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PERSPECTIVE

From gene networks to brain networks

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The brain's structural organization is so complex that 2,500 years of analysis leaves pervasive uncertainty about (i) the identity of its basic parts (regions with their neuronal cell types and pathways interconnecting them), (ii) nomenclature, (iii) systematic classification of the parts with respect to topographic relationships and functional systems and (iv) the reliability of the connectional data itself. Here we present a prototype knowledge management system (http://brancusi.usc.edu/bkms/) for analyzing the architecture of brain networks in a systematic, interactive and extendable way. It supports alternative interpretations and models, is based on fully referenced and annotated data and can interact with genomic and functional knowledge management systems through web services protocols.

This year marks the 50th anniversary of Watson and Crick's famous three-dimensional structural model of DNA[1] (**Fig. 1**). The elegantly simple linear genetic code it predicts sparked the molecular biology revolution that ultimately allowed sequencing the human genome. One fundamental insight from the genome map is the boundary condition that 30,000 or so discrete functional units (genes) form our chromosome set. Over the last quarter century, we have learned much about how individual gene expression is regulated combinatorially by transcription factors. Now attention is shifting to understand the more difficult problems of how genes interact cooperatively in hierarchical networks[2,3].

In contrast, it has been just over 450 years since Vesalius presented his global structural model of the human body. (**Fig. 1**). For centuries, there has been consensus about the fundamental arrangement (and names) of the body's parts (muscles, bones, blood vessels) and functional systems. And since the cell theory's introduction in the 19th century, basic tissue histology (cell type identification, morphology and topographic distribution) also has been established. Molecular biology is the next frontier.

Considering the historical importance of reliable structural models for understanding biological function, it may be surprising that there is no consensus about the brain's basic parts and cell types, or about how they are interconnected as a functioning organ—the mind[4]. Whereas the cell biology of the brain's individual units (neurons) and their functional links (synapses) are thoroughly understood, the basic wiring diagram of the brain as a three-dimensional biological computer—the actual arrangement of its cellular building blocks—remains obscure. There is no physically accurate model of fundamental brain network organization analogous to the Watson-Crick DNA model. This is not for lack of effort; increasingly sophisticated methods for analyzing brain structure have been applied since classical antiquity[5,6]. It reflects the extreme complexity of brain tissue architecture. A truly vast and rich—yet confusing and contradictory—literature has accumulated over the centuries, but fundamental organizing principles of brain circuitry remain vaguely understood.

Here we outline major problems associated with establishing the fundamental structural architecture of brain circuitry and introduce a prototype knowledge management system (KMS) for organizing legacy data ('the literature') and analyzing neural network organization based on it. Revolutionary new insights into how the brain generates behavior and mind will inevitably emerge from a global understanding of how the nervous system actually functions as a system, like the global understanding provided by Harvey for the circulatory system, or Pavlov for the digestive system.

Reliability of connectional data

Much legacy information about neural connections is inaccurate or is misleading because it is vastly oversimplified—once pathways beyond the relatively straightforward craniospinal nerve nuclei are considered. This conclusion is illustrated by evaluating limbic system connections (**Table 1**), which control fundamental motivated and emotional behaviors essential for survival of the individual and the species[7]. A connection is defined in the classical sense of an axonal pathway (projection) from one cell group (region) to another cell group, as from retina to tectum.

About 55 hypothalamic cell group connections were considered valid in 1940, although subsequent experimental analysis indicates that over 80% of these were false-positive technique artifacts. By 1970, Nauta's more powerful method[8] identified about 75 hypothalamic connections, although today's criteria indicate that half were false-positive artifacts (due mostly to interrupted fibers of passage). Axonal transport pathway tracing methods revolutionized experimental neuroanatomy[9] in the 1970s, and by 1987 some 450 hypothalamic projections were described, of which perhaps 90% are reliable. Even more powerful methods have since been introduced (**Table 1**), and today about 3,000 hypothalamic projections are described. A similar trend applies to hippocampal, amygdalar and septal projections, so now more than 4,000 projections are known for the four regions combined.

Although this qualitative reliability analysis involves some degree of circular reasoning (reliability measures based on current methodology), it is nevertheless clear that a huge database of relatively reliable information about brain connections now exists, taking just one forebrain component as an example. It is equally clear that legacy data about connections must be evaluated critically because brain cir-

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Figure 1 Portraits of discovery. Left, 1543 portrait of Vesalius[39] demonstrating human body structure, with special reference to the muscles and tendons of the hand, a uniquely human specialization. Right, 1953 photo of Watson and Crick[40] admiring the large physical model they built to predict the structure of DNA (Copyright A. Barrington Brown/Photo Researchers, Inc.).

cuitry has been examined with a succession of increasingly reliable methods. Furthermore, the vast amounts of reliable connectional data accumulated recently have not been synthesized in a form immediately useful to the neuroscience community. A popular neuroscience textbook[10] mentions only about 335 connections for the whole CNS, whereas Brodal's classic (1948) neuroanatomy textbook[11], by comparison, lists a total of 195 known CNS connections (in both, about 15% involve the limbic system).

The success of bioinformatics technology in the genomics arena indicates that the time is ripe to develop fresh approaches for analyzing and understanding the structural and functional organization of brain circuitry based on the huge new database of relatively reliable information—and on vast new data about brain gene expression patterns expected soon.

How many brain connections are there?

The basic strategy for understanding the brain as a system was articulated by Nicolaus Steno[12] in 1669. "There are two ways only of coming to know a machine: one is that the master who made it should show us its artifice; the other is to dismantle it and examine its most minute parts separately and as a combined unit." How many basic cell groups ('parts') of the brain are there, and how many connections does each have? What is the basic structure of brain circuitry at this macroconnection level of analysis? What are the boundary conditions for systems neuroscience?

Over 150 years of detailed microscopic analysis has parcelled the mammalian brain into 500–1,000 cell groups (also called regions, centers, nuclei, or nodes in the circuitry), each with a unique set of axonal projections to other cell groups. Familiar examples include cranial nerve nuclei and cortical areas (**Fig. 2**). They are like a continent's countries, providing a convenient and conventional—though sometimes arbitrary—way to describe position and topographic relations. However, brain cell groups typically contain multiple neuronal cell types. Unique combinations and distributions of neuronal cell types allow brain region identification in the first place.

Three basic criteria traditionally define neuronal cell types: shape (morphology), spatial distribution and connections. Since Golgi's first broad division of neurons into those with a short axon (local circuit) or a long axon (projection) in 1873 (ref. 13), axon distribution has been the single most important feature in determining cell type. The reason is simple—the axon is the neuron's output device and in this sense determines its function, or 'what it does.' Motor neurons innervate muscle cells, and retinal ganglion cells transmit photic information to the brain (**Fig. 3**).

How many neuronal cell types are there? Only rough estimates are possible, but in the few places like cerebellum and retina (**Fig. 3**) where consensus exists, there are on the order of five. If five is the average in each of the roughly 500-1,000 regions, then the brain has 2,500–5,000 neuronal cell types. This is only a crude approximation, though, because of subtypes. In the retina, there are many subtypes of photoreceptors and bipolar, ganglion, amacrine and horizontal cells. Each subtype accounts for part of a cell type's overall projection pattern, and conversely the projections of all subtypes together account for the overall pattern.

Next, how many different cell types does each neuronal cell type innervate? This is a direct measure of overall complexity in brain macrocircuitry—the basic organization of connections between cell-type populations (in contrast to microcircuitry, which is the absolute number, distribution and strength of synapses associated with individual neurons). The single axon of each neuron generally branches (collateralizes) extensively to innervate multiple cell types, producing major information flow divergence in neural networks. Again, only crude approximations are possible now, but experience over the last decade suggests that the average cell type innervates 10–20 distinct cell types (ranging between two and hundreds). Assuming 2,500–5,000 cell types, each innervating 10–20 cell types, brain circuitry has about 25,000–100,000 macroconnections, which is roughly comparable to the number of mammalian genes!

100,000 may seem like a large number of macroconnections, but it is a far cry from the 25,000,000 possible macroconnections among 5,000 cell types. Common assertions that "everything in the brain is connected to everything else" are obviously untrue—actual brain connectivity is very sparse compared to potential brain connectivity. The boundary condition of 100,000 brain macroconnections also makes dealing with huge numbers of neurons (about 10^{11} in humans and 10^{8} in rats)[14], and even more overwhelming numbers of synapses (about 10^{14} in humans and 10^{11} in rats)[14], less intimidating and more amenable to experimental and theoretical analysis.

Taxonomy of brain parts, connections and systems

Interest in systematically arranging brain parts began with Aristotle and at least five different schemes for hierarchical arrangements have

Table 1 Limbic system connections (cell group to cell group)

Era	Major techniques	Total	Valid today	% Valid
Hypothalamus				
1940	Normal silver/myelin degeneration[31,32]	55	10	18%
1969	Axon degeneration (Nauta method)[33,34]	75	38	51%
1987	Axonal transport (autoradiography/HRP)[35]	450	400	90%
2002	PHAL/dyes/histochemistry[a]	3,000	2,850	95%
Hippocampus-amygdala-septum				
1969	Axon degeneration[36–38]	57	40	70%
2002	PHAL/dyes/histochemistry[a]	2,000	1,900	95%
Hypothalamus + Hippocampus-amygdala-septum				
1969	Axon degeneration	116[b]	70	60%
2002	PHAL/dyes/histochemistry	4,000[b]	3,800	95%

[a]Estimate based on informal survey of the literature. [b]Some connections of the hypothalamus are with the hippocampus-amygdala-septum.

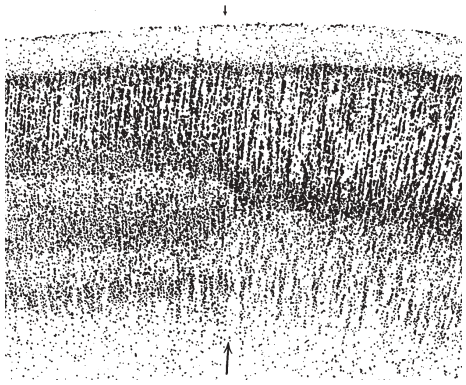


Figure 2 Borders of brain regions are defined by recognizable changes in cell type distribution patterns. This principle is illustrated here for the vertical border (arrows) between cerebral cortical areas 17 (primary visual) and 18 (secondary visual) in humans. From Brodmann[41].

emerged: dual brain, segmental, developmental, evolutionary and genomic. Convincing evidence favoring one or another model is lacking[15]. This approach has obvious theoretical importance for understanding fundamental brain organization, especially its fundamental wiring diagram. Ultimately the latter will emerge from a systematic taxonomy of the 2,500–5,000 neuronal cell types that form the brain's macrocircuitry.

Ethics precludes analysis of human brain circuitry using current experimental methodology. What we 'know' about it is mostly inferred from animal research, so comparative neuroanatomy becomes indispensable. Various criteria for establishing brain part homologies have been discussed, and algorithms for evaluating degrees of similarity have been developed[16].

The nomenclature nightmare

Calculations mooted above were crude because very limited data exist for neuronal cell types throughout much of the brain, and this information gap is reflected in today's complex, unsystematic, poorly defined brain parts nomenclature. Nevertheless, functional localization is the most notable historical trend in brain research[17,18], so the definition of terms used to describe brain structure/location is fundamentally important—arguably even a mandatory starting point for systematic analysis.

For circuit description, brain parts include differentiations or regionalizations of gray matter (cell groups) and white matter (pathways between cell groups). Most names for them are vaguely defined for two reasons: lack of decisive structural data and pervasive disinterest in nomenclature—largely because journals do not require definitions or reference to historical precedence. As a result, the goal of a standard neuroanatomy nomenclature is currently undesirable because it would be arbitrary and actually impede determining true brain structure[19].

Figure 3 Neuronal cell types defined by connections. Cajal's[42] circuit diagram illustrates three basic retinal cell types, defined by spatial distribution, morphology or shape, and especially projections. Photoreceptors (a, A, b, B) detect light and send a short, local circuit axon to innervate bipolar cells (c, C, d), which in turn send a short, local axon to innervate ganglion cells (D, e, E). The latter's long axon courses through the optic nerve and tract to the midbrain tectum (g, G, H, S). Arrows indicate postulated information flow direction. Cajal noted that connections are more important than position for determining neuronal cell types, using the example of displaced ganglion cells, whose cell bodies lie in the bipolar cell body layer. The two other retinal neuronal cell types, amacrine and horizontal cells, are not shown. They are local circuit neurons that spread information tangentially, not radially (like bipolar cells).

Instead, precise definitions of terms and relationships between them—an ontology of neuroanatomical terms—are needed.

Only a small fraction of recognized brain parts have universally accepted boundaries, so different authors often draw borders around the same part differently (changing the definition of all bordering structures in the process). In fact, there are alternative parcelling schemes for most larger brain areas (**Fig. 4**). In reality, borders of most brain parts are difficult to define (they are 'fuzzy').

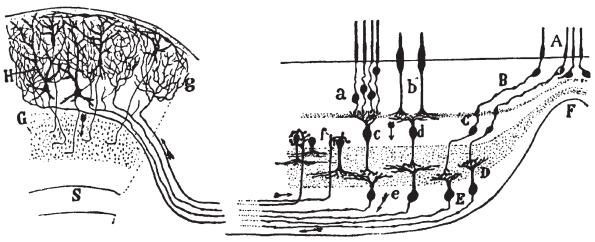
From the nomenclature standpoint, there are thus alternative definitions for the same term (when boundaries are different), and the same term has even been used for two different structures. Furthermore, there are synonyms, alternative spellings, translations in multiple languages and partial correspondences. The total number of brain-part terms is unknown, but probably on the order of 100,000. By 1888, there were already over 10,000 (ref. 20), and during the period 1885–1894 alone, some 1,300 CNS anatomy papers were published[21].

It is essential to know the meaning of terms (key words) when referring to specific brain locations (**Fig. 4**, asterisk) in database searches. When constructing network models from multiple literature sources, one must convert (index) term definitions in individual articles to an internally consistent parcelling scheme or nomenclature (*e.g.*, either **a** or **b** in **Fig. 4**).

A brain circuitry KMS

Considering these general principles, we designed and implemented a Brain Architecture Knowledge Management System (BAMS) for storing and manipulating structural data about the nervous system in text- and table-based formats (see **Supplementary Fig. 1** online). Ultimately, it is important for a KMS to display and manipulate spatial information, such as neuroanatomical data mapped in two and three dimensions[22,23]. However, because neuroanatomical data and literature are so complex, we chose to formulate basic strategies with textual abstractions before extending into the technically and conceptually more difficult problems of web-based spatial KMSs. Earlier approaches to computational analyses of neural circuits are reviewed elsewhere[24–26].

BAMS has four basic modules: brain part *nomenclatures* (regions/cell groups and pathways), *relations* between parts from different nomenclatures, neuronal *cell types* and *connections* between regions and cell types. All information in BAMS is annotated with references to source and collator. The object-relationship structure of BAMS centers on the object 'brain part,' as defined in various nomenclatures and uniquely identified by name, species, atlas (nomenclature scheme) and atlas version. Any brain part record in BAMS may be captured in a hierarchy defined by the authors or constructed in BAMS from other information.



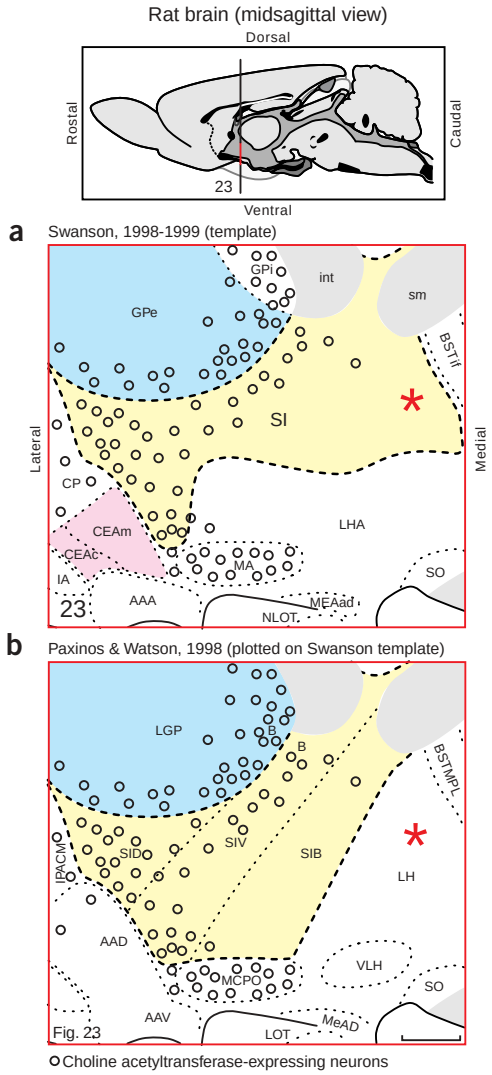


Figure 4 Alternative brain region parcelling schemes. (**a**) Regionalization (Swanson[19]) of a part of the basal forebrain centered around the substantia innominata (SI) in a transverse rat brain histological section (rostrocaudal position shown in midsagittal view at top of figure). (**b**) Paxinos and Watson's[43] alternative regionalization scheme transferred onto the Swanson template for direct comparison. Note major differences between the two schemes: borders of the SI and other regions are often different, some regions are present in one map but not the other, different names are sometimes used for the same structure, and different abbreviations for the same structure are common. Different regionalization schemes lead to major difficulties when interpreting the literature. For example, data localized to the position of the large red asterisk might be described as lying medially in the SI (Swanson atlas), or as lying dorsally in the lateral hypothalamic area (LH; Paxinos and Watson atlas). The situation is much worse when gene expression patterns are mapped. The approximate distribution of cholinergic neurons (expressing the synthetic enzyme choline acetyltransferase) is plotted on the maps, based on literature reports[44,45]. Note how greatly pattern descriptions differ, depending on which atlas (nomenclature scheme) is used. Other abbreviations: AAA, anterior amygdalar area; AAD/AAV, dorsal/ventral parts of anterior amygdaloid area; B, basal nucleus (Meynert); BSTif, bed nuclei stria terminalis, interfascicular nucleus; BSTMPL, bed nucleus stria terminalis, medial division, posterolateral part; CEAc,m, central amygdalar nucleus, capsular, medial parts; CP, caudoputamen; GPe,i, globus pallidus, external and internal parts; IA, intercalated area amygdala; int, internal capsule; IPACM, interstitial nucleus posterior limb anterior commissure, medial part; LGP, lateral globus pallidus; LHA, lateral hypothalamic area; LOT, nucleus lateral olfactory tract; MA, MCPO, magnocellular preoptic nucleus; MEAad, MeAD, medial amygdalar nucleus, anterodorsal part; NLOT, nucleus lateral olfactory tract; SIB, SID, SIV, substantia innominata, basal, dorsal, ventral parts; sm, stria medullaris; SO, supraoptic nucleus; VLH, ventrolateral hypothalamic nucleus. Scale bar, 0.5 mm.

BAMS inference engines relate stored information about parts nomenclatures, cell types and connections. If a brain nomenclature is hierarchically organized as a superstructure tree that includes the searched region, then adjacent regions as well as the searched region's position in the entire hierarchical tree is viewed dynamically. To relate different parcelling schemes for the same brain areas (**Fig. 4**), we adapted a qualitative inference algorithm[27] using the set of all possible topological relations between two brain regions.

The cell type module is constructed in a many-to-many (m:n) relation with 'brain parts'. A neuronal cell type may be distributed in multiple brain regions, and a brain region typically has multiple cell types (**Fig. 4**). A cell-type profile of any region can be reconstructed if the required nomenclature is hierarchically organized. Because region *x* is captured in a superstructure tree, its cell type set is represented in any other regions (higher in the tree) that include region *x*. Thus, hierarchical trees reconstructed in BAMS can be extended to cell types and subtypes. BAMS allows insertion of qualitative and numerical data about cell types collated from different references. Currently, gene expression profile and functional (electrophysiological, behavioral) information about cell types can be obtained by linking to other databases (*e.g.*, http://senselab.med.yale.edu/senselab/).

A neural connection in BAMS is a direct relation between two brain regions (or cell types) in an internally consistent nomenclature scheme. Customized connection matrices from all regions in such a scheme are constructed by the inference engine. The connection module accepts qualitative and quantitative connection data (40+ parameters) and supports statistical analyses related to individual atlas levels.

The 'networks' function in the 'evaluate' menu of the BAMS web interface is a key feature of the connection module. It constructs potential neural circuits based on connection data in the system (**Fig. 5**). How, for example, might information reach the primary visual cortex from the retina? Current rat data in the system indicates 2 possible routes involving one relay, 22 involving two relays and 302 involving three relays. Alternatively, the retina projects directly to the lateral

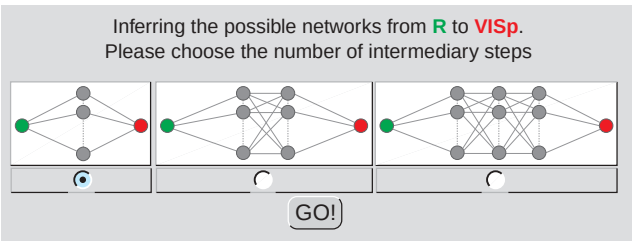


Figure 5 Inferring possible networks between two brain regions or cell types with one, two or three intermediate connections. Brain regions or cell types are indicated by green (the starting point) and red (the endpoint) dots, whereas intermediate connections are indicated by gray dots. R, retina; VISp, primary visual area.

hypothalamic area (arousal, feeding and other functions), and there are 6 possible routes involving one relay, 89 involving two relays and 1,473 involving three relays. Inference of potential neural circuits from connectivity data in BAMS is the first step toward constructing expert systems for investigating the functional organization of brain networks. Next we plan to combine inferences performed in BAMS about possible networks with electrophysiological, behavioral and genomic data provided by other databases, using various web services protocols.

The BAMS web interface allows searching by region name, species and references (author, source, year). Author searches return lists of all publications by that author, and information associated with each retrieved reference may be viewed. For example, the system can reconstruct a connection matrix for the brain region set abstracted from the associated reference.

In addition to the public part of BAMS just described, there is a personal part where unpublished neuroanatomical data can be entered and manipulated by registered users.

BAMS was created in MySql with the scripting language PHP. Initially it contains 6,000 brain part names from ten human, monkey, cat, rat and mouse nomenclatures ('atlases'); 5,000 connection reports from rat visual and navigational systems[28,29]; and 4,500 limbic system connection reports from L.W.S. and colleagues since 1974.

Perspective

Widespread interest in biology is returning at last to functional systems analysis, whether gene networks or neural circuitry, and general strategies for reverse engineering any complex biological system may emerge[30]. One ultimate goal of systems neuroscience is to determine the formal relationship between gene networks and brain networks in controlling behavior. The vast complexity involved requires mathematical modeling supported by comprehensive knowledge management systems.

Useful links

Brain atlases: http://www.loni.ucla.edu
Cell types: http://senselab.med.yale.edu/senselab/NeuronDB/default.asp
Nomenclatures: http://braininfo.rprc.washington.edu/mainmenu.html

Note: Supplementary information is available on the Nature Neuroscience website.

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