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Using Genetic Mutations to Study the Neural Basis of Behavior

Minireview

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Progress in neurobiology is often driven by advances in technology, and it is hard to imagine an advance that has captured more attention than targeted genetic mutations in mice (Chen and Tonegawa, 1997; Silva et al., 1997). In principle the concept is simple: study the contributions of a molecule to behavior by eliminating its gene, or by introducing a gene whose product interferes with the molecule in some way (see Capecchi, 1989, 1994; Zimmer, 1992). But systems-level neuroscientists know that using molecular or anatomical lesions of the brain is a tricky business. It is, after all, studying the brain by breaking its parts. Now that the dust from the initial stampede may be settling somewhat, it seems useful to take a close look at gene targeting as a tool for studying the neural basis of behavior, particularly the mechanisms of learning and memory. We will consider the strengths and weaknesses of genetic mutations relative to older and less exotic methods, and we will suggest features that could make the use of mutations even more effective for the study of neural system function.

Behaviors are generated by collections of neural components (cells, synapses, etc.) interacting in ways that constitute a system with certain input/output properties. As such, identifying the list of essential components is a key first step in analyzing a system. Yet ironically, even this is made extremely difficult by possible interactions between components. A brain system is a little bit like an ecosystem—it's hard to affect one component without producing effects that cascade through the rest of the system. Thus, analysis is always plagued by a potential confound: does a behavioral deficit indicate a specific contribution of the removed component, or is it a relatively uninformative consequence of odd interactions between the remaining components?

The ability to overcome these difficulties is related in large part to thoughtful behavioral analyses and to the specificity of the lesions that can be made. We will suggest here that the utility of different lesion techniques relates to the extent to which three goals can be achieved—component specificity, temporal specificity, and behavioral specificity. We will consider why each is important and will use these ideas to outline an approach to the use of targeted mutations that enhances the study of neural systems.

Component Specificity

The conclusions that can be drawn from any lesion or mutation study are equivocal to the extent that components other than the target are damaged or otherwise significantly affected. In reality, due in part to the remarkable capacity of neural systems to compensate, it is unlikely that any lesion or mutation inherently achieves a desirable level of component specificity. As such, the goals are to minimize the collateral effects and to employ behavioral analyses that can effectively exclude alternatives. Attempts to demonstrate component specificity via histological analysis are less satisfying. While such strategies can provide assurance about missing or damaged tissue, they can less assuredly verify the physiological health of remaining tissue. As all slice physiologists know, healthy looking slices can display a complete absence of synaptic transmission.

Targeted mutations appear to offer great promise for component specificity, but they suffer from the same difficulties. On the plus side, there is generally only one gene that differs between wild-type and mutant mice. Moreover, the growing list of known cell-specific promoters means that the primary effects of mutations can increasingly be limited to a single cell type. However, mutations are not only subject to criticisms related to possible compensatory changes, but also to concerns regarding developmental abnormalities. Thus, as with conventional lesions, it may be tempting to conclude that the absence of a molecule is directly responsible for observed behavioral deficits, but such conclusions are almost always relatively unsatisfying.

Mutations Versus a Mutation Approach

In sum, component specificity is fundamentally important, but a number of factors conspire to make it extremely difficult to achieve and to verify. Yet there are many examples in which neural components mediating particular behaviors have been identified using brain lesions. The resolution of this apparent contradiction stems from the distinction between the limitations of each single lesion experiment and what can be accomplished with a "lesion approach" that has evolved over the years. Two cornerstones of this approach are careful selection of experimentally advantageous behaviors and careful behavioral studies that almost always require temporal control over the lesions. We believe that a successful "mutation approach" could follow a parallel development, thus the importance of considering temporal and behavioral specificity.

Temporal Specificity

Techniques for making mutations inducible or reversible in the adult brain are improving rapidly (see for example, Mansuy et al., 1998b). A primary motivation for these efforts is to improve specificity through the elimination or exclusion of developmental problems. The need for these improvements is so fundamental that it alone justifies the energetic efforts that are underway.

There are additional and perhaps equally compelling reasons to endow targeted mutations with temporal specificity. Making mutations inducible opens the door to a wide variety of important behavioral manipulations that further enhance component specificity. In a nutshell, the ability to induce the mutation either before or after learning often permits behavioral designs that help achieve a reasonable level of component specificity.

As a convenient example, we consider how lesions have been used to determine whether the cerebellar

cortex is required for the acquisition of conditioned eyelid responses. In eyelid conditioning, repeatedly pairing a relatively neutral stimulus, such as a tone, with a reinforcing stimulus, such as a puff of air in the eye, leads to the acquisition of a learned response: the animal closes its eye in response to the tone. Some data showed that lesions of cerebellar cortex have no effect (McCormick and Thompson, 1984), and other data indicated that responses are abolished (Yeo et al., 1984). The crux of the problem was to provide assurance of component specificity. Were responses abolished in one study through inadvertent damage to nearby pathways known to be required for the expression of responses, or was learning spared in the other studies because the lesions missed a critical region of cerebellar cortex?

One solution involved behavioral studies that exploit the temporal specificity available with the lesions. By initially making lesions *after* training, it was possible to determine whether the cerebellar cortex is required for the expression of the responses. As it turns out, the lesions do not abolish responses, but they have a characteristic effect on response timing (Perrett et al., 1993; Garcia and Mauk, 1998). This result indicated that pathways required for response expression were still intact, but that the lesions had damaged regions of the cerebellar cortex critical for response timing. With these assurances, it was then possible to show, by training with different stimuli, that these same lesions prevent learning of new responses in the same animals.

These studies illustrate how careful behavioral studies and temporally specific lesions can be combined as a "lesion approach" to rescue an acceptable level of component specificity. The details might differ for other behaviors and other brain regions, but the systematic approaches possible with temporal control of lesion, or mutations can greatly enhance component specificity through the exclusion of alternatives. Thus, the importance of temporal specificity, especially the ability to induce mutations in adult animals, extends well beyond the ability to exclude possible developmental abnormalities. It opens the door to a rich repertoire of behavioral tricks that can make all the difference.

Behavioral Specificity

We use the phrase "behavioral specificity" to refer to a variety of issues involved in establishing links between neural manipulations and a target behavior. Whereas with component specificity the challenge is showing that a behavior is affected because of damage to the target component, with behavioral specificity the challenge is to exclude the possibility that behavioral deficits following a lesion or mutation occur because of nonspecific, uninformative actions. In the analysis of learned behaviors, for example, it is necessary to separate performance effects from specific learning effects. A mutation-induced deficit in finding the hidden platform in the Morris water maze could indicate a host of deficiencies unrelated to problems with spatial learning; the animal could be disoriented, blind, afraid, unmotivated, sick, inclined to swim near the edge, or countless other possibilities. Simple controls sometimes employed, such as comparing swimming speed between mutants and wild types, only scratch the surface of the potential confounds. Different behaviors vary in their ability to provide controls for performance effects. In eyelid conditioning, for example, the effects of lesions can be specific to one side of the body, allowing training on the opposite side to be used as a within-animal control for performance effects of unilateral lesions.

Some of the most common problems with behavioral specificity are exemplified by the growing use of brain region–"dependent" behaviors to screen mutant mice. There are many examples of behaviors that depend on a particular brain structure. Lesions of the hippocampus disrupt certain spatial and contextual learning tasks (Squire, 1992; Abeliovich et al., 1993). Similarly, lesions of the cerebellum affect simple motor tasks such as walking on a rotating rod (the rotorod). In some sense then, these behaviors are hippocampal dependent or cerebellar dependent. The pitfall is that these relationships are not commutative. That is, deficits in spatial learning or in rotorod performance do not, in turn, imply problems with hippocampal or cerebellar function. The same controls used to establish behavioral specificity in the original lesion studies would need to be repeated to establish behavioral specificity of the mutations.

State of the Art Mutations

A novel application of transgene techniques recently reported in *Cell* (Watanabe et al., 1998) offers an early glimpse of the enormous potential for major advances in systems neuroscience from the use of mutations. This technique involves the expression of a transgene encoding the human interleukin-2 receptor α subunit (IL-2Rα). In this study, the expression of this gene was initially limited through the use of a promoter for a metabotrophic glutamate receptor (mGluR2). The mice appear to develop normally. Cells expressing this transmembrane fusion protein can then be killed through the infusion of a recombinant protein comprised of the variable heavy and light chains of a monoclonal antibody against human IL-2Rα fused with an exotoxin (PE38). Because the mGluR2 promoter is fairly specific to Golgi cells in the cerebellum, a relatively complete, and apparently specific, lesion of Golgi cells was obtained with intrathecal injection of this protein. Within 5 days, virtually all Golgi cells in the cerebellar cortex were destroyed. Thus, immunotoxin-mediated cell targeting represents a first generation technique for inducible, cell-specific lesions through genetic mutations. Presumably, this amazing technique could be applied to any cell for which there is a relatively specific promoter.

These results should be of interest to cerebellar research in part because so little is known about the function of Golgi cells. In the context of his theory of cerebellar motor learning, Marr (1969) proposed that Golgi cells provide negative feedback that helps keep the number of cerebellar granule cells that are active relatively constant despite possible variations in overall input to the cerebellum. He showed mathematically how this constancy could optimize the number of different cerebellar inputs that can be encoded by different patterns of granule cell activity. We have used computer simulations of the cerebellum to suggest that Golgi cells additionally help encode time during stimuli in a way that is important for the cerebellum's ability to time movements (Buonomano and Mauk, 1994; Mauk and Donegan, 1997). Because Golgi cell recordings are difficult, these ideas

and others remain untested empirically. The data from Watanabe et al. (1998) show that after injections of the antibody/toxin protein, the mice display severe cerebellar ataxia with acute and chronic phases as distinguished in part by different deficits in rotorod performance. Results of this sort illustrate the importance of Golgi cells to overall cerebellar function and will provide the foundation for testing specific ideas about what Golgi cells contribute to cerebellar information processing.

Ironically, given the inducibility and cell-specific targeting of the lesions, Watanabe et al. (1998) do a careful job of identifying an apparent compensatory change, produced by the Golgi cell lesion, in NMDA-mediated currents in cerebellar granule cells. Thus, even the best of lesions can be a reminder that establishing component specificity of lesion effects is always a challenge. A reversible, Golgi cell–specific inactivation might help to determine the precise role of Golgi cells in cerebellar function.

The technology for rapidly inducing and reversing the expression of transgenes in brain appears to be improving rapidly. Mansuy et al. (1998b) have recently described a technique that provides an initial glimpse of how incredibly powerful targeted mutations will become for the study of systems-level neuroscience. This technique, which provides temporal control over the expression of the calcineurin transgene, allowed Mansuy et al. (1998b) to probe distinct components of memory such as acquisition, consolidation, and retrieval as well as to demonstrate a greater degree of component specificity. Initially, this group used a brain-specific promoter (CaMKIIα) to restrict expression of the reverse tetracycline-controlled transactivator (rtTA) to the forebrain of the mouse. Subsequently, by placing a calcineurin transgene under the control of a tTA-responsive promoter (tet0) in these same mice, Mansuy et al. (1998b) showed that doxycycline induced calcineurin transgene expression in a dose-dependent manner. Maximal expression was obtained in the CA1 and CA2 areas of the hippocampus after 6 days of doxycycline treatment administered in the food, while 2 weeks after doxycycline removal no transgene mRNA could be detected.

Although it was already known that overexpression of calcineurin in transgenic mice interferes with spatial memory (Mansuy et al., 1998a), the temporal control provided by the rtTA system allowed these investigators to assess whether calcineurin overexpression affects the acquisition, consolidation, or retrieval of spatial memories. During expression of the calcineurin transgene, spatial learning was impaired, as was the induction in the hippocampus of long-term potentiation, a form of synaptic plasticity (changes in strength) possibly involved in spatial learning. In addition to this learning and memory deficit, these investigators were able to demonstrate impairments in retrieval of hippocampal-dependent spatial memories by training mice in the Morris water maze before overexpressing calcineurin. Moreover, the most interesting result that Mansuy et al. (1998a) report is the apparent recovery of normal retrieval after discontinuing treatment with doxycycline. This illustrates how temporal specificity can be used to improve the degree of component specificity through exclusion of confounds such as gross developmental abnormalities. Since it seems that greater rapidity in turning gene expression on/off will be achieved in the (near) future through the use of more potent doxycycline derivatives, the power of the behavioral studies that will be possible with these type of mice should increase dramatically.

Fantasy Mice

These examples clearly portend a bright future for the use of genetic mutations to address the function of neural systems. Commenting on his tendency to dream about future scientific technologies, the late Carl Sagan suggested, “Dreams are maps.” Here, in the spirit of Sagan’s comment, we will ignore limitations or apparent feasibility and will dream about types of mutations that could help revolutionize systems-level neuroscience. We do not imagine that these particular mice comprise a list that is either comprehensive or optimal, nor do we imagine that they are possible in the near future. They are simply our maps.

One mouse is inspired by the immunotoxin-mediated cell targeting technique of Watanabe et al. (1998). The goal is to achieve functional inactivation of a particular cell type with extremely rapid onset and offset. An approach of this type has been employed with great success in certain invertebrate systems where particular cells can be functionally removed from the circuit via direct hyperpolarization through a microelectrode. The cell is still alive, but it cannot communicate with other neurons because the hyperpolarization prevents action potentials. Although this technique affords superb temporal specificity, its application is limited to the number of cells that can be simultaneously impaled with microelectrodes.

Perhaps entire classes of cells could be functionally removed from a circuit with cell type–specific expression of a recombinant protein comprised of a known potassium or chloride channel and a receptor for an artificial ligand. If the appropriate artificial receptor–ligand combination could be developed, cells expressing the transgene could be functionally removed from the circuit via hyperpolarization that is activated by injection of the ligand. Combining such a transgene with a cell type–specific promoter could produce a rapid onset and reversible variant of the immunotoxin-mediated technique. Cells could be rapidly removed and, with an artificial competitive antagonist, rapidly reinstated in the circuit. By potentially eliminating compensatory changes such as those characterized in the cerebellum by Watanabe et al. (1998), such a “hyperpolarization” mouse would provide a tremendous degree of component and temporal specificity.

Another type of futuristic mouse might move away from using targeted mutations as lesions (see Siegel and Isacoff, 1998). Perhaps a recombinant protein could be expressed to record activity of cells. Such a mouse could be used, for example, to determine the cells that are active during a particular form of learning. The recombinant protein might be constructed with three properties: (1) the ability to bind some activity-dependent molecule such as elevated free calcium, (2) the ability to bind an initiator molecule, and (3) when these two binding sites are occupied to undergo a detectable

conformational change or to generate a measurable product.

The idea would be that the initiator molecule could be injected or otherwise delivered in a temporally discrete manner, and that this would initiate the "recording" process. From that time on, binding at the second, activity-dependent site would generate a measurable product in the cell. The keys would be the temporal specificity of the initiator molecule, as well as the specificity and stringency of the binding site detecting neural activity. If the expression of this molecule could be limited to synapses, the resolution of activity-dependent accumulation of the marker molecule could be at the level of synapses rather than cells. In this way mutant mice could be used not only to identify the neural components responsible for a particular behavior, but also to study how those components interact to produce the input/output properties of the system.

Finding synapses that undergo plasticity during learning is a fundamental but technically demanding goal for neurobiologists studying the neural basis of learning. As more is learned about the molecules required for particular forms of synaptic plasticity, it might even be possible to limit the accumulation of marker to synapses that have undergone that form of plasticity since the injection of the initiator molecule. Such a mouse could be used to establish links between forms of plasticity and forms of learning at a level of certainty not currently approachable. A mouse could be injected with the initiator molecule and then trained in a specific task. Subsequent histological analysis could reveal synapses with marker accumulation, and thus synapses that had undergone plasticity during that form of learning could be identified.

As systems-level neuroscientists, it is hard to guess whether mice like these are many decades away or just around the corner. Either way, there is no doubt that continuing advances in gene targeting technology, when combined with robust behavioral analysis, will help revolutionize our ability to study and to understand brain systems.

Selected Reading

Abeliovich, A., Paylor, R., Chen, C., Kim, J.J., Rosenmund, C., Stevens, C.F., and Tonegawa, S. (1993). Cell *79*, 365–375.

Buonomano, D.V., and Mauk, M.D. (1994). Neural Comp. *6*, 38–55.

Capecchi, M.R. (1989). Science *244*, 1288–1292.

Capecchi, M.R. (1994). Sci. Am. *270*, 52–59.

Chen, C., and Tonegawa, S. (1997). Annu. Rev. Neurosci. *20*, 157–184.

Garcia, K.S., and Mauk, M.D. (1998). Neuropharmacology *37*, 471–480.

Marr, D.J.P (1969). J. Physiol. (Lond.) *202*, 437–470.

Mansuy, I.M., Mayford, M., Jacob, B., Kandel, E.R., and Bach, M. (1998a). Cell *92*, 39–49.

Mansuy, I.M., Winder, D.G., Moallem, T.M., Osman, M., Mayford, M., Hawkins, R.D., and Kandel, E.R. (1998b). Neuron *21*, 257–265.

Mauk, M.D., and Donegan, N.H. (1997). Learn. Mem. *3*, 130–158.

McCormick, D.A., and Thompson, R.F. (1984). Science *223*, 296–299.

Perrett, S.P., Ruiz, B.P., and Mauk, M.D. (1993). J. Neurosci. *13*, 1708–1718.

Siegel, M.S., and Isacoff, E.Y. (1998). Neuron *19*, 735–741.

Silva, A.J., Smith, A.M., and Giese, K.P. (1997). Annu. Rev. Genet. *31*, 527–546.

Squire, L.R. (1992). Psychol. Rev. *99*, 195–231.

Watanabe, D., Inokawa, H., Hashimoto, K., Suzuki, N., Kano, M., Shigemoto, R., Hirano, T., Toyama, K., Kaneko, S., Yokoi, M., et al. (1998). Cell *95*, 17–27.

Yeo, C.H., Hardiman, M.J., and Glickstein, M. (1984). Behav. Brain Res. *13*, 261–266.

Zimmer, A. (1992). Annu. Rev. Neurosci. *15*, 115–137.