Mini review

Connectional neuroanatomy: the changing scene

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Abstract

This article gives a brief overview of cortical connectivity, as this has been investigated by anatomic tracer studies over the past 25 years. Open questions are discussed, from the perspective that connectivity studies are in a transition period when both techniques and conceptual frameworks are rapidly changing.

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1. Introduction

On the occasion of the BRES volume 1000, I would like to briefly comment on the current state of cortical connectivity studies. More and more clearly, these seem poised to enter a new era, characterized by new techniques from molecular biology, and a greater attention to cellular and regional diversity [4,5,12,22]. The promise is that this will lead, not to overwhelming detail, but to a more complete synthesis across the molecular, cellular, and functional domains.

It is interesting to look back and take as reference point an early paper I co-authored with D.N. Pandya [25]. In that paper, by using the newly developed HRP tracer, we were able to demonstrate neurons of origin for several sets of cortical connections. By also using complementary injections of anterograde tracers, we demonstrated, for the early visual areas in macaque monkeys, a certain laminar signature of what became known as “feedforward” and “feedback” connections. Subsequent tracer experiments by many groups contributed data on laminae of origin and termination for different connections in different areas and species. These were often referenced to three categories, feedforward, feedback, and lateral, in what was to be an enormously influential model of hierarchical architecture of cortical areas [9,20]. In retrospect, this model was almost too successful, in that it tended to overshadow many other questions related to microcircuitry and network organization [2,24]. Some of these are recapitulated in the next two sections, along with comments on limitations and open questions in the field.

2. Background

Conventional retrograde and anterograde tracers are well suited for establishing the basic “wiring diagram,” or what is connected to what; but connections are not just routes by which “area A projects to area B.” Factors that need to be considered include convergence and divergence, connectional efficacy, and anatomical and functional subtypes. Tracer studies have been less effective in addressing these more detailed factors; but examples of some results are given below.

Retrograde labeling in vivo [19] or combined with intracellular fills in slice [16] demonstrated that neurons giving rise to different projections had distinctive patterns of dendritic and axonal arbors. Whether the patterns are stereotyped within each projection system requires further criteria, and remains actively debated [4,17]. Retrograde tracers combined with other markers were another approach to identifying projection-specific characteristics. In contrast with interneurons, however, relatively few markers have been available that differentiate among pyramidal cells. One study of visual cortical connections combined immunohistochemistry for neurofilament protein, labeled by antibody to SMI32, with retrograde labeling of several groups of efferent neurons [14]. A distinct distribution of neurofila-
ment protein was indicated for some feedforward and feedback projections.

The high-resolution anterograde tracers (PHA-L, biocytin, and the biotinylated dextran amines) produced Golgi-like images, which allowed further assessment and quantification of connections. Here, too, the evidence pointed to further subtypes [23,24]. For example, the distinction between feedforward and feedback connections was reinforced by differences in arbor size (Fig. 1): feedback connections to layer 1 are widely divergent (>1.0 mm), while individual arbors of feedforward axons are smaller (~0.25 mm each). Correlative information on parent neurons would be important.

In a few instances, anterograde labeling was able to show terminations with signature distinct morphology. The clearest example to date may be the two types of corticopulvinar terminations [23]. So far, morphological criteria alone have not been widely successful in this regard. There is some indication, however, that pathway-specific differences may be definable by other means, such as receptor combinations [18].

Intracellular [15] or juxtacellular [33] filling improved on extracellular injections in that both revealed the cell of origin, as well as axon arborization. This allows fuller evaluation of diversity or uniformity among neurons within a defined projection, or even between neighboring neurons. Further advances in this respect might be expected from newer techniques producing Golgi-like images, such as “DiOlistics” [10] or viral tracing techniques [7,31].

Other data from conventional tracers are as follows. Estimates can be made of connectional density, either by the number of retrogradely filled neurons [6], or by the density per unit area of anterogradely labeled terminals. These are a useful estimate of connectional weight (or efficacy), but need to be corroborated by other criteria.

Double retrograde tracer experiments provide evidence for a subpopulation of neurons that branch to several areas. This is confirmed by single axon reconstruction, which also shows the occurrence of collaterals to several areas [23,30]. These “manifold neurons” [29] presumably make up a subpopulation of many if not all projection systems; and their other characteristics and functional roles need to be more fully investigated.

Laminar patterns, especially outside the early visual areas, actually show major inter-area variability. Studies using double anterograde tracers dramatically show that converging connections have differential overlapping or interdigitating patterns in different target areas [26]. Comparison of single tracers across areas supports the same conclusion. To give one example, corticothalamic terminations are commonly considered to arise from neurons in layer 6, but there area specific “disjunctive features” (Fig. 12 in Ref. [11]). Further systematization of these patterns is needed, especially in correlation with other markers, such as area-specific gene expression.

Retrograde tracers have been used to label pyramidal neurons in combination with electron microscopy, in order

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Fig. 1. (A) and, higher magnification from arrow, (B) Feedback terminations in area V1, anterogradely labeled by an injection of fluororuby in area V4. Two adjacent sections have been merged in Adobe Photoshop (at the asterisk in (A). Note divergent arbor, measuring over 0.8 mm. (C) An injection of biotinylated dextran amine in area V1 shows anterogradely labeled terminations in layer 4 of V2 and (acting as a bi-directional tracer for this pathway) retrogradely labeled feedback-projecting neurons. Arrows emphasize the bistratified distribution of the neurons, primarily in layers 2, upper 3, and 6. Modified from Ref. [24]. Scale bars = 200 μm (A, C), 50 μm (B).
to map aspects of their synaptic connectivity. Populations of efferent cells receive rather characteristic numbers of axoaxonic or axo-somatic inhibitory inputs [8]. More complete maps of excitatory as well as inhibitory inputs are still needed.

2.1. Open questions

Despite years of work, the field of cortical connectivity is at an early stage. There are open questions remaining at almost every level. Why do many connections originate from neurons in different layers? There have not even been laminar-specific anterograde injections to clarify the likely differences in terminal axons (Fig. 1).

What are the postsynaptic pools for different connectional systems in different areas? How do these compare in different layers, especially when axons have collaterals in multiple layers? Is the same neuron contacted twice on different portions of its dendritic tree (routinely? sometimes? never?)?

Is the glutaminergic pyramidal cell population really more homogeneous than GABAergic interneurons, or is the relative homogeneity largely apparent, a consequence of the small number of differentiating markers available for pyramidal cells?

How do connections converge and interact at the level of single neurons? This requires two or more connections to be simultaneously labeled, in combination with reliable Golgi-like filling of postsynaptic targets. The first requirement is, in principle, now feasible, especially with dextran amines tagged with different chromogens or fluorochromes. The reliable simultaneous filling of postsynaptic neurons is more difficult and is something still to be attained.

Another problem is more related to experimental design; that is, which connections to emphasize among the large set that converges on any area. The early concentration on a feedback–feedforward dichotomy has arguably been too restrictive (see also Refs. [2,23,24]). Coincidently or not, there have been years of neglect of the thalamocortical connectivity [28] and some over-emphasis on a duality of “driving” vs. “modulatory” inputs [2,23].

3. Conclusions

A recent review states, with a large amount of plausibility, “The field of interneuron research has come of age” [21].

The same cannot in any way be said for cortical connectivity; but after years of some decline, the field does seem on the verge of an exciting renaissance. In part under the impetus of functional imaging studies in humans, there is great interest in cortical areas and regionalization. Important results have already been contributed by neuroanatomical studies of area characteristics and organization [35]. The developments in DT MRI, one of the few noninvasive techniques suitable for investigating connectivity in human subjects, may give further impetus to a badly needed new generation of studies in human neuroanatomy [3].

A distinct cause for optimism is the development of new techniques and markers from molecular biology, and their application to neuroanatomical questions. Electroporation, one of several means for introducing exogenous genes in the intact animal, permits imaging of detailed cell morphology using GFP fluorescence [13]. In addition, patterns of gene expression are being correlated with architectonic areas, with some instances of area-specific expression [32]. In the rodent, latexin, a carboxypeptidase A inhibitor, has been shown to be expressed in the infragranular layers. More particularly, expression is selective for corticocortical rather than corticosubcortical neurons [1].

Additional advances are likely to be rapid with the sponsorship of large-scale screens to create atlases of gene expression at the cellular level. These have the self-proclaimed mission of providing vectors and transgenic mouse lines to offer experimental access to CNS regions, cell classes, and pathways [12].

One technique with immense impact would be an intracellular transneuronal retrograde or anterograde marker. This would dramatically demonstrate the actual composition of neuronal groups, in terms of numbers, subtypes, and laminar distribution of interconnected populations, and directly advance information on neuronal networks. This may still be in the future, but genetic approaches are already available to visualize multisynaptic neural pathways in rodents [34].

Finally, there have been important changes in the research climate as regards connectional neuroanatomy. One is a recent emphasis on the role of intrinsic factors in cortical areas, layers, and modules [22,27]. The emerging evidence for molecular gradients and signaling centers provides new interpretations for some of the connectivity questions discussed here. A second change is increased attention to species diversity and to diversity of cell types and areas. This opens an immense challenge and opportunity for new integration under the rubric of molecular neuroanatomy.

References