as follows. In unbiased worlds, females expressed a stronger laterality than males (Fig. 1b). Thus, when mice are placed into a biased world, it may be expected that males would adapt more readily than females to a bias opposite to their native laterality. Sex differences in expressed laterality are routinely reported in human populations. Left-handedness is more frequent in human males (*9*). The order of sex differences for mice and humans is reversed. This is somewhat puzzling, and I have no ready explanation for it.

One explanation for the origin of paw preference in mice is that it is created de novo, is learned, in the very act of testing. On the basis of the following, I conclude that this explanation is not correct.

All 320 mice of the preceding experiment were assigned randomly to three groups for further testing in R, L, or U worlds. If we denote the first and second biased world tests as R-R and L-L, we now concern ourselves with the paw preference scores of mice tested in worlds of the opposite bias, or "antiworlds" (test sequences R-R-L and L-L-R) (*11*). All mice of the replicate experiment were assigned to biased antiworlds. Data for mice tested in sequences R-R-R, L-L-L, R-R-U, and L-L-U are less germane to the central question and are not presented here.

The biased antiworld paw entry (BAWPE) score refers to the number of paw entries for a mouse tested in a world biased opposite to that in which it was originally tested. Figure 1f illustrates the BAWPE scores for 215 mice tested. The distribution is somewhat ragged. An increase in the number of mice is seen in the direction of the antiworld bias.

We should expect that in any large sample of C57BL/6J mice, half would be right-handed and half would be left-handed (Fig. 1a). If these mice were placed in a right-biased world, the "native left-handers" would be pressed in the dextral direction. Later, if the population was observed in a left-biased world, one might expect the "native left-handers" to gravitate in the sinistral direction. Mice that gravitate toward the antiworld bias (group A) and those that resist the antiworld bias (group B) can be identified by means of the distribution in Fig. 1f. The scores of group A and group B animals on the original biased world tests (Fig. 1, c and d) can then be partitioned. If the original population of mice tested in biased worlds was composed of equal numbers of "native right-handers" and "native left-handers," the biased world scores for A and B animals should give different distributions. If mice learn their paw preferences in the initial tests themselves, the biased world scores of A and B animals should not differ significantly.

Accordingly, Figure 1f scores were partitioned at the BAWPE score of 25. Group A ($N = 108$) comprised individuals with BAWPE scores of 26 to 50, and group B ($N = 106$) included those with BAWPE scores of 0 to 24; one mouse was not classified. Figure 1, g and h, illustrates in cumulative percentages the partition of test 1 and test 2 scores, respectively. In each case group A differed statistically from group B (for test 1, $\chi^2 = 45.48$, d.f. $= 2$, $P < 1 \times 10^{-9}$; for test 2, $\chi^2 = 49.00$, d.f. $= 2$, $P < 1 \times 10^{-10}$). Group A, considered to be mainly left-handed mice tested in right-handed worlds and right-handed mice tested in left-handed worlds, had some difficulty in adapting to the world bias; most of them could be described as being rather ambilateral. But group B, considered to be mainly right-handed mice tested in right-handed worlds and left-handed mice tested in left-handed worlds, readily adapted to their initial biased worlds. In fact, approximately 54 percent of mice in test 1 and 84 percent of those in test 2 exhibited the highest degree of lateralization possible, a score of BWPE 48 to 50. While paw preferences in the original biased worlds were not learned therein, experience in

Fig. 1. Distributions of paw preference scores for C57BL/6J inbred mice tested in unbiased and biased worlds. Fifty food reaches were observed for each mouse. Data were cast into 17 classes of three paw reaches. Hands point to the direction of the world bias in each case. (a) Distributions of paw preference scores, expressed as right paw entries (RPE's), in test 1 (dashed line) and test 2 (solid line) are shown for 709 mice tested in the unbiased or U world. (b) Distributions of average RPE's are shown for 368 male mice (dashed line) and 341 female mice (solid line) tested in the U world. (c) Distributions of RPE's in test 1 (dashed line) and test 2 (solid line) are shown for 218 mice tested in the left-biased or L world. (d) Distributions in RPE's in test 1 (dashed line) and test 2 (solid line) are shown for 217 mice tested in the right-biased or R world. (e) Distributions of paw preference scores, expressed as number of entries consistent with the world bias [biased world paw entries (BWPE's)], are shown for 220 male mice (dashed line) and 215 female mice (solid line). (f) Distribution of biased antiworld paw entries (BAWPE's) is shown for 215 mice retested in a world that was a mirror image of their original biased world. Group A consists of mice that exhibited paw preference scores in the direction consistent with the antiworld bias; and group B, those with scores in the direction opposite to the antiworld bias. (g and h) Biased world scores originally plotted in (c) and (d) have been partitioned for groups A and B. They are replotted as cumulative percentage distributions on test 1 (g) and test 2 (h).

the original worlds substantially influenced behavior in the antiworlds.

On the basis of these results, I propose that manifest laterality arises from the following five sources of influence: genetic variation, outcomes of an "asymmetry lottery," environmental biases, maturation and experience, and "noise."

The differences in expressed laterality between sexes in mice indicate that sources of genetic variation can affect the degree of lateralization. To show similar effects for autosomal variants, other experimental studies would be required. That such effects could influence lateral sense by indirect means is shown in the following. If genetic variation influences the abilities of individuals to adapt, and if individuals are placed into environments demanding lateral uniformity, then almost any gene substitution or chromosomal abnormality that influences adaptive ability would lead to changes in the proportions of expressed dextral and sinistral forms. For example, the proportions of left-handers in human populations characterized as "mentally retarded" are consistently enlarged (*12*).

I believe it necessary to postulate the existence of a seemingly random process, the outcome of which, a "random tilt" or "accidental nudge," directly affects initial lateral sense. This "asymmetry lottery" might reside in the individual's genome. If so, one admissible genetics of laterality would have as its motto, not "one gene, one enzyme," but "one gene, two enzymes" or, better, "one gene, one flip of a coin." Mice tested in the U world possessed the minimum genetic variance and exhibited the maximum phenotypic variation (Fig. 1a). Furthermore, that this phenotypic variation arose from a residue of heritable genetic variation remaining unfixed after prolonged inbreeding is argued against by the consistent failure to increase dextral and sinistral forms by selective breeding experiments (*13*). Another admissible genetic hypothesis might be that a random selection process is impressed upon right and left alternatives strictly coded in the genome. For precedent, consider the Lyon hypothesis which states that in females, one or the other X chromosome becomes inactivated in each cell early in embryonic development (*14*). Alternatively, genetic variation may simply maintain the

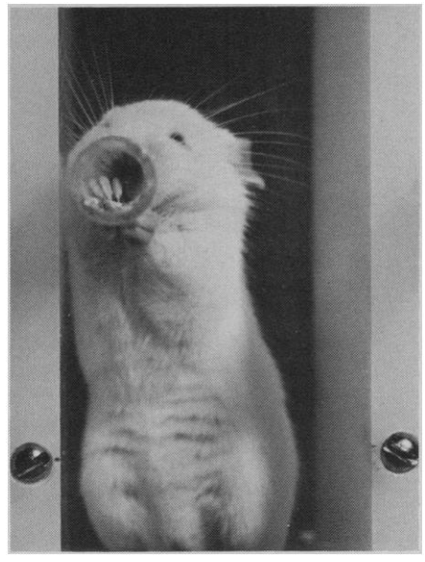


Fig. 2. A view, directly through the feeding glass, of a BALB/c-Col mouse in a right-biased or R world retrieving a food flake with its right paw. After additional testing, the mouse was considered to have been a "native left-hander."

"asymmetry lottery" and thereby engender lateral sense, the "tilt" outcomes being reserved for the "traffic accidents" of neurogenesis (*15*) or the buffetings of early postnatal life. A finely tuned equilibrium may be inherently unstable. The "asymmetry lottery" need not harbor equally likely outcomes. Indeed, the probabilities for alternative forms could vary for each bilaterally organized modality within species, as well as across species.

Environmental biases, due either to asymmetric worlds or cultural standards, can strongly modify lateral preferences. Distributions of paw preference scores for mice tested in biased worlds clearly demonstrate these effects. Effects of maturation and prior experience are important. Mice retested for paw preference in each world exhibited stronger lateral scores than on initial testing. The blurring effects of "noise," the effects of yet unidentified processes, and the traumatic accidents of life necessarily exert effect upon manifest laterality.

The origins of human right- and left-handedness are somewhat puzzling. Lay explanations are often grounded in superstition (*16*), and scientific explanations are frequently not without controversy (*17*). Yet, I believe that sound solutions to the understanding of the origins of this primitive and fundamental behavioral dimorphism are discoverable through studies such as these: studies of the functional bilateral asymmetries of genetically defined mice tested in bias-defined environments.

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3. A strain may be regarded as being inbred if it has been derived by single pair brother-by-sister matings for at least 20 consecutive generations, or by parent-by-offspring matings for the same number of generations if each mating is made to the younger parent. After 20 generations of inbreeding, approximately 99 percent of genetic loci are considered to be in a homozygous condition. The C57BL/6J inbred strain has been inbred for more than 100 generations [J. Staats, *Cancer Res.* **28**, 391 (1968)].
4. The test apparatus was fabricated of Plexiglas and consisted of five in-line testing cubicles whose inside dimensions were 3.8 cm wide by 5.5 cm deep by 11.5 cm high. A 9-mm glass feeding tube was attached to the front wall of each cubicle 5.75 cm from the floor.
5. Two-tailed statistical evaluations of the data were performed by using Kolmogorov-Smirnov two-sample tests. Unless otherwise specified, all statements regarding distributional differences are referred to $P < .0001$.
6. For female mice in the U world, the correlation of test 1 and test 2 scores was $r = .912$ ($t = 40.96$, d.f. $= 339$); for male mice, $r = .904$ ($t = 40.43$, d.f. $= 366$). In each case, $P < .0001$ that $\rho = 0.0$.
7. "J"-shaped is used as an approximate descriptor; the "toes" are missing.
8. In the L world, 27 of 218 mice in test 1 and 19 of 218 mice in test 2 scored in the region of 26 to 50 RPE's. In the R world, 32 of 217 mice in test 1 and 23 of 217 mice in test 2 scored in the region of 0 to 24 RPE's.
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10. For female mice tested in biased worlds, the correlation of test 1 and test 2 scores was $r = .853$ ($t = 23.84$, d.f. $= 213$); for male mice, $r = .793$ ($t = 19.24$, d.f. $= 218$). In each case $P < .0001$ that $\rho = 0.0$.
11. The antiworld of the R world is the L world. The antiworld of the L world is the R world. The U world and its own antiworld, as the neutral pion and its own antiparticle, are indistinguishable.
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18. In memory of John Papaioannou. I thank N. T. Noyes for dedicated and brilliant technical assistance. Supported in part by NIMH grant MH 11327 and by institutional funds of the Jackson Laboratory. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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