Plants Talk—But Can They Listen?

K. BROWN'S ARTICLE "SOMETHING TO SNIFF at: unbottling floral scent" (News Focus, 28 June, p. 2327) highlights the spectacular advances recently made in the field of plant volatiles through a combination of ecological, molecular, and evolutionary techniques. The sidebar within the article ("Plants 'speak' using versatile volatiles," p. 2329) attempts to tie these advances to the question of what have been called "talking trees." Several researchers, including J. Tumlinson and I. Baldwin, have demonstrated that insects have exquisite abilities to detect volatile compounds emitted by plants and that both herbivorous and predatory insects can respond quite strongly to certain phytogenic volatiles. Such results fit well with research on the physiology and neurobiology of insect olfactory systems.

If a tree talks, is anyone listening?

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What is not yet known, however, is whether plants growing under natural (or agricultural) conditions respond directly to volatile signals from other plants. Many of the recent experiments on this question have been conducted under laboratory conditions that artificially (and, possibly, artifactually) raise the concentrations of the volatile compounds under consideration. Simple calculations of biogenic flux and turbulent diffusion rates suggest that most plants growing outdoors see concentrations of biogenic volatiles several orders of magnitude lower than those commonly used in lab and growth chamber experiments. We still lack convincing evidence that plants respond to volatile signals from other plants when turbulence conditions are realistic and concentrations approach those seen in nature. In contrast to insects, plants appear to lack highly evolved reception and transduction systems for volatile signals. The question about plants is not whether they can talk. The question is, do they listen?

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Something in the Eye of the Beholder

BECAUSE OF THE INTENSE INTEREST IN THE stem cell field, even reports of failure to replicate previous findings have appeared in prominent journals. For example, two recent reports question whether adult bone marrow–derived cells contribute to central nervous system (CNS) neurons because the authors failed to see markers of such cells in brains ("Failure of bone marrow cells to transdifferentiate into neural cells in vivo," R. F. Castro *et al.*, Brevia, 23 Aug., p. 1299; "Little evidence for developmental plasticity of adult hematopoietic stem cells," A. J. Wagers *et al.*, Reports, 27 Sept., p. 2256; published online 5 Sept.; 10.1126/science.1074807). These conclusions are in marked contrast with previous reports by us and others (*1*–*3*) that found that bone marrow–derived cells transit to the CNS in adult mice, express proteins typical of neurons in the olfactory bulb, and contribute to well-defined subsets of neurons such as Purkinje cells in the cerebellum. Efforts to replicate discoveries are critical to the scientific process, and convincing failures to do so are important contributions to the literature. The fundamental issue is defining what makes a study convincing and, therefore, what should be the criteria for overturning previously published findings. The findings by Castro *et al.* and Wagers *et al.* underscore the need to establish criteria for publishing negative reports.

First, a prerequisite for proving a null finding is the clear ability to detect a positive control. A case in point is Castro *et al.*, who fail to detect not just neurons but also bone marrow–derived microglial cells within the CNS. At least 20 reports over the past 15 years have shown that bone marrow transplantation results in readily detectable replacement of a large proportion of microglial cells in the brain (*4*–*8*). Moreover, following a stab wound, the presence of such cells in the brain would be impossible to miss, as they are localized in great abundance at the site of the wound (*9*). Thus, the lack of detection of microglia by Castro *et al.* suggests that their system was unable to detect marrow-derived cells that should have been present in the brain.

If such controls fail, the fidelity of an assay must be questioned. Perhaps the major problem in the findings of Castro *et al.* lies in the use of ROSA26 transgenic mice that constitutively express β-galactosidase (β-Gal) in most cells. The expression of β-Gal by these mice is very weak at the single cell level and can be difficult to distinguish from endogenous mammalian β-Gal activity, especially in the brain in cells at high magnification. We know this from personal experience, as we, like Castro *et al.*, used ROSA26 bone marrow donors for an entire year to track marrow-derived cells within the brains of recipient mice. We ultimately rejected the ROSA26 approach because it lacked specificity and sensitivity in the brain and, therefore, took pains to redo all of our experiments for 2 subsequent years with a marker that has no endogenous counterpart, green fluorescent protein, before publishing our report (*1*).

The report by Wagers *et al.* exemplifies another concern regarding the publication of negative findings. In the absence of an adequate description of the methodologies used, the experimental results are difficult to interpret or compare with previous results. Indeed, scientists may well be comparing apples with oranges. For example, in Wagers *et al.*, in the case of the brain, it is unclear what regions were assayed, and a different marker was used from those published previously (*1*–*3*). In the case of skeletal muscle, the particular muscles sampled were not identified. This choice could have profound effects on the results obtained, as there are hundreds of

muscles, which differ markedly. Although the general findings by Wagers *et al.* are consistent with the conclusions of previous reports that transdifferentiation is a rare event in the absence of damage, comparisons with future publications will be difficult. Detailed descriptions of methodologies to allow for replication and comparison of results should be a prerequisite for publication, especially now that space limitations have been alleviated because of the ability to put supporting material on the internet.

Given the massive interest this subject has generated, it is particularly important that experiments be rigorous, with well-defined methods for identifying cells in new locations (*10*). To question a positive finding, a system of comparable sensitivity is required. Nonseeing is not the same as nonbeing.

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References

1. T. R. Brazelton, F. M. V. Rossi, G. I. Keshet, H. M. Blau, *Science* **290**, 1775 (2000).
2. É. Mezey, K. J. Chandross, G. Harta, R. A. Maki, S. R. McKercher, *Science* **290**, 1779 (2000).
3. J. Priller *et al.*, *J. Cell Biol.* **155**, 733 (2001).
4. W. F. Hickey, H. Kimura, *Science* **239**, 290 (1988).
5. D. W. Kennedy, J. L. Abkowitz, *Blood* **90**, 986 (1997).
6. W. J. Krall, P. M. Challita, L. S. Perlmutter, D. C. Skelton, D. B. Kohn, *Blood* **83**, 2737 (1994).
7. W. Krivit, J. H. Sung, E. G. Shapiro, L. A. Lockman, *Cell Transplant* **4**, 385 (1995).
8. P. Rezaie, D. Male, *Microsc. Res. Tech.* **45**, 359 (1999).
9. K. Imamoto, C. P. Leblond, *J. Comp. Neurol.* **174**, 255 (1977).
10. H. M. Blau, T. R. Brazelton, J. M. Weimann, *Cell* **105**, 829 (2001).

Response

IN OUR BREVIA, WE REPORTED THAT BONE marrow cells (BMC) failed to differentiate into neural cells, challenging earlier reports (*1*, *2*). In their letter, Blau and colleagues state that our failure to observe BMC-derived neural cells perhaps resulted from weak expression of β-Gal in ROSA26 cells that limits their detection in host CNS. They reveal that they too used ROSA26 donors in initial experiments but switched to the green fluorescent protein (GFP) marker when they did not obtain the expected results, which they attribute to a weak β-Gal signal. To our knowledge, there are no studies that demonstrate that GFP is a more robust marker than β-Gal. The chromogenic substrate X-gal is continuously hydrolyzed by β-Gal, so the signal is amplified over time and should be more intense when compared with the GFP signal. In our study, cells in ROSA26 mice stained robustly for β-Gal activity, and we observed rare β-Gal–positive donor cells in perivascular locations in the CNS and in injured muscle of transplant recipients. Moreover, in recent experiments, we detect ROSA26 BMCs that are directly transplanted into the CNS. On the basis of these positive controls and the extensive cell marking literature, ROSA26 cells are unequivocally detected in the CNS if they are present. It is not the β-Gal marker that is in question; rather, it is the specificity of the GFP marker that can give rise to false positives from cell fusion, close juxtaposition to neighboring cells, engulfment of protein and cellular debris, and autofluorescence.

Blau and colleagues also question why we did not observe large numbers of host-derived microglia in animals that had received injury. We analyzed the recipients ≥1 month after trauma, when the injury had largely resolved and when others have shown that few microglia have had time to move into the brain (*3*, *4*). However, our capacity to discern donor-derived cells in the CNS was validated by their ready detection in perivascular locations. Their globular morphology was not, however, consistent with a neural phenotype.

We agree with Blau and colleagues that a proposal as interesting and as important as bone-to-brain transdifferentiation should be tested with numerous rigorous and well-defined methods. Wagers *et al.* (*5*) and our own report indicate that their claim remains to be corroborated. In stem cell science, evidently, seeing may not always establish being.

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References

1. T. R. Brazelton, F. M. V. Rossi, G. I. Keshet, H. M. Blau, *Science* **290**, 1775 (2000).
2. É. Mezey, K. J. Chandross, G. Harta, R. A. Maki, S. R. McKercher, *Science* **290**, 1779 (2000).
3. D. W. Kennedy, J. L. Abkowitz, *Blood* **90**, 986 (1997).
4. W. J. Krall P. M. Challita, L. S. Perlmutter, D. C. Skelton, D. B. Kohn, *Blood* **83**, 2737 (1994).
5. A. J. Wagers, R. I. Sherwood, J. L. Christensen, I. L. Weissman, *Science* **297**, 2256 (2002); published online 5 Sept. 2002 (10.1126/science.1074807).

Response

BLAU *ET AL.* RAISE SEVERAL ISSUES REGARDING the rigor required of studies that report negative results. In regard to our Report, they express concern that the level of detail given in the original online publication regarding the tissues analyzed in our study is insufficient to allow comparison with other published data. We certainly agree that adequate information regarding the methods used is essential to allow the reproduction of scientific results by independent laboratories, and we have endeavored to redress this oversight both in this

letter and by modifying the online supplemental material of our paper to include a more specific description of the regions of the brain and muscle groups that we examined. In the case of skeletal muscle contributions, we analyzed sections of three different muscle groups, the tibialis anterior (TA), diaphragm, and abdominal muscle. Through ongoing experiments, we have, to date, examined 6544 myofibers in TA, 7428 myofibers in diaphragm, and 7216 myofibers in abdominal muscle of single hematopoietic stem cell (HSC) transplanted mice, and 59,557 myofibers in TA, 5786 myofibers in diaphragm, and 9719 myofibers in abdominal muscle of control partners of long-term parabiotic pairs, but we have seen 0 GFP-positive HSC-derived or cross-engrafting myofibers. As there are no differences in the engraftment rates of these different muscle groups in our analysis, we combine the data under the heading "skeletal muscle" (0/21,188 for single HSC transplanted animals and 0/75,062 for parabionts).

In the case of brain tissue, our analysis of sagittal sections included cells of the olfactory bulb, cortex, and cerebellum. We did employ a different marker than that used by Brazelton *et al.* (*1*) to identify donor-derived neurons; however, the marker we employed, MAP2, has been used previously by other investigators as a sensitive and specific marker for neurons (*2*–*4*). Furthermore, our analysis included staining with the pan-hematopoietic marker CD45, which clearly demonstrated the hematopoietic commitment of the majority of HSC-derived GFP-positive cells in the brain. Our experiments were not designed to replicate precisely the work of other investigators, but to clarify and extend their observations by establishing, through the transplantation of single, prospectively isolated, GFP-marked HSC, whether or not the production of nonhematopoietic cell types is a true, robust, physiologic function of HSC. Importantly, our data do not necessarily contradict the observations of other investigators that bone marrow cells maintain the potential to generate both hematopoietic and nonhematopoietic cells. However, our results do suggest that other cell populations in marrow, not HSC, are likely responsible for the generation of nonhematopoietic tissues after transplantation of unfractionated bone marrow cells into otherwise uninjured animals. Clearly, increased efforts at defining cell populations within the marrow capable of robustly generating muscle, skin, brain, and so forth will be an appropriate and important target for future research.

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References
1. T. R. Brazelton, F. M. V. Rossi, G. I. Keshet, H. M. Blau, *Science* **290**, 1775 (2000).
2. R. B. Maccioni, V. Cambiazo, *Physiol. Rev.* **75**, 835 (1995).
3. Y. Matsuoka *et al.*, *Eur. J. Neurosci.* **11**, 3617 (1999).
4. C. A. Messam, J. Hou, E. O. Major, *Exp. Neurol.* **161**, 585 (2000).

Revisiting the Science/Political Mix

AS DONALD KENNEDY POINTS OUT IN HIS Editorial "When science and politics don't mix" (7 June, p. 1765), the boycotting of Israeli scientists by non-Israeli scientists is in essence anathema to the idea and practice of "open science" and also engenders anger, which is already in surfeit in that area of the world. A more effective proposal would be for the non-Israeli scientists to somehow get together with the Israeli scientists who oppose their country's disastrous "military only" policy (and from my own experience, I know that many do) to see what can be done to aid the Israeli sci-