Nobel hopes are clarified. The existing smallpox vaccine is defended. And the significance of a study of behaviors measured across three laboratories using the same mouse strains is discussed: "We should...use these results to highlight that scientific progress is a changing mosaic of overlapping studies that correct, build, and expand on earlier findings," say one set of letter writers.

Brazilian Nobel Hopes

The article "Brazil lobbies for first Nobel" by Cassio Leite Vieira (News Focus, 27 Aug., p. 1346) is most timely. Yet small clarifications are in order: (i) It is implied that the candidacy has attracted extensive media attention because of my efforts, when many other people are working hard to promote chemist Otto Gottlieb's research. Some very fine science journalists deserve credit for drawing the media's attention to highly complex topics and stressing their implications for sustainable development of Brazilian biodiversity. (ii) There is no lobby, only a sincere attempt to overcome two enormous handicaps: the interdisciplinary nature of the work and the language in which some important aspects were reported (had the underlying principles been closer to biochemistry than to botany or a larger part of the results communicated in English instead of Portuguese, these efforts would probably not be necessary).

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Smallpox Vaccine

I find Donna E. Shalala's justification for retaining, and experimenting with, viable variola (smallpox) virus (Editorial, *Science*'s Compass, 13 Aug., p. 1011) misleading. The existing vaccine is entirely satisfactory and was the basis of the successful World Health Organization eradication campaign. Its present short supply could easily be remedied by manufacturing more. The small number of immunocompromised individuals, and, more important, pregnant women, for whom it is contraindicated could be protected by vaccination of contacts and herd immunity.

New vaccines cannot be licensed because their efficacy cannot be proved in the absence of humans exposed to smallpox. If smallpox reappeared, it would be unethical to offer an experimental vaccine in place of the traditional vaccine, known to be effective.

Manipulation of viable variola will always involve a risk of escape; containment systems can fail, most likely by human error. If such experiments are done, it is essential that all involved be currently vaccinated, as has been standard practice.

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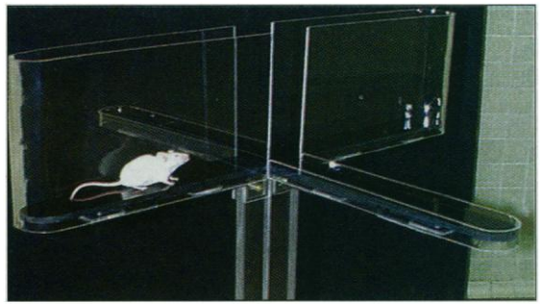
Testing the Genetics of Behavior in Mice

The important study by J. C. Crabbe *et al.* of behaviors measured across three laboratories using the same mouse strains (Reports, 4 June, p. 1670) demonstrates clearly what is widely known in the neuroscience field: behavior is a complex phenomenon that is strongly affected by both genetics and environment. News reports about this study have glossed over the rather striking finding that the majority of behavioral measures were consistent between laboratories. Indeed, the data largely confirm many strain-dependent differences reported in the literature. Although the study demonstrates that differences in investigators and unforeseen environmental factors from laboratory to laboratory can alter behavioral results, these phenomena are not limited to studies in behavioral neuroscience, or even to the biological sciences for that matter. Far from precluding scientific advances, interlab variability generates controversies that stimulate further investigation, resulting in methodological improvement over time. No single, exciting finding, whether at the behavioral, physiological, or molecular level, can stand on its own.



Results in this elevated plus maze varied from lab to lab.

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The interlab differences reported by Crabbe *et al.* illustrate that techniques for behavioral analysis are still evolving. It is imperative that strong communication exist between researchers in many disciplines so that advances in unraveling the complexities of behavior can be rapidly incorporated into experiments using newly engineered mice. An important message conveyed by this study is that several different approaches should be used, either within or between laboratories, before a definitive interpretation of a behavioral change is made. We should not conclude from this study that behavioral analysis is beyond rigorous scientific investigation or that genetic engineering will not help elucidate the molecular basis of complex behaviors. We should instead use these results to highlight that scientific progress is a changing mosaic of overlapping studies that correct, build, and expand on earlier findings.

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The laudable attempt of Crabbe *et al.* to standardize behavioral testing conditions for studies of genetic determinants of behavior has significant ramifications. One issue not completely addressed in their discussion is the use of group housing. There can be significant individual differences in behaviors of group-housed rodents, depending on their social rank within the colony. Rank-related differences in behaviors are expressed in such paradigms as the open field, and these behaviors also differ when one compares rats from aggressive and nonaggressive groups. Second, it is likely that there were differences in the handling of mice by the experimenters and also in maintenance-related conditions in the vivariums. Although acclimatization of rodents to repeated human handling is important, it is difficult to control for idiosyncratic differences in picking up and in handling rodents during behavioral testing. Moreover, the care and behavior of personnel involved in the maintenance of the animal room is also important. We have noted significant behavioral differences in the behavior of rats associated with certain cleaning activities carried out by the caretakers. Also, for health or other personal reasons, caretakers are substituted from time to time, which can introduce further unexpected, and most likely unknown, variability in the behavioral response of experimental ani-

mals. These issues and possibly still others, in addition to those controlled for in the authors' report, contribute to the diversity and challenges involved in behavioral research.

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Crabbe *et al.* reiterate important issues that bear on the proper conduct and interpretation of behavioral studies in general, primarily, the principle that the establishment of robust, reliable effects on behavior comes from converging evidence obtained by using different approaches. They express their main concern as being that, where small genetic effects exist, they might be lost in the noise arising from acute environmental factors. Of course, this can easily happen where the experimental variables are ill defined; however, such considerations are not specific to behavioral measures, and they apply to any situation where small (but potentially important) effects are examined. The main source of variation Crabbe *et al.* report in one laboratory was where the experimenter was highly allergic to mice. Not surprisingly, experimenters who have allergies handle animals in very different ways from those who do not. More important, the experimenter in question wore an Airstream helmet to protect him/herself from the allergens. The fan motors in these helmets emit ultrasound, which can profoundly affect rodent behavior unless prevented from doing so by extensive habituation of the rodents.

The conclusion of Crabbe *et al.* is that one test should not be relied on to determine a phenotypic difference in behavior. This is not novel, particularly when unconditioned tests (such as the elevated plus maze and open-field arena) are used, where the controlling variables are often obscure. Without a stable baseline—which can be achieved by using a conditioned task—it is difficult to control fully for variations in test performance. Our own practice is never to rely solely on the results of one test, but to apply multiple tests, covering spontaneous and conditioned behaviors.

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Crabbe *et al.* specifically note that they are unable to replicate a preference for alcohol in knockout mice lacking the 5-HT_{1B} receptor reported in an earlier study (*1*).

The disappearance of the alcohol-preferring phenotype is probably a valid result, because the original finding was replicated four times in Crabbe's laboratory and the recent lack of alcohol preference was demonstrated in three different laboratories, including Crabbe's. This change in phenotype over time appears to be quite specific, as other behavioral characteristics (including other components of the response to alcohol) remained unchanged in Crabbe's laboratory (*2*) and in others (*3, 4*). Then what explains the change in alcohol preference over time within Crabbe's laboratory? Although it is impossible to exclude the possibility of an unrecognized environmental factor, a more likely hypothesis is genetic drift in the knockout or wild-type colonies, or both.

The genetic background of knockout colonies often evolves over time because different sources of embryonic stem cells were derived from different 129/Sv substrains. For example, our initial stem cells are derived from the 129/SvPas substrain, but our current stem cells are derived from

the 129/SvEvTac substrain (*5*). Therefore, there has been a gradual introduction of 129/SvEvTac genes into our wild-type and knockout colonies, although not necessarily at the same pace. Such a genetic drift may be responsible for the observed phenotypic drift, suggesting that the 129/Sv substrains differ in one or several genes that have an effect on alcohol consumption. In keeping with this idea, 129/Sv-ter mice were shown to drink significantly more alcohol than the 129/SvEvTac mice (*6*).

Although embryonic stem cell–induced genetic drift within colonies is largely unavoidable, it is important and feasible to ensure that knockout or transgenic strains do not drift away from their wild-type controls. This is easily achieved by periodically interbreeding mutants and controls (*7*). The first generation will yield heterozygotes, and subsequent breeding of these heterozygotes will produce the necessary wild-types and knockouts. Such heterozygote breeding is often not performed because it requires genotyping of the offspring, which many laboratories are not equipped to do. However, it is the only rigorous way to study the effect of a specific mutation, because it ensures that mutants and controls have similar genetic backgrounds. An additional advantage of heterozygote breeding is that it controls for maternal effects, as wild-type and knockout littermates have the same parents (*4*).

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6. J. C. Crabbe, D. Wahlsten, B. C. Dudek, *Science* **284**, 1670 (1999). The 5-HT_{1B} knockout mice used by Crabbe *et al.* in their initial study (*1*) were a mixture of 129/SvPas and 129/Sv-ter substrains, but the mice used in the recent study are a mixture of 129/SvPas, 129/Sv-ter, and 129/SvEvTac. Frozen embryos corresponding to the original 5-HT_{1B} knockout substrain are available and should enable us to test the hypothesis that the phenotypic drift that resulted in a loss of alcohol preference results from the introduction in the knockout colony of a modifier gene coming from the 129/SvEvTac substrain.
7. Banbury Conference on Genetic Background in Mice, *Neuron* **19**, 755 (1997). This is the gold standard for the maintenance of knockout colonies.

Crabbe *et al.* report that, despite rigorous attempts to control husbandry and test procedures, genetically identical mice tested at different sites differed in behavior. A factor that the investigators may not have controlled was the animals' diet. All the mice were fed a commercially available chow. According to the manufacturer, this diet varies in composition depending on the natural ingredients available (*1*). Handling and storage conditions also influence chow pellet size and water content. Food composition, texture, and moisture can have substantial effects on growth, nutrient choice, and other behaviors (*2*). It seems a worthwhile precaution in future studies to use rigorously defined semisynthetic diets, such as those recommended by the American Society for Nutritional Sciences (*3*). If, as the saying goes, we are what we eat, then it follows that, if we don't know what the mice eat, we don't know what they are.

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Response

Our colleagues make important points about the stability of genetic differences in mouse behavior across laboratories.

We agree completely with the points raised by Picciotto and Self. We find the stability of several effects reassuring. Also, there were virtually no effects of shipping animals, which is exceedingly good news and should facilitate validation of results, including characterizations of knockout mice, across laboratories.

Pohorecky identifies several important variables that are likely to influence rodent behavior under test conditions like those we used. Social dominance is clearly an important variable, unmeasured in almost all such studies. However, if it were crucial for the particular behaviors we measured, we might have expected to see more frequent effects of sex, as agonistic behavior is generally more pronounced in male than in female mice. Unlike common practice with laboratory rats, mice are typically housed with like-sexed individuals in small groups, usually with littermates in those laboratories that maintain breeding colonies. Thus, group housing is the

standard protocol in most mouse laboratories. Group-housed and isolated male mice differ in the dynamics of the patterns of their dominance hierarchies, as well as in their aggressive behavior, and these differences are strain-dependent (*1*). Moreover, individual housing can lead to increased anxiety-like behavior in an elevated plus maze (*2*). Just how housing practices might interact with laboratory site to affect strain differences is not readily predictable from literature of which we are aware, nor for our data were there differences in housing—all mice were grouped.

Dawson *et al.* agree that multiple tests using different approaches should be used to solidify inferences about the genetic structure of behavior, although they are hardly unique in adopting this practice. They imply without directly asserting that the fact that our measures were unconditioned, as well as ill defined, may have led us to be unable to isolate small genetic differences reliably. Only some behavioral domains are best tapped with conditioned responses, and we avoided these in our study for practical reasons. We do not agree that our responses were ill defined. For example, on our water escape task, group differences were not particularly large (multiple $R^2 = 0.18$), but the intertrial consistency of behavior, as indicated by Cronbach's coefficient alpha, was reasonably high ($\alpha = 0.81$ over the first four trials). Such consistency is not the hallmark of ill-defined tasks. They also err in their assertion that "the main source of variation...in one laboratory was where the experimenter was highly allergic to mice." We offered this as an example of a laboratory difference, but there are no data suggesting it was the "main source of variation"—this appears to be Dawson *et al.*'s opinion. There was probably ultrasound emitted from the motor of the Racal Airmate 1 device strapped to the small of the back of the Edmonton experimenter at waist level. However, this was a constant: the experimenter wore the unit whenever working with the mice, from the day they arrived in the colony until the end of testing, and there was ample time for habituation. Whether wearing the Airmate apparatus had any effect on mouse behavior in standard tests can only be addressed with a controlled study using people not allergic to mice who wear or do not wear the filter unit. Data relevant to this question are needed before the effects can be called "profound."

Hen notes that his knockout mouse colony has been maintained on a genetic background involving multiple 129 substrains. This, we suspect, is true for many other knockout colonies as well. To explain the loss of alcohol drinking phenotype in the 5-HT$_{1B}$ knockouts over time, he proposes that an increasing influence of modifier genes from the 129/SvEvTac strain reduces alcohol preference. This hypothesis can be tested definitively by rederiving cryopreserved embryos from the original population. The stability of reduced alcohol-induced ataxia in the knockouts suggests that the effects of such modifier genes are trait-specific, which is consistent with our other findings. Hen elaborates a breeding strategy that can protect against such modifier gene effects; maintaining knockouts on fully inbred rather than segregating populations also will accomplish this.

Tordoff *et al.* suggest our results may have been influenced by differences among labs in the composition of Purina diets. This is quite feasible because there were modest but statistically significant differences among our three labs in mouse body and brain weights. We agree that it would be interesting to run further experiments of this nature using rigorously defined semisynthetic diets. Our study rigorously equated the behavioral test apparatus and testing protocols, and we sought to restrict variation in many aspects of the lab environment. We did not seek to *equate* the lab environment, however. We wanted to know whether commonplace variations in lab environments would modify the pattern of genetic effects, and we found that for certain behaviors they did, whereas other behavioral tests yielded substantially the same results in all three labs, despite the differences among diets and drinking water. It is doubtful that differences between labs can be explained by a single environmental factor; instead, both the environmental and genetic contributions are probably multifactorial and complex.

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CORRECTIONS AND CLARIFICATIONS

It should have been made clear in George B. Dyson's letter "Darwin in Kansas" (*Science*'s Compass, 27 Aug., p. 1355) that Erasmus Darwin's warning was one that could have been given to today's Kansas Board of Education. It was not literally given to the Board in 1794, as the state of Kansas did not then exist.

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[6] **Genetics of Mouse Behavior: Interactions with Laboratory Environment**

John C. Crabbe; Douglas Wahlsten; Bruce C. Dudek

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