Essay

Form and function in systems neuroscience

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'Form follows function' is an architectural philosophy attributed to the great American architect Louis Sullivan [1], and later taken up by the Bauhaus movement. It stresses that the form of a building should reflect its function. Neuroscientists have used the connverse of this dictum to learn the functions of neural circuits, believing that if we study neural architecture, it will lead us to an understanding of how neural systems function. New tools for studying the structure of neural circuits are being developed, so it is important to discuss what the old techniques have taught us about how to derive function from the form of a neural circuit.

The past 30 years have produced breathtaking successes at the two extremes of our knowledge about brain function. At the cellular level, molecular genetics and biophysics have provided explanations of neuronal and synaptic function that are complete and satisfying, from the molecular structure of ion channels to the mechanisms of synaptic release and plasticity. At the other extreme, brain imaging techniques — primarily functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) — have made possible investigations of our own cognitive functions so that we can begin to catch glimpses of such processes as perception, making judgments, paying attention, and thinking. This raises the hope that we will one day understand the mind as completely as we now understand the synapse. To have such an unbroken knowledge from molecules to the mind, however, we will need to understand the large gap in between: how do networks of neurons operate to produce behavior? This intermediate realm, usually called systems neuroscience [2], has a long and hallowed tradition with many current practitioners, but its progress has been slow, in part perhaps because the work is so laborious given the available techniques. Systems-level explanations tend to be complicated, incomplete and often specific to the brain region or the organism being studied.

If the excitement buzzing around a recent meeting (Neuronal Circuits: From Structure to Function Meeting, Cold Spring Harbor Laboratory, New York, March 9–12, 2006) is any indication, however, all this is changing. This meeting centered around the question: how much can be learned about the function of a neuronal circuit from its anatomical architecture? The discussions focused mainly on new techniques for determining neuronal connectivity using molecular genetics and imaging. Not surprisingly, with this emphasis, the animals primarily represented were the ones whose genetics are best developed: the fruit fly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, the zebra fish *Danio rerio* and the mouse *Mus musculus*, though these techniques can also be applied in animals that are not genetic models, such as primates, through the use of viral vectors [3]. But there is a big question surrounding this work: does the connectivity of a neuronal circuit adequately explain how it functions?

A humbling example shows some of the difficulties that need to be faced in deriving function from connectivity. Consider the simple two-neuron network in Figure 1A: neurons a and b are reciprocally inhibitory and receive non-patterned excitatory input, x and y. This network is essentially the 'half-center' model proposed by Graham Brown [4] nearly a century ago to explain rhythmic

alternation of flexor and extensor motor neuron pools during walking. This circuit architecture has been shown to be used in a number of central pattern generator circuits to generate rhythmic movements (Figure 1B) [5]. So, if we find such a network using anatomical techniques, do we know that it produces an oscillatory pattern of activity? The answer, unfortunately, is 'No'. The rhythmic alternation between flexion and extension requires that neurons a and b have either a synaptic mechanism that releases them from inhibition or a membrane property that allows them to escape from inhibition [6]. If the neurons in the network lack the appropriate values for properties such as postinhibitory rebound, spike frequency adaptation or synaptic depression, then the same network architecture could lead to a variety of non-oscillatory outputs. For instance, the circuit could act as a bistable persistant memory device such that, if neuron a is active, it will continuously inhibit neuron b until an external input activates neuron b and causes the system to flip states (Figure 1C) [7]. In fact, an architecture of mutual inhibition has been proposed as a way for the nervous system to make and sustain choices among incompatible behaviors [8]. And with different cellular and synaptic properties, the same circuit architecture can produce synchronized firing (Figure 1D) [9]. Thus, even this very simple circuit architecture cannot be used to infer function; cellular and synaptic properties are also important for determining the dynamics of circuit function.

But maybe these are not real problems; after all, in many cases where reciprocal inhibition has been found — in worms, molluscs, lobsters, insects, frogs, fish, and mammals — the

circuits are, in fact, oscillatory [10]. So, in those cases, if we had found reciprocal inhibition and had guessed that these neurons are involved in central pattern generation, we would have been correct. Then, what about more complicated circuitry? How much can we learn about the function of a neural circuit from its wiring diagram? Surprisingly little, unfortunately.

Consider, for instance, the elegantly simple central nervous system of *C. elegans*; with only 302 neurons in its entire nervous system, it is more complicated than the half-center architecture, but considerably less complicated than a slice of cortex. Every connection in the *C. elegans* nervous sytem has been obtained from serial electron microscopy 20–30 years ago [11,12]. Yet, despite this exquisitely detailed knowledge, not a single behavior has been successfully inferred from looking at the connectivity pattern alone. Killing individual neurons using lasers or molecular genetics has helped the effort greatly [13], but we do not know the activity patterns of each of the neurons because their extremely small size has made electrophysiological recordings extremely difficult. So, if we could determine the pattern of activity, would we understand how the circuits function? Not necessarily, given the experience of researchers on the lobster stomatogastric ganglion (STG).

The activity patterns of the neurons in the lobster STG can be recorded and the connectivity in this ganglion of 30 neurons has been ascertained using electrophysiology [14]. Yet the STG does not have just one behavioral output; instead, it is capable of producing a wide range of motor patterns. There are many neuromodulatory inputs to the ganglion, more than two dozen at last count, which modify connection strengths and ionic currents in STG neurons. By altering cellular and synaptic properties, these neuromodulators induce the system to produce a variety of very different rhythmic motor
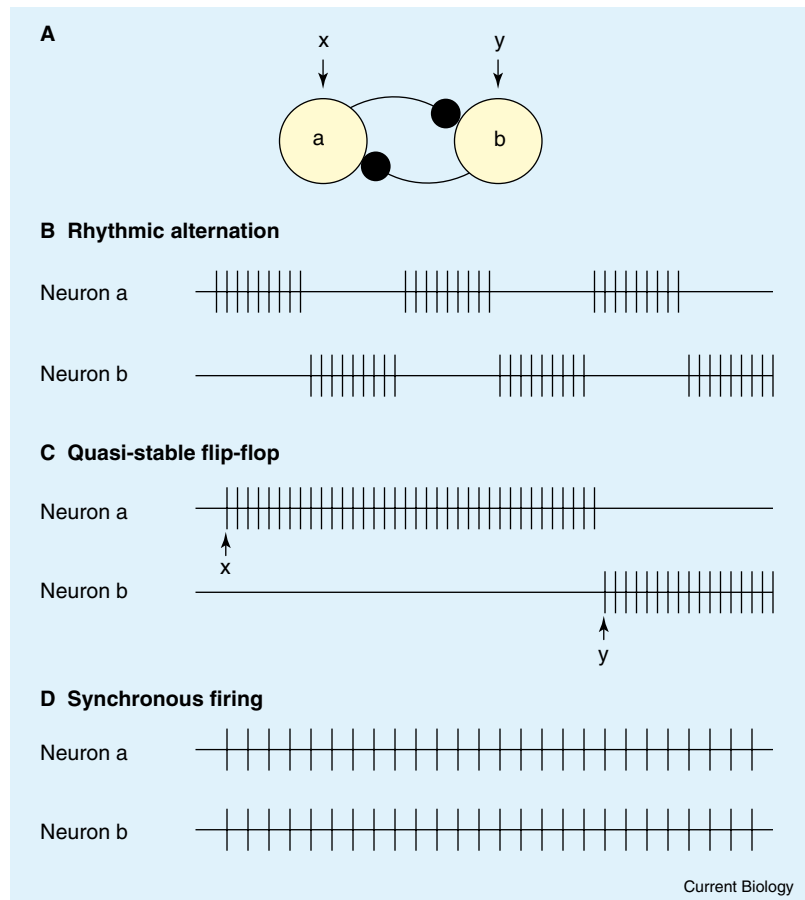

Figure 1. Form and function of reciprocal inhibition.

(A) Neurons a and b are reciprocally inhibitory and receive excitatory input from x and y respectively. Depending upon the properties of the neurons and their inputs, this simple circuit configuration can produce a number of different activity patterns: rhythmic alternation (B), quasi-stable flip-flop (C) or synchronous firing (D).

patterns [15], none of which is an obvious outcome of the pattern of the connections among neurons. Thus, the wiring diagram does not predict the behavioral output; a single, anatomically defined neural circuit can exist in different states and thus produce very different outputs depending upon the type of neuromodulatory input that it receives.

So, what if we could work out the neural circuitry, record from all the neurons, and know the different states that a circuit can assume? Would we then understand the function of the neural circuit? While it is tempting to assign a function to a structure, as in 'the function of the hippocampus is to store long term memory', this approach may run into trouble when you consider the role of the circuitry in other behaviors.

From a systems perspective, the 'function' of a neural circuit is to transform input or generate a pattern of activity. A neural circuit might transform its inputs in a special way so that it can be used to perform different functions. The cerebellar circuitry, for example, does the same transformation on vestibular input as it does on cortical input [16]. So, if you tried to understand the cerebellum in terms of specific functions, your answer would depend upon which end of the proverbial elephant you were examining.

Similarly, assigning a function to a neural circuit or its components can lead to confusion when comparing different animal species. Species-specific behaviors appear to derive from small changes in the neural circuits

controlling those behaviors. For example, closely related voles can exhibit vastly different social behaviors, including pair-bonding. Some of the difference in the behavior can be attributed to the localization of vasopressin V1A receptors in the nucleus accumbens in the monogamous species [17]. Does this mean that the nucleus accumbens is in the 'pair-bonding circuit' in one species but not in the other? The nucleus accumbens is involved in a variety of other types of behaviors and pathologies including drug abuse [18]. Thus, it is important to consider what the circuitry in this brain area does with its inputs in order to understand how it functions. Even at the single neuron level, the functions of homologous identified neurons can differ in species with different behaviors [19].

With these caveats in mind, it is still an important task to understand how neural circuits produce behavior. Although knowing the wiring diagram is not sufficient to predict the output of a neural circuit, it is unquestionably an important prerequisite for developing an understanding about how that neural circuit functions. Thus, the new molecular genetic techniques for circuit exploration will certainly lead to a tremendous advance in our basic knowledge of neural circuits. In addition, those genetic techniques may also allow the activity of large numbers of neurons to be recorded and modified.

One of the biggest problems in studying the functioning of neuronal circuits is that electrophysiological investigations are generally limited to recording from one or two neurons at a time. It is difficult to understand the dynamics of a neuronal circuit if the activity of the components cannot be monitored simultaneously. New tools are being developed to allow particular neurons or classes of neurons to be monitored optically. Voltage-dependent and Ca^{2+}-dependent dyes have been around for awhile now, but their limitation was that they had to be loaded into neurons individually or bulk-loaded into all neurons in a region. The former method made it impractical to study circuits of neurons, whereas the latter method did not easily allow individual neurons to be identified. The latest generation of genetically encoded Ca^{2+} sensors can be loaded using retroviruses or expressed in particular classes of neurons using genetic manipulations [20–22]. This approach may also allow imaging of activity in genetic model animals with small neurons such as *C. elegans* and *Drosophila*. Thus, it will be possible to monitor the activity of many neurons at a time even in neural circuits that have not been amenable to electrophysiology. In addition, two-photon imaging has made possible the imaging of many neurons simultaneously in the intact mammalian brain at depths not possible previously [23].

Recording the dynamics of activity does not, however, show which neurons are necessary for the functioning of the neural circuit. Ablating parts of the nervous system to test the behavioral function of a particular structure has a cherished history in experimental psychology, pharmacology, and neuroscience. Modern optical and molecular techniques are adding great spatial and temporal refinement to this approach. For instance, small numbers of targeted neurons can be ablated by specific expression of a gene that either kills the cells or blocks their activity. Ideally, activity in the targeted neurons can be turned off transiently, for example by using either ligands or temperature-sensitive mutants [21]. These techniques recently have been applied to particular neurons involved in locomotory movements in the mouse spinal cord [24]. Another exciting possibility is to use light-activated, membrane-bound channels that open inhibitory channels, thereby silencing the illuminated neurons [25].

Recordings and ablations show a correlation with the behavior, but to study the dynamical functioning of a neural circuit, one would like to selectively activate components, not just inactivate them. In small circuits, this can be done by injecting depolarizing current into individual neurons with a microelectrode or patch electrode. However, single neuron stimulation is not always feasible because of the size or accessibility of the neurons. Furthermore, in large circuits, stimulation of a single neuron is not likely to contribute to the overall processing of the circuit. The same kinds of techniques used for ablation or expression of genetically encoded sensors can be used to specifically activate certain classes of neurons [3,26]. These techniques are a good complement to the classic stimulation techniques using electrical currents or focal drug application. Caged neurotransmitters are another means of probing neural circuits; glutamate can be uncaged in a spatially and temporally confined fashion, providing synaptic activation of particular components of the neural circuit [27].

In general, after all the correlation, necessity and sufficiency tests have been performed, one more step is required: to propose an explanation, a model, for how the system works. Increasingly, because the systems and the interactions are so complex, this means a computational simulation [28]. Having a simulation that reproduces the data is a minimal requirement; the next step is to test the model on novel inputs. Furthermore, it is important that the model suggests new physiological experiments.

Minimally, the advances in technology that we have briefly reviewed will greatly speed up the rate of progress in systems neuroscience: the new techniques enable us to track down the neuronal participants and to show how they interact at a much greater rate. In addition, we now can do things that we never were able to before such as monitor or perturb the activity

of many neurons of a particular class at a behaviorally relevant time frame [29,30]. Some day, these kinds of techniques might be able to test directly whether some of these circuit features underlie the capabilities that we consider peculiarly human. Cognitive capabilities — making judgments, formulating plans, making decisions — may use the same circuit features that are used for sensory processing, working on the abstracted information in the same way that sensory areas act upon primary sensory input. However, the important lessons of how to find function from form in neural circuits must be remembered or these new techniques won't give anything more than pretty pictures.

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Evolvability

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Increasing numbers of biologists are invoking 'evolvability' to explain the general significance of genomic and developmental phenomena affecting genetic variation. What exactly is evolvability, and how important is it likely to be for our understanding of evolution? Definitions of evolvability are almost as numerous as the papers and books that have been written on the subject. All definitions agree that evolvability has to do with the capacity of populations to evolve — no surprise there. In actual use, however, evolvability can be a rather slippery concept with a variety of meanings and implications. The goals of this primer are to try to pin down some of the meanings of evolvability and to explain why evolvability is a controversial subject.

Evolvability and heritability
First, it is important to point out a basic way in which populations can vary in their capacities to evolve that is not controversial. A population with a large amount of heritable variation for fitness can certainly be considered more evolvable than one with very little heritable variation for fitness. Similarly, a population with a larger amount of heritable variation for a phenotypic character will respond more quickly to natural or artificial selection on that character than one with a smaller amount of such variation. Evolvability of this kind is central to our established quantitative genetic understanding of phenotypic evolution.

Evolvability and the generation of new variation
Most recent ideas about evolvability, however, focus on the capacity of populations to