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Networks of Neurons, Networks of Genes

Minireview

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The "genetic networks," composed of proteins that regulate gene expression and the promotor elements to which they bind, show similarities to other kinds of networks, especially networks of neurons. Both neuronal and genetic networks have been described as information-processing devices and compared to electrical circuits and computers. Neurons communicate across synapses, which may be either excitatory or inhibitory; genes can communicate through the proteins they express, which may ultimately cause either increased or decreased expression of another gene. Positive and negative inputs are integrated in each neuron to determine its electrical activity, and at the promoter of each gene to determine the level of gene transcription. The same set of inputs can have different effects depending on the recipient neuron, due to its constellation of ion channels, or gene, due to its enhancers and promoter. In fact, an individual input sometimes increases the activation of one neuron or gene and reduces that of another. Neuronal networks and genetic networks can include massive convergence and divergence: a single neuron may receive inputs from a large number of connected neurons; a single gene's promoter may form a complex with multiple bound proteins. In turn, a single neuron may produce effects on many other neurons; a single gene that codes for a DNA-binding protein may modify the expression of multiple genes.

Like most new ideas, the analogy between neuronal and genetic networks is not new at all. In 1930, Karl Lashley wrote: "Many lines of evidence show a close parallelism between the facts of morphogenesis and those of the organization of the nervous system. In both we have given as the fundamental fact an organization which is relatively independent of the particular units of structure and dependent upon the relationships among the parts. In both there is a capacity for spontaneous readjustment after injury, so that the main lines of organization are restored."

Why list parallels between nervous system operation and gene regulation? Neuronal and genetic networks not only operate similarly; they also have been studied similarly. Researchers in each field may be able to gain insights from the obstacles and successes of the other field. Organizational principles that apply to neuronal and genetic networks may turn out to be general properties of a still wider variety of networks. In this minireview, I will sketch out a few generalizations from research on neuronal networks and note analogous aspects of genetic networks.

An initial goal in studying a neuronal or genetic network is to identify the elements that play important roles in a particular set of functions. One would like to know where each element is, when it is active with respect to the functions studied, and which other elements it interacts with. Elements are often localized by neuroanatomical tract tracing and single neuron dye filling in the study of neuronal networks and by genome mapping in classical and molecular genetics. The timing and level of activity of individual elements can often be studied by monitoring electrical activity in neuronal networks and the expression patterns of proteins from genes in genetic networks. In favorable cases, one can monitor the electrical activity of each neuron during approximately normal functioning of a neuronal network, in vivo. An analogous approach in molecular genetics would measure changes in the level of expression of a gene with respect to ongoing functions in vivo. It is sometimes possible to activate an individual neuron artificially (by electrical stimulation) and to observe the effect on the neuronal network; a comparable genetic approach involves inducible and time-limited gene activation in vivo, such as by insertion of the protein-coding region of interest under the control of an inducible promotor. An interaction between two neurons can be identified by filling each neuron with a dye; the sign and strength of an interaction can be quantified by monitoring the electrical activity of each, and especially by electrically stimulating one neuron and observing the response in the other. Interactions between two molecules in a genetic network can be identified by creating animals containing mutations in both genes or by measuring the binding and effect of one protein on a second protein or stretch of DNA.

Another important method of identifying elements involved in a particular function is to perturb a network by destroying or inactivating an individual element or set of elements and to observe the effect on the network as a whole. This approach is exemplified by nervous system lesions or single neuron kills in neuronal networks and by mutations or targeted gene disruptions in genetic networks. Lesion experiments have been used extensively in the study of both neuronal and genetic networks and so may provide the most useful analogies. In neuronal network research, lesion studies have led to the concepts of ordered sensory maps, central pattern generators, and lateralization of cerebral cortical functions (Grobstein, 1990).

After much trial and error in interpreting the results of nervous system lesions, one can now discern several generalizations, which may apply to genetic networks as well. The simplest and by far the most common use of nervous system lesions has been to identify which functions are missing or altered following a lesion, and then to assume that the destroyed region is normally required for the missing function. This assumption may turn out to be correct for some cases, but may be misleading or wrong in others. The reason that this assumption is unreliable is illustrated by the following example. Removal of the visual cerebral cortex on one side of the brain in cats eliminates visually guided behavior in one spatial hemifield; such animals are effectively blind on one side. But Sprague (1966) showed that a certain additional lesion (cutting fibers that enter the ipsilateral

superior colliculus through the commissure of the superior colliculus) restores visually guided behavior in the affected hemifield. He suggested that the first lesion disrupts a balance of excitation and inhibition between structures on the two sides of the brain, without eliminating structures necessary for the visual behavior; the second lesion restores the balance. The processes and anatomical pathways underlying this recovery of visuomotor function are still being investigated (Wallace et al., 1990), but the basic point is clear and well-established: in this case, an appropriate second lesion can reverse a deficit caused by the first lesion. Thus, the alteration or elimination of a behavior does not necessarily indicate that the missing elements are necessary for the behavior (Grobstein, 1990). In molecular genetics, some mutations may analogously eliminate a cellular function indirectly, by removing an activator or repressor protein that affects the level of expression of other genes; these other genes might be the ones truly required for the missing function.

On the other hand, if a particular lesion has no noticeable effect on a given function, one can legitimately conclude that the lesioned element is not necessary for that function (Grobstein, 1990). Thus, if a behavior continues to occur normally immediately after a region of the nervous system is destroyed, that region is not necessary for the observed behavior. This approach may be exploited in molecular genetics by using targeted mutations to rule out hypotheses about which genes are necessary for a given function, rather than to verify hypotheses about the necessity of a gene. An element that is not necessary for a network function, however, may nonetheless contribute substantially to that function in the intact network. Thus, a weak or undetectable effect of a lesion or mutation does not imply that the missing neuron(s) or gene has no related function. This has been demonstrated most clearly in very small neuronal networks, in which each neuron is individually identifiable. One can selectively kill one or more neurons and compare the activity of the network before and after this lesion (Selverston and Miller, 1982). For example, the stomatogastric nervous system, which controls digestion in crustaceans, can produce several oscillatory patterns. One such pattern, the pyloric rhythm, was thought to require the activity of the AB neuron, which, using intrinsic ion channels, can oscillate even when all synaptic activity is blocked. When the AB neuron was eliminated, however, the pyloric rhythm continued, provided that sufficient tonic (nonrhythmic) excitation was supplied to the network. The fact that the pyloric rhythm continued without the AB neuron did not demonstrate that the AB neuron does not contribute to the function of the intact network, however. In fact, the AB neuron normally affects the frequency of the rhythm and the phase relationships among the other neurons in the network. The frequency is lowered and the phase relationships subtly altered in the absence of the AB neuron (Selverston and Miller, 1982).

If two or more mechanisms normally make similar contributions to a given behavior, these mechanisms may be redundant. Redundancy may reinforce important functions and make them more flexible and less sensitive to perturbations or injuries. For example, escape movements in animals as diverse as fish and crayfish are mediated by a small number of neurons with giant axons; if the giant axons are lesioned, however, the animals produce similar, though not identical escape movements, using a set of nongiant neurons instead (Eaton, 1984). Genetic networks may also mediate functions via multiple mechanisms; this may account for the apparent lack of effect of many targeted mutations in mice (Gerlai, 1996).

What we initially regard as a single function may actually include two functions mediated by separate networks. On the other hand, what we regard as separate functions may be mediated by a single network. The use of lesions in an experimental design called double dissociation can demonstrate that two functions can be mediated by separate networks. In a double dissociation, ablation of one element disrupts function A, but leaves function B unchanged; ablation of a second element (in a second animal or subject) disrupts function B, but leaves function A unchanged. For example, people having a lesion within one set of cortical structures (the so-called dorsal stream) can correctly describe the size and orientation of an object, although they cannot correctly adjust their hand posture and orientation to grasp the object. On the other hand, a person having a lesion within another set of cortical structures (the ventral stream) can correctly calibrate her grip and orient her hand to grasp each object, although she cannot describe the size or orientation of the object (Goodale, 1993). This double dissociation reveals the nonintuitive fact that the human nervous system separately processes the conscious perception of object size and orientation and the use of object size and orientation to guide movements. A genetic analog of double dissociation involves biochemical mechanisms underlying memory formation in Drosophila: a protein synthesis inhibitor prevents formation of long-term memory, but not anesthesia-resistant memory; mutation of the *radish* gene prevents formation of anesthesia-resistant memory, but not long-term memory. Thus, what were thought to be two characteristics of the final stage of memory formation are really two independent types of memory (Connolly and Tully, 1996).

In all types of lesion studies, the interpretation is simplest if functions are assessed soon after a lesion is made, because, given time, many networks can reorganize in response to the perturbation. For example, bodily injuries, or even repeated sensory-motor tasks, can substantially alter the organization of neuronal networks over a period of weeks or less (Kaas, 1991). Compensation for a lesion may change the original effect or make it invisible in the reorganized network. In genetic networks, a mutation that exists throughout development, when networks are probably most malleable, may have no noticeable effect or a misleading effect by the time of adulthood (Gerlai, 1996). This suggests that targeted mutation approaches should focus on methods of inducible gene inactivation that can be applied at any time (Kühn et al., 1995). A mutation that exists throughout development may instead have an obvious effect on adult functions, but only because it disrupted developmental processes. For this reason, it is important to

demonstrate in knockout mice that the normal phenotype can be "rescued" in the adult by addition of the missing gene product (e.g., Patterson et al., 1996).

Suppose we reach the point where nearly all the elements in a network and their interactions have been identified and quantified. We could then draw a "circuit diagram" of the network, as has been done for some small and intensely studied neuronal networks. In these cases, however, it has been found that activity within the network or inputs to the network can alter the functional (but not anatomical) connectivity. Getting and Dekin (1985) coined useful terms to describe the flexibility inherent in such a system: the neurons are "multifunctional" and the network is "polymorphic." In at least some cases, functional reconfiguration of a network is induced by release of neuromodulators, substances that alter the properties of ion channels (Selverston, 1995). A neuromodulator can effectively alter several individual elements or interactions without itself forming a network connection. In genetic networks, a protein or enhancer often can indirectly change the level of expression of several genes; in some cases, such effects may be akin to those of neuromodulators.

In cases where a network is sufficiently understood for a circuit diagram to be drawn, the network's operation may be simulated using a computer-based model. Then one can delete or alter one or several elements or interactions at will and observe the effect on the simulated network. Such manipulations are analogous to lesions but are much easier to apply. Simulations also can confirm or preclude the sufficiency of a particular network for producing a known behavior. Computer simulations can lead to greater understanding of the role(s) of each element or interaction in the operation of the network, and can suggest new, experimentally testable hypotheses. Computer modeling has become a widespread and important tool in the study of neuronal networks (Marder and Abbott, 1995) and promises to be equally useful for the study of genetic networks (e.g., McAdams and Shapiro, 1995).

Let us suppose we know everything we would like to know about the elements, interactions, and modulation of a network. Is the ability to draw a complete circuit diagram sufficient to allow us to understand how the network works? What will be our criteria for saying we understand? Even for very small neuronal networks, the interactions are generally too complex for us to predict how the network (or a computer simulation of the network) will respond to a particular perturbation (Selverston, 1980, and commentaries therein). In other words, a functioning network may have emergent properties that cannot be predicted by studying its elements and interactions in isolation.

Even if we already know the effect of perturbing a particular element, we may find it difficult to conceive the relationship between the element and the output or function of the network. Are particular elements or subsets of the network dedicated to particular functions, or is processing distributed, with each element contributing to a wide variety of functions? In the study of neuronal networks, the notion of dedicated circuitry originated in the 19th century, when clinical neurologists first correlated disruptions of human behavior, especially in the use of language, with particular, localized, cerebral cortical brain lesions caused by injury or stroke. The notion of distributed circuitry was put forth by Lashley (1950), who removed parts of the cortex in trained animals and found that behavioral performance was more closely related to the amount of brain tissue removed than to its location. Many would now say that both local mediation of function and distributed organization occur in neuronal networks, depending on the network and the function (Ojemann, 1991; Morton and Chiel, 1994).

In the study of both neuronal and genetic networks, we have tended to focus on those elements that, when activated abnormally or removed, can have dramatic effects on the animal. Neurophysiologists have described "command neurons," which can evoke a complex behavioral sequence when individually activated by electrical stimulation (Kupfermann and Weiss, 1978). Analogously, geneticists have described "master control genes," such as homeobox genes that, when mutated, can lead to body part substitutions. Such elements are easier to study because they produce clearly observable effects. The language used to describe these elements suggests that they are among the highest elements in a hierarchy. They may be considered necessary and sufficient for a given function (Kupfermann and Weiss, 1978). There is a temptation to extrapolate from these few examples to view each category of network function as the responsibility of an individual element or a small number of elements. But it is much more likely that individual elements normally participate in multiple network functions, and that command neurons and master control genes are exceptions. Moreover, even these exceptions may require more complex interpretations. For example, the Mauthner neuron has long been considered the command neuron for escape movements in fish, but escape movements have been shown to occur even after elimination of the Mauthner neuron (Eaton and DiDomenico, 1985). Mutation of the Drosophila *eyeless* gene or its mouse homolog can lead to the absence of eyes, and targeted expression of its complementary DNA can induce ectopic eyes in Drosophila, suggesting that it is the master control gene for eyes; however, it is also normally expressed in the mouse spinal cord, where eyes do not develop (Halder et al., 1995).

In recent decades, there has been a rush from research on animal behavior to neuronal networks to sets of ion channels to genes. As a result, many genes have been located and identified, but their functions and partners within molecular networks often remain mysterious. Reverse genetics, the method of mutating or abnormally expressing genes within the context of the entire animal, has provided some fascinating results, but sets for itself an especially challenging task: learning the functions of a network by starting with a knowledge of its components. There may be parallels in the study of neuronal networks: Loeb and Marks suggested that "the cerebellum is an example of a structure whose basic components and circuitry were made available before there was any real experimental evidence regarding its function, with the consequence that the literature is now full of things that the cerebellum should be doing but is not" (commentary to Selverston, 1980). In the case of neuronal networks, it is often easiest to understand the

network's function if one pays attention to the natural behavior of the animal. For example, many neurons in the auditory cortex of the mustached bat respond selectively to pairs of sounds, each of which is a frequency-modulated sweep that begins at 30 kHz or one of its harmonics; this might never have been discovered, much less understood, if the researchers had not been aware that these bats navigate by emitting such sounds and listening for the echoes (Suga, 1988).

It is useful to study animal or human behavior to understand the functions of neuronal networks. To understand the functions of genetic networks, however, animal behavior may not be the most fruitful type of function to study. One reason, of course, is that the behavioral phenotype is not generally determined by the genotype alone; interactions with nongenetic factors are often both numerous and important. A second reason is that, even if genotype did determine behavioral phenotype, genes and behaviors represent such distant levels of organization that a correlation between the two may provide little or no understanding of the causative processes involved. For example, it is now known that *weaver* mice have a mutation in a gene coding for a potassium channel, which leads to degeneration of certain types of neurons in the cerebellum and the substantia nigra (Hess, 1996). Despite a long period of study, analysis of behavioral abnormalities in *weaver* mice shed little light on the underlying genetic abnormality. Similarly, future studies of the potassium channel that is mutated may shed light on important developmental processes, but are unlikely to aid our understanding of the neural control of movement. Thus, to understand the functions of genetic networks, it may be more useful to focus on the intracellular and intercellular functions that genetic networks directly perform (e.g., Artavanis-Tsakonas et al., 1995) than on genes apparently associated with complex behaviors but for unknown, and potentially quite indirect reasons.

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