accounted for by the correlation is about 18% (r^2). The remaining 82% could be some other source of variation that is not explained by pleasantness.

From the title of their paper, and from the figures, it appears that Lapid *et al.*² prefer to use pleasantness as the independent (x) variable and EOG activity as the dependent (y) variable. Previously, they concluded that pleasantness was a property of the odorant, or was at least determined in part by it. According to this line of thinking, odorant pleasantness is the cause of the EOG rather than vice versa. A different description of the relationship is to consider the EOG to be the independent variable that produces an effect on the dependent variable, the perception of odorant pleasantness. This would not change the statistics, but would change the emphasis from response coding to traditional stimulus coding.

The discovery of a hedonic feature representation in the olfactory epithelium raises the obvious question of how odorant pleasantness is represented in the olfactory bulb,

the next step in processing odor information. The primacy of odorant pleasantness as a coded attribute represented in EOG recordings suggests a possible underlying structure that is not currently evident in the receptors or in the organization of the olfactory epithelium and olfactory bulb. Even if olfactory stimuli can be considered to be broadly pleasant or unpleasant, probably an equally important feature is the convergent projections of receptor subtypes onto the olfactory bulb that represent the quality distinctions determined by receptor binding and activation patterns. The distribution of receptors in the epithelium, whether patchy, scattered or random, is completely restructured by segregation of the projections of receptor subtypes to particular glomeruli in the olfactory bulb. Does each glomerulus represent a point on a quality axis? How many dimensions would it take to represent all of odor space? Is odor quality representation in the olfactory bulb sparse, combinatorial or diffuse? It will be

interesting to find out how the patchy hedonic features in the epithelium are woven into the glomerular features that define odor character. That study will require an animal model and considerable human ingenuity.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Seeing more clearly with Scale

Current approaches for imaging deep into tissue are limited. Mechanical sectioning techniques, such as array tomography or serial block-face scanning electron microscopy, allow for high optical resolution, but are labor intensive, costly and require sophisticated data reconstruction procedures. Optical sectioning techniques are potentially faster and less expensive, but are limited by tissue opacity and light scattering. Standard laser-scanning confocal microscopy can penetrate only to about 150 μm below the brain surface, and two-photon excitation fluorescence microscopy cannot image more than 500–800 μm below the brain surface. To image entire projections and cell populations in the intact brain, a much greater depth of penetration is needed.

Optical clearing, in which tissue is rendered transparent, thereby achieving refractive uniformity and reducing light scattering, increases the depth at which tissue can be imaged. Previously, several clearing solutions have been described that, when applied to fixed specimens, allow for optical sectioning, three-dimensional imaging and reconstruction. On page 1481, Hama and colleagues improve on the solutions that are presently available for optical clearing, presenting a new aqueous clearing reagent, Scale, which preserves fluorescent signals and allows for imaging at unprecedented depths.

Scale is a urea-containing reagent that renders whole, fixed mouse brains transparent. The authors show that tissue treated with Scale does not absorb any light with wavelength above 276 nm. Scale also causes a roughly twofold expansion in tissue volume, but the expansion is isotropic and homogenous. Notably, Scale does not affect the fluorescence intensity of GFP or YFP, a substantial improvement over similar reagents. Both one- and two-photon microscopy benefit from tissue clearing, and the authors are able to image to a depth of 2.0 mm, which is limited only by the working distance of the objective. In fact, a customized objective designed by Olympus with a longer working distance (4 mm), as well as a sufficiently high numerical aperture, allowed them to image even deeper into the brain.

Using Scale, the authors three-dimensionally reconstruct a number of brain structures. As shown in the picture, the authors determine, in a YFP-expressing mouse line, the three-dimensional architecture of cortical and hippocampal networks, in which individual dendritic spines can be resolved. The authors also use this technique to measure, from a three-dimensional perspective, the proximity of neural stem cells to blood vessels in the dentate gyrus of the hippocampus, indicating that neural stem cells are closer to blood vessels than mature neurons. The exact makeup of Scale can be optimized for specific applications, suggesting that this technique will be widely useful in allowing interrogation of three-dimensional structures in the brain and throughout the body.

Brigitta Gundersen