

Editorials

The splendour of unthresholded brain maps

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Brain imaging technologies offer unparalleled potential to understand brain organization. Initial efforts sought to attribute functions to individual regions by applying strict statistical thresholds to isolate locations with the greatest effects. Despite prominent theories about the nature and importance of integration among regions, analysis and reporting of unthresholded maps used to be relatively uncommon. A new trend has recently emerged that forgoes thresholds in favour of whole-brain topography. Focusing on continuous unthresholded maps of brain structure and function opens fundamentally new biological questions, encourages links with adjacent fields in neuroscience, and prompts deeper appreciation for classical neuroanatomy. A broad framework is taking shape to realize the possibilities of brain imaging more fully, to strike a balance between the study of functional specialization and integration, and to lengthen the stride of neuroscientific inquiry.

The term *brain imaging* implies consideration of the whole brain. Since their inception, techniques such as CT, MRI, PET and others have offered the capacity to measure a wide array of biological features *in vivo* with varying extent and resolution. As the techniques became more established, they could be applied in progressively larger and ever more sophisticated experiments, often featuring multiple participants and manipulations.

Despite the fact that this exciting technology yields spatial maps of the whole brain, in the first few decades the weight of emphasis was on circumscribed regions and how they are associated with cognition, age and disease. Confronted with unfavourable ratios of features (often in thousands of voxels) to samples (often in tens of participants), numerous analytical procedures were developed to artificially reduce the whole-brain search space and focus more narrowly on questions about individual regions. These include statistical procedures specifically geared for brain imaging, such as cluster-based correction¹⁻³ and searchlight analysis,^{4,5} as well as focused region-of-interest (ROI) studies. In a sense, the methodology assumed something of the nature of single-location recording techniques rather than wide-field brain imaging. The enduring images from that era are of heavily thresholded maps peppered with so-called “blobs”—isolated islands of significance seemingly breaking forth from an implicitly irrelevant background. Indeed, reporting of peak activations became so commonplace and abundant in the literature that it was possible to design automated meta-analytic procedures to relate regional coordinates with experimental terms.^{6,7}

Practical constraints aside, there is also the fact that imaging allowed an unprecedented opportunity to pursue questions about *in vivo* functional specialization hitherto only possible with lesion studies. The natural course of action therefore was to embark upon a programme of cataloguing associations between regions and experimental manipulations.⁸ Amidst this combination of statistical constraints and inveterate localizationism, the field became somewhat unmoored from the original premise of the technology and increasingly drifted towards functionally annotating discrete locations with less regard for their collective action.

And then, something wonderful happened: we stopped thresholding maps. Perhaps somebody, somewhere, forgot to turn the threshold slider all the way. Whatever the reasons, the past 5-10 years have seen a shift of emphasis from a patchy, often binary and inherently piecemeal model of the brain to coherent, continuous-valued maps that can be readily analyzed from a more integrative perspective ([Fig. 1](#)). The change has been positive, prompting entirely new questions about the brain, such as its global organization, the links between its microscale and macroscale features, and its physical geometry. Many of these notions are not new; they have been eloquently articulated by scores of authors, such as the idea of regions operating in a broader, network-mediated “neural context”,⁹ or the balance between functional specialization and integration.¹⁰ What is striking and noteworthy however, is how deeply they have taken root and how habitually they are now practiced.

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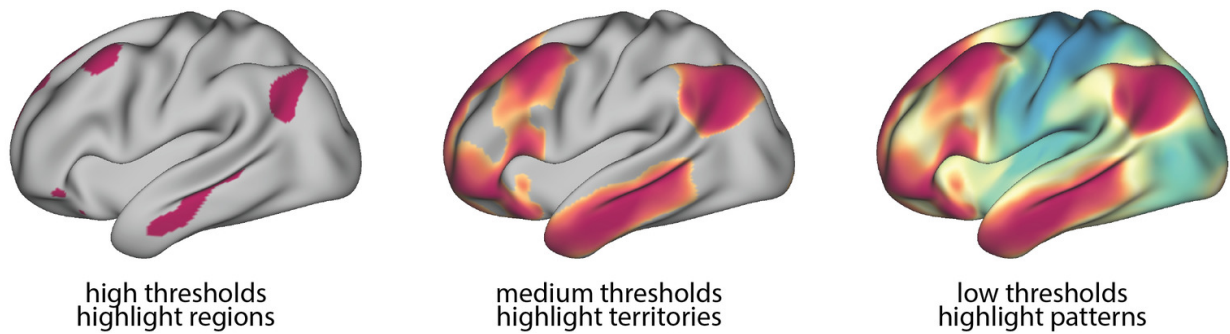


Figure 1. Different thresholds, different perspectives

The figure shows the now-classic unimodal-transmodal functional connectivity gradient reported by Margulies et al.¹¹ Applying a conservative threshold emphasizes high-valued regions and the resulting map gives the impression of isolated pockets of vertices. Intermediate thresholds include more adjacent vertices and the map appears to have more structure, but the highlighted territories are still isolated and it is ambiguous if or how they are related to one another. Removing the threshold altogether emphasizes the natural continuous space in which the vertex values reside and allows the gestalt to emerge, clearly showing smooth transitions between transmodal and unimodal cortex.

What new knowledge has been gleaned from this approach? A notable example is the ubiquitous differentiation and polarization between sensory-motor cortex and association cortex. Largely predicted based on synaptic connectivity¹² and observed in resting fMRI,¹¹ this broad pattern can be readily recovered using other measurements and other biological features, such as T1/T2w maps of intracortical myelin,¹³ cell staining maps of laminar differentiation¹⁴ and microarray gene expression.¹⁵ The prolific research in this domain is matched by an equally rich and colourful terminology to describe the basic phenomenon, including the “unimodal-transmodal hierarchy”,¹¹ the “natural axis”,¹⁶ the “sensory-fugal gradient”,¹⁷ and the less roseate but probably most apt term, the “sensorimotor-association axis”.¹⁸ Although the situation is slightly convoluted by inconsistent nomenclature, looming through the fog is the general impression of a subtle but perceptible undercurrent of organization that manifests at virtually all levels of description.

This (now empirically accessible) thread linking molecular and cellular features to imaging measurements has galvanized interest in integrative biological questions. Reference maps of genes,¹⁹ synaptic proteins,²⁰ neurotransmitter receptors,²¹ neuropeptides,²² cell types,²³ laminae,²⁴ metabolic pathways^{25–27} and oscillations²⁸ are now available in toolboxes such as *NeuroVault*,²⁹ *neuromaps*³⁰ and *JuSpace*,³¹ and routinely used to study the biological origin of observed maps and to guide the design of future experiments. Examples include identifying the transcriptomic and cellular origin of macro-scale organizational features observed with *in vivo* imaging, such as cortical folding,³² intrinsic networks,²³ meta-analytic task activation^{33, 34} and disease vulnerability.¹⁵ Indeed, it is rapidly becoming clear that brain mapping as a field is no longer confined to practitioners of select brain imaging techniques, such as MRI, PET and M/EEG. It now encompasses the broader enterprise of building a vertically coherent picture of the brain, with methodological and increasingly conceptual links to histology, genomics and proteomics.

With increased grounding in basic biological questions, modern brain imaging papers may appear to be, in one way or another, rediscoveries of classical neuroanatomy. But the

strands that connect our present thinking to older ideas are far from trivial and can be instructive. In many instances, imaging offers the opportunity to assess the extent to which observations made using invasive techniques in specific locations (often only in animal models) constitute truly general principles at the whole-brain level. At the extreme, original data from the distant past can be reanimated and incorporated into present workflows. For example, several classical atlases have been digitized over the past 10 years and are now routinely used, such as Vogt-Vogt myeloarchitecture atlas³⁵ and the Von Economo-Koskinas cytoarchitectonic atlas.^{36–38} Ultimately, access to diverse maps of brain organization has inspired renewed appreciation for classical neuroanatomy, encouraging deeper, recurrent links with past theories and results.

A corner has been turned, but what of the road ahead? Access to reference maps of biological features is greater than ever, but the shape and extent of this search space is largely conditioned by what features can be measured using existing imaging technology. At present, the corpus of maps in libraries such as *neuromaps* is more eclectic and miscellaneous than truly comprehensive, with greater representation of maps originating from research cultures that are particularly enthusiastic about data sharing (e.g. fMRI) or from techniques that generate many simultaneous measurements (e.g. gene expression). The composition is of course less important for its own sake than as a prelude to a more systematic exploration and biological assaying of newly-generated maps. With time libraries of reference maps will inevitably be more complete and better reflect the sheer biological richness of brain organization.

A related challenge is how to navigate this expanding and multifarious search space. If you have an input brain map and you wish to understand its molecular and cellular origins, should you correlate it with every reference map available? To what extent should priors help guide the process of associating maps? At present, many investigators opt for a kitchen sink approach as a sort of judicious shaking of the tree, but of course this strategy has the potential to uncover as many spurious associations as meaningful ones. While more concrete statistical issues—such as controlling for the background influence of spatial autocor-

relation—appear to be at least nominally understood,^{39–43} these broader strategic questions are more opaque and reflect the need to strike a balance between exploratory and confirmatory analytic mindsets.

More challenging still are the disparate origins of reference maps of biological features. Not surprisingly in view of how expensive, time-consuming and difficult data collection is, one is often forced to mix and match reference maps from multiple sources, yielding datasets that are marked by pronounced differences in sample size, sample composition (e.g. age, sex), and image resolution. While there exist some datasets in which multiple ante- and post-mortem modalities were measured in the same individuals,⁴⁴ they are the exception rather than the norm. And the day may dawn when diverse features can be simultaneously measured in large representative samples; meanwhile, we must resign ourselves to the pragmatic practice of stitching together independently-collected datasets, with the tacit but increasingly empirically supported assumption that variance in biological features across brain regions is greater than variance across individuals.⁴⁵

Taking a wide angle perspective on brain organization does not obviate anatomical precision or rigour. Initial forays in this domain emphasized commonalities between levels of organization captured by diverse maps, seeking instinctively for principles that can be represented by the broadest brush. Less work has been done in the other direction, to understand instances in which modalities systematically diverge. One notable example is how many maps are placed under the umbrella of the ostensibly universal unimodal-transmodal gradient. This is broadly correct but over-simplified, in the sense that several biological features, such as gene PC1 and intrinsic timescale, follow a decidedly more ventromedial-dorsolateral gradient.^{14,15,46,47} In other words, they are quantitatively correlated with the prototypical functional connectivity unimodal-transmodal gradient,¹¹ but obviously anatomically distinct.³⁰

Tempting as it may be to see this incipient paradigm as a clean break from localizationist endeavours, there is a clear and logical progression from one to another. Arealization, response selectivity and functional specialization

are all well documented and constitute a valuable reference point,⁴⁸ and the field continues to benefit from a well-developed foundation of methods to study the brain from this perspective.⁴⁹ Understanding the balance between functional specialization and functional integration requires an equally balanced methodological approach that can accommodate both perspectives. It is therefore less likely that a whole-brain, unthresholded-map approach will eclipse and supplant the careful and statistically precise attribution of functions to individual regions, but rather complement it.

Altogether, there is a discernible new note in modern brain imaging discourse. Propelled by a theoretical appreciation for the biological basis of whole-brain organization and a vibrant culture of Open Science, a compelling direction has emerged. Shifting emphasis from cloistered blobs to the connective tissue between them encourages us to think about the brain in the more natural continuous space it inhabits, opening new scientific vistas and fulfilling the potential of brain imaging.

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CONFLICTS OF INTEREST

The author declares no competing interests.

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