The Pharmacogenomics Journal (2004) 4, 219

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Old principles in new clothes

The Pharmacogenomics Journal (2004) **4,** 219. doi:10.1038/sj.tpj.6500253

In a previous issue, Nakatani and co-workers[1] examine gene expression differences in the frontal cortex and hippocampus of learned helpless rats compared to untreated controls and after treatment with antidepressants. It is a very interesting and informative study providing comprehensive analysis of gene expression changes, particularly regarding antidepressant-regulated genes, in an animal model of depression. However, the appropriateness of one of the control groups is called into question, which reduces the impact of the study. Why is this an important issue that deserves a note of caution?

In recent years, the use of microarrays changed the discovery phase of molecular genetics. Instead of studying the changes in the expression of individual genes in response to a treatment or in a pathophysiological state, researchers can study the activity of thousands of genes at once. At the same time, many frustrated scientists trying to replicate the experiments in their own system find that controlling the number of variables at every step of the experimental design can be forbiddingly expensive and virtually impossible. So, it seems that everybody, or almost everybody, is cutting corners when it comes to designing a microarray experiment. The real question is what are the crucial variables that every experiment should control for and where can there be more flexibility? Open letters and reviews appearing in the best journals suggest standards that a microarray experiment should follow, such as the Minimal Information About a Microarray Experiment guidelines[2] and related statistical issues.[3] The reviews deal largely with the technical and statistical components of the experimental design of a microarray study, since these aspects are novel, leaving the classic experimental design, the bread and butter of scientists, less emphasized. When a classic experiment does not control for all the relevant variables, the reader can interpret the limitation of the study. In contrast, when a microarray experiment is designed without all the relevant controls, the vast quantities of data generated can cloud the study's limitations. How are we to see the forest for the trees?

This is the specific quandary regarding articles like that of Nakatani *et al*[1]. In this paper, the authors are using the learned helplessness (LH) rat model of human depression. In the experimental design, LH is elicited by exposing rats to inescapable footshock and then 24 h later determining the number of escape failures as measures of depression-like behavior. However, only 40% of animals express LH in this study; the rest seem to resist the stress of inescapable shock and do not express LH behavior. Where does this bimodal distribution come from?

The study described above employed the outbred Sprague–Dawley (SD) rat strain that shows a substantial degree of genetic heterozygosity, which could be responsible for the bimodal distribution of the LH phenotype. Indeed, others selectively bred SD rats leading to congenitally learned helpless and congenitally nonlearned helpless animals[4] that differ in multiple physiological and behavioral measures. Further biochemical and genetic differences exist between animals that show LH behavior and those that are resistant to it.[5] So, when the scientific goal is to determine the gene expression changes induced by LH, helpless animals need to be compared to nonhelpless animals who have been subjected to the same training and testing routine, so that the differences reflect those systems that are specifically altered in helpless behavior.[5] Instead, in the present study, the comparison is made between LH animals and a control group that did not receive an inescapable shock 24 h prior to the escapable shock. Prior stress is known to affect expression of stress-related genes to differing degree, and some of these effects last up to 4 weeks.[6] Thus, the study in question identifies a number of genes that are potentially involved in the long-term effects of inescapable shock, disregarding the presence of the learned helpless phenotype.[7] These genes, therefore, do not relate directly to those involved in the learned helpless behavior. Since LH is a depression model, antidepressants that reverse the LH behavior should also normalize the expression of those genes that are altered in the learned helpless animal. However, in the present study, gene expression changes in LH animals relative to controls were expected to be reversed by antidepressants, and not expression differences related to developing the LH phenotype. As a consequence, false negative results are likely and thus the study may not have fulfilled its potential.

The choice of the right comparison is particularly important in studies of behavioral traits. The authors are aware of the difficulties in applying microarray techniques to study gene expression changes between behavioral phenotypes when they quote Mirnics *et al*:[8] true gene expression changes in psychiatric traits are small and psychiatric diseases may result from cumulative subtle changes. In an animal model of depression that relies on a stress-induced behavioral phenotype, the magnitude of changes in gene expression are also likely to be subtle since the tight homeostatic balance that rules in the nervous system works against them. Thus, microarray studies of the molecular determinants of behavior are particularly burdened by the necessity of precise experimental design.

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