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Comment on “Magnetic Resonance Spectroscopy Identifies Neural Progenitor Cells in the Live Human Brain”

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Manganas *et al.* (Reports, 9 November 2007, p. 980) used a metabolic biomarker identified in vitro for the detection and quantification of neural progenitor cells in vivo. Although their detailed experiments and general approach are laudable, aspects of their magnetic resonance spectroscopy data and analyses raise questions about their results.

Manganas *et al.* (1) reported on a metabolic biomarker identified in vitro for the detection and quantification of neural progenitor cells (NPCs) in the human brain in vivo. Although the authors' expertise in microbiology is clear, their apparently less experienced use of magnetic resonance spectroscopy (MRS) may have resulted in invalid in vitro data and unintentionally opportunistic analytic processing in vivo.

The in vitro NPC spectrum reported by Manganas *et al.* [figure 1A in (1)] has a broad peak labeled as N-acetyl-aspartate (NAA) near 2.02 parts per million (ppm). However, this resonance does not have corresponding aspartate groups (at 2.48 and 2.67 ppm) (2), which suggests that little NAA is present. Accurately setting the spectrum shift is critical for identifying the frequency position of the suspected biomarker. If the narrow choline peak at 3.2 ppm is used to position the spectrum, the labeled NPC biomarker (1.28 ppm) aligns closely with lactate (1.33 ppm). This consideration, and the other broad peaks in the spectrum, likely mobile lipids (3), suggest that few viable cells were sampled. Because separation of neurospheres with trypsin can cause massive cell death (4), and previous studies evaluating pluripotent cells show very different spectral profiles having narrow lines (5–7), whereas cells undergoing necrosis demonstrate nearly identical spectra (3), this possibility seems likely. Without invoking a specific NPC biomarker, mobile lipids would behave similarly as a dependent measure, scaling to cell numbers, being discriminatory ver-

sus saline, and increasing after electroconvulsive therapy (8), as examples.

Manganas *et al.* then examined the raw in vivo data for the presence of the NPC biomarker, concluding that “[t]he Fourier transform did not reveal the 1.28 ppm biomarker in any of the voxels.” Although this conclusion is likely correct, the authors then created a singular value decomposition (SVD) approach that they claim clearly detects the biomarker. Although SVD is commonly employed to remove the residual water signal when the water shoulder is not modeled in the line fitting, the use of SVD to remove frequency components from a broad resonance with the opportunistic aim of retaining a fixed peak is highly atypical. The approach appears to have been applied variably (“iteratively,” as described by the authors) without appropriate constraints [e.g., using a priori parameters (line width, frequencies, coupling) from known biological resonances to inform SVD editing]. Different magnetic field strengths and acquisition parameters between in vitro/animal/human will not yield identical data surrounding 1.28 ppm.

Even if SVD editing were theoretically valid, the line fitting used to derive the experimental results shows little correspondence to the retained data. For example, if the fitted peak at 1.28 ppm is lipid, then it is too narrow (large molecules relax at a faster rate after excitation yielding broad lines), whereas the fitted lines for choline, creatine, and NAA are too broad, incorporating the remaining entire lipid and macromolecule baseline (9). Although modeling known resonances additively is conceptually easy to understand, the selective removal of some signals, followed by line fitting that results in signal overlap (1.28 ppm and NAA), confounds what is

being measured. This point is underscored by a recently performed study in rats comparing the hippocampus and corpus callosum (10) [synonymous to figure 3A in (1)]. At higher magnetic field, using greater spectral and spatial resolution, and employing a shorter acquisition delay (e.g., leaving more signal available to be measured), there was no evidence, either by visual inspection or detailed chemical fitting, of a differential 1.28-ppm peak in the hippocampus (10). Thus, it appears that this resonance can only be revealed by analysis.

This leads to questions in the range of philosophy. If an algorithm creates a numerical representation of a biomarker that cannot be observed in the raw data, can logical results ever substantiate the presence of that biomarker? Can logical results substantiate the method? Although the premise of characterizing NPC activity in the human brain has unquestionable merit and urgency, the data and method described in (1) will likely unleash a multitude of studies attributing any fitted peak at 1.28 ppm to NPCs. Unfortunately, no derived results will be falsifiable or verifiable by the raw data. For future studies attempting to detect NPCs in the living human brain, a detailed integration of pluripotent cell biochemistry (5–7), consideration of spectral errors (11), and attention to measurement sensitivity (12) may provide helpful guidance toward defining what NPC signal criteria may be accepted as measured signal. Without such reins, hypotheses paired with data processing can be a compelling daemon, as evidenced by the cautionary tale found in studies employing silicone magnetic resonance spectroscopy (13).

References and Notes

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