Caught in the Act

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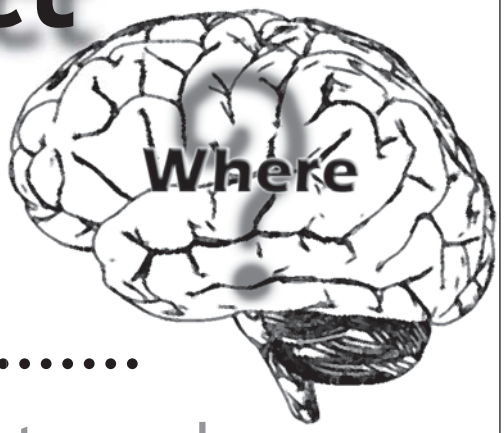

mapping the brain using immediate early genes

By Eva Nong

"Roll over!" As soon as you speak, your dog Spot lowers his head, kneels on the floor, and rolls into a ball. It all seems simple, right? Inside Spot's brain, however, something much more complex is taking place, something that remains one of the greatest challenges to neuroscience—understanding the basis of learning and behavior. Although parts of the brain are known for their general roles, it is far more challenging to bridge the gap between specific targets in the brain that are responsible for learning and the outward action of rolling (1). One way of solving this puzzle may require bridging the gap between the dynamic neural interactions that relay and integrate information in the brain. This would allow the identification of the definite neuronal components that promote response to a stimulus, in Spot's case, your command.

This is by no means an easy task. Not only must scientists differentiate between neural targets specifically activated during rolling, they also must single out the individual neurons that actually cause the action. Recently, a team led by John Guzowski, of the Department Of Neuroscience at the University off New Mexico, has made advances in detecting immediate early gene activation (2). Using fluorescent technology, we can finally obtain a more detailed picture of the changes that occur in the brain, from Spot learning a new move to studying for your latest exam.

credit: Pimkwan Jaru-ampornpan, HSR

Visualizing Learning: Immediate Early Genes

What are these bold new indicators of neuronal activity? Simply put, they are genes located in the brain that are the first to turn on in response to new environmental stimuli. The name "immediate early gene" is fitting; these genes are turned on faster than any new proteins can be made. While several groups of genes are turned on by neural activity, immediate early genes are considered distinct from "late response" genes, because they are the only ones that do not require new protein synthesis. Most IEGs are proteins that relay signals that cause the cell to change how and whether certain genes are expressed (2,3) The downstream products of IEGs often play an important role in promoting the formation of new synapses, which is thought to underlie learning and memory. Studies that use RNA interference technology to turn off certain IEGs show that their expression is instrumental in stabilizing early changes in synapses and hence in learning and memory formation (4).

Immediate early genes have long been heralded as early markers indicating the formation of new synapses, in responses to external stimuli, a process of changing communication between cells which is considered the cellular basis for learning. These genes are especially indicative of learning because they are rarely expressed in resting, unstimulated cells but rapidly increase during both brain activity and new synapse formation (5,6). This dramatic activity-dependent response makes immediate early genes a unique and ideal candidate in reporting the synaptic changes that occur during the process of learning (7).

However, merely identifying the immediate early genes as targets is not sufficient; it is also challenging to pinpoint these genes and their protein products in an accurate manner. The conventional method of identifying IEG activation during learning has been to either label the protein products of the IEG genes within cells with antibodies (immunohistochemistry), or to add RNA (in situ hybridization)

which pairs with the IEG RNA (8). The problem with these approaches lies in their lack of specificity and dependence on large number of IEG-containing cells in order to show evidence of learning a task.

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However, what gene is being turned on often matters much more than how much a group of genes is expressed. Furthermore, these processes require that we put molecules into fixed, dead cells, which means that we cannot pinpoint either the time or the exact location of activity in the entire organism. Fortunately, recent breakthroughs in fluorescence technology, such as "catFISH" and whole-brain imaging, have allowed scientists to see a more detailed picture of the cellular basis of learning—by visualizing the activation patterns of immediate early genes that keeps track of both space and time.

Fishing for IEGs

A new method of florescence imaging, cellular compartment analysis of temporal activity by fluorescence in situ hybridization, or catFISH, has allowed scientists to detect the presence of immediate early genes. CatFISH works by adding a RNA probe that specifically binds, or hybridizes, to the complementary RNA expressed by IEG during activation. What makes catFISH superior to other fluorescence in situ hybridization (FISH) is its ability to detect the RNA both in the nucleus and the cytoplasm. In other words, tracking the course of RNA in and out the nucleus allows catFISH to discover the sequential timeline of IEG activation.

More specifically, catFISH employs time-specific IEGs, like Arc, as the "genomic timers" of neuronal activation. For about 1-15 minutes after the presentation of a stimulus, ArcRNA can be immediately detected in the neuron nuclei (2). This burst of Arc activity is quickly followed by the disappearance of the RNA signal in the nucleus (7). This brief peak of Arc activity indicates that the animal's brain is processing its experience of a novel stimulus. After the stimulus is removed, another peak in ArcRNA levels is seen in cell cytoplasm after 20-60 minutes (7,8). The transient but precise nature in the appearance and disappearance of ArcRNA in each cell compartment allows us to gauge the specific phases of neural processing from learning to behavior. CatFISH also integrates information about gene expression and neuronal activation, and charts the waves of rapid, transient IEG expression in specific groups of active neurons during and shortly after the introduction of a new learning experience.

Since the nucleic and cytoplasmic peaks occur at two distinct times, ArcRNA can be used to infer the neuronal activity of the animal during the course of learning. Several other IEGs, such as Homer, show similar patterns, with two distinct phases of timed activation, and thus can be used in the same manner (8). Using catFISH, researchers can also look for other genes which are expressed during the various peaks of IEG expression, allowing us to learn more about what goes on in the neuron when it is called upon to process information.

catFISH Goes 3-D

With catFISH, we can figure out when genes are expressed during learning, but imagine expanding that process to the entire brain! Conventional FISH analysis is limited to small regions in the brain because it is very time-consuming to manually process the information from multiple nuclei. Recently, however, automated three-dimensional cell nuclei segmentation and FISH quantification for monitoring gene activity, or 3D-catFISH, has established itself as a promising whole-brain imaging approach to acquiring spatial information during learning (9). In its simplest form, 3D-catFISH employs a comprehensive software imaging tool to analyze and integrate three-dimensional, multi-channeled catFISH input from individual nuclei in the brain. Unlike conventional analysis by human experts, 3D-catFISH is much faster, less tedious, and more accurate (1,9).

3D-catFISH, an image analysis soft-

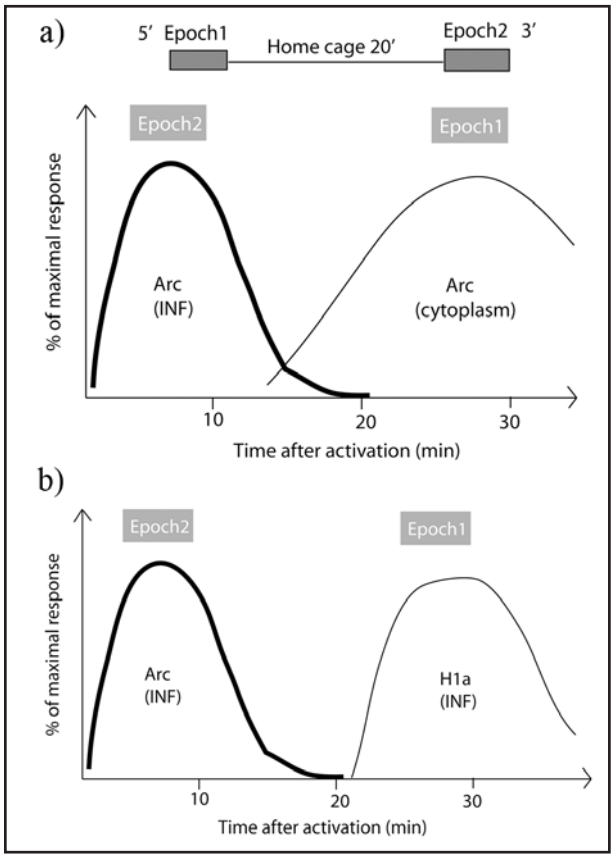

Figure 1. a) and b) show the rise and fall of Arc RNA expression in nucleus and cytoplasm.

credit: Pimkwan Jaru-ampornpan, HSR

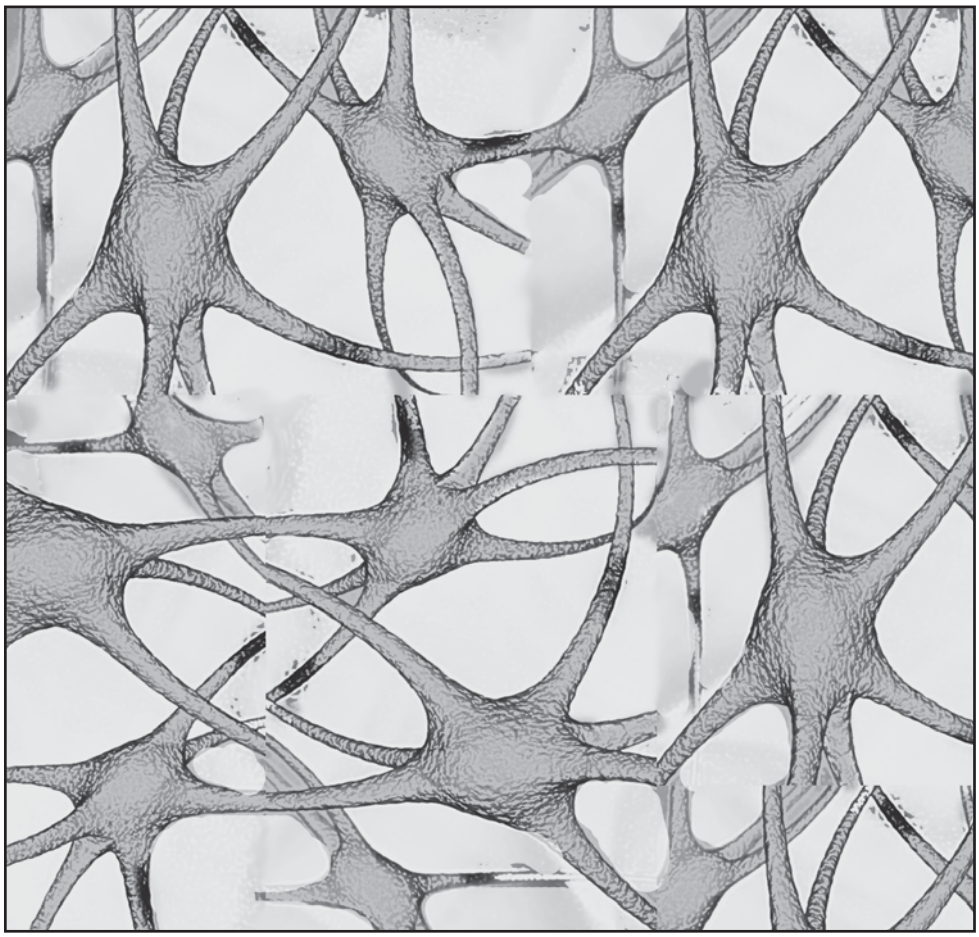

Figure 2. The 3-D visualization software catFISH will allow researchers to connect brain coordinates to their corresponding behaviors.

credit: Pimkwan Jaru-ampornpan, HSR

ware package developed by a research team at Johns Hopkins University, reads 3-D confocal microscope images from separate catFISH experiments and simultaneously detects both the number of neurons expressing IEGs and the amount of IEGs in each cell. Moreover, this process can also detect multiple genes, and then isolate individual signals from different IEG RNAs, allowing it to integrate information about different genes into a more complete picture of where IEGs are active (9).

Several studies have already used catFISH to strengthen the correlation between IEG activation and information processing in specific brain regions, such as the three major subfields of the hippocampus (10,11). The integration of 3-D "whole brain" imaging with catFISH has allowed researchers to make larger strides forward. By incorporating many distinct pieces of information, such as the location, number, and intensity of neuronal activity, this new technique creates a comprehensive and overlapping map of brain activity (9). Taking a step further from previous IEG technology, this advance allows scientists to find the location of activated genes and to chart what cells are activated when during the process of learning (9). This information can form a precise summary that connects the brain coordinates to their corresponding behaviors. This combinatorial approach will help us to outline the neural circuits and their related functions. Defining these circuits may one day tell us which parts of the brain need extra exercise when grasping a new concept.

The Future of 3-D FISHing

While these new techniques offer scientists a unique opportunity to visualize learning, there is still a lot of progress that must be made before we get a complete picture. Better imaging will require better fluorescent probes in order to label the gene of interest. An advance in custom-designed fluorescent agents might achieve maximum cell type specificity and obtain clearer fluorescent pictures (12). While this technology is still undergoing development, there have been many tantalizing improvements. For example, using "quantum dots," or semiconductor nanocrystals, to label the IEG DNA or RNA probes might increase the rate of mapping and allow greater specificity, as they are small enough not to interfere with the binding of the probe (13).

These innovations in large data processing and visualization will advance our understanding of the dynamic neural mechanisms underlying learning and memory. With the aid of these novel technologies, the key molecular and cellular cues behind the brain's new activity may finally come to light. Perhaps, one day, it will be as easy to train your brain to ace an exam as it is to train Spot to roll over. H

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References

1. Cole, AJ, *et al. Nature* 340 (1989): 474-476.
2. Guzowski, JF, *et al. Current Opinion in Neurobiology* 15 (2005): 599-606
3. Buke, SN, *et al. Neuron* 45 (2005): 667-674
4. Guzowski, JF, *et al. Hippocampus* 12 (2002): 86-104.
5. Guzowski, JF, *et al. Nat Neurosci* 2 (1999): 1120-1124.
6. Cole, AJ, *et al. Nature* 340 (1989): 474-476.
7. Bottai, D, *et al. J Neurosci* 22 (2002): 167-175.
8. Vazdarjanova, BL, *et al. J Neurosci* 22 (2002): 10067-10071.
9. Chawla, MK, *et al. J Neurosci Methods* 139 (2004): 13-24.
10. Chawla, MK, *et al. Hippocampus* 15 (2005): 579-586
11. Vazdarjanova, BL, *et al. J Neurosci* 24 (2004): 6489-6496.
12. Sinclair, MB, *et al. Appl Opt* 43 (2004): 2079-2088.
13. Matsuno, A, *et al. J Histochem Cytochem* 53 (2005): 833-838.