NIH Celebrates 100

Technically speaking, the National Institutes of Health (NIH) are not really 100 years old, but that did not stop more than 400 alumni from returning to celebrate the centennial. From 15 to 18 October, organizers capitalized on the fact that NIH's predecessor—a one-room Laboratory of Hygiene at the Marine Hospital on Staten Island—was established a century ago. The laboratory moved to Washington, D.C. in 1891, and in 1930 Congress renamed it the National Institute of Health. Not until 1948—when there were four major institutes instead of the current twelve—did the research facility become the National Institutes of Health. The following are highlights from symposia on neuroscience and developmental biology.

Patterns and Processes Mark Brain Activity

"We knew precious little about the brain in 1951," says Seymour Kety of the National Institute of Mental Health (NIMH), who came to the institute in that year as its scientific director. "Many of the attempts to bridge the gap between the brain and mental illness had been premature, unsuccessful, and often absurd." Today, he says, researchers know more about the basic aspects of brain function, but explanations that link it to mental illness are "few and meager."

Julius Axelrod, also of NIMH, is one of the researchers whose work begins to bridge that gap. In 1970 he earned a Nobel Prize for his studies on the inactivation of catecholamines—a group of neurotransmitters and hormones that includes norepinephrine, epinephrine, and dopamine—and the effects of psychotropic drugs on this process. His interests now lie in the complex interaction of catecholamines with multiple receptor types and the cellular responses that result from their interactions.

Much of the current excitement is over G proteins, named because they bind guanosine triphosphate. The G proteins often control the linkage between receptors and intracellular enzymes by stimulating or inhibiting the enzymes. Axelrod summarized their importance in the responses of cultured rat thyroid cells to two different chemical signals, thyroid-stimulating hormone (TSH) and norepinephrine. TSH triggers the production of cyclic adenosine monophosphate (cAMP), a so-called second messenger that induces the thyroid cells to synthesize new receptors for norepinephrine. But norepinephrine binding triggers two different chemical pathways that ultimately decrease the cell's sensitivity.

"The α-adrenergic receptor that binds norepinephrine is linked to two signal transducing systems," says Axelrod. "First, it is linked to phospholipase C via one G protein. This pathway results in the production of thyroid hormone. Second, the receptor is linked to phospholipase A_2 via another G protein. This pathway causes cell growth."

These two pathways are also linked with each other inside the cell, however. With activation of phospholipase A_2, the cell makes arachidonic acid, a fatty acid that stimulates protein kinase C activity. This intracellular enzyme, in turn, adds phosphate groups to the adrenergic receptor. Phosphorylation depresses receptor sensitivity to the original signal, norepinephrine. As a result, arachidonic acid release is halted and the thyroid cells stop growing.

"How do you explain lithium, a drug that is both an upper and a downer?" asks Solomon Snyder of the Johns Hopkins University School of Medicine. Perhaps lithium dampens nerve cell responses to the neurotransmitters that induce the highs and lows of manic depression. The key pathway may be phosphatidylinositol (PI) metabolism, which is triggered when certain neurotransmitters stimulate the breakdown of this membrane phospholipid.

In his review of recent advances in the field, Snyder noted that today researchers can map both the enzymes and the receptors that mark brain locations of PI metabolism. Snyder reports that the receptor for inositol trisphosphate (IP3) is phosphorylated by the enzyme dependent on cAMP, an example of "cross-talk" between two different second messenger systems. In addition, he notes that low concentrations of calcium ions inhibit the activity of the IP3 receptor and that calmedin, a recently discovered calcium-mediator protein, may regulate this interaction.

Other drugs that change brain activity affect different synapses. "At synapses where GABA [gamma-aminobutyric acid] is a neurotransmitter, there is a peptide we call DBI that may be important in the tolerance to some benzodiazepines and also important in alcohol toxicity," says Erminio Costa of the Fidia-Georgetown Institute for the Neurosciences in Washington, D.C.

Costa updated information that both DBI (diazepam-binding inhibitor) and a peptide fragment of DBI induce conflict activity when they are injected into the animal's brain. Both block the action of benzodiazepines, including diazepam or Valium, and both seem to occur naturally in the brain. Costa proposes that the peptides act at $GABA_A$ synapses to reduce the likelihood that GABA will exert its usual inhibitory effect on nerve cells.

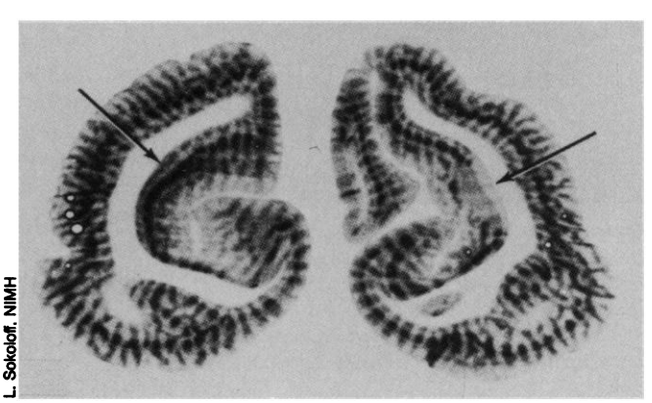
Some animals become resistant to benzodiazepines, but the mechanism for this tolerance is not known. Costa attributes it to an increased turnover of DBI in specific brain regions. He also says that a similar phenomenon occurs in alcohol-treated rats that have been maintained on a vitamin B–deficient diet.

Recent advances in understanding brain function sometimes serve to rewrite scientific history, says Floyd Bloom of the Scripps Clinic. He reviewed several kinds of synaptic interactions that at one time were not conceivable, but today are being demonstrated in many laboratories.

One is that a single neurotransmitter can have simultaneous multiple actions on responding cells. Another is that one transmitter, acting at the same synapse, will cause different responses in sequence. Bloom also identifies a third variation on the synaptic

2-Deoxyglucose Image

Of the brain of a monkey that has the right eye patched. The dark stripes are metabolically active neurons in the visual cortex stimulated by the open left eye. Arrows mark areas where the blind spots of the retina are represented.



L. Sokoloff, NIMH

theme—namely, that one neurotransmitter can act at multiple synaptic sites to influence the effects of a different neurotransmitter.

For example, in the parts of the cerebral cortex that process sensory information, norepinephrine and vasoactive intestinal peptide mutually enhance each other's affects. This interaction is thought to occur when many sensory signals are carried in parallel neural pathways via the thalamus into the sensory cortex. Norepinephrine generally inhibits the firing of cortical neurons during behavioral arousal. Vasoactive intestinal peptide acts locally within the cortex to enhance the action of norepinephrine. Both stimulate cAMP production, and norepinephrine also activates phospholipase A_2, which then decreases the sensitivity of norepinephrine receptors. The net effect is a self-enhancing and, at the same time, self-regulating system.

Noting that there are aspects of brain function other than synaptic transmission, Louis Sokoloff of NIMH described how energy metabolism can be mapped by using the 2-deoxyglucose method. "Unlike the liver, in which all cells have about the same level of activity at any one time, the level of activity in the brain varies from one region to another," he says. By studying the changing patterns of metabolic activity, researchers can identify what brain regions are necessary for certain functions, or analyze certain brain abnormalities associated with disease.

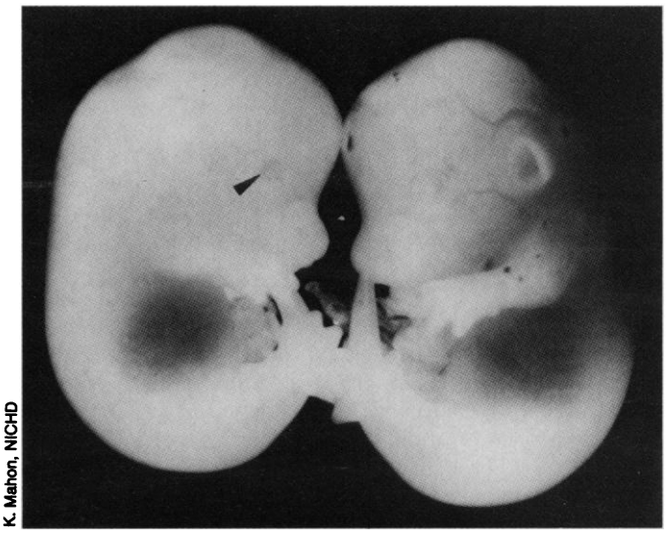
Today researchers can measure the local cerebral glucose utilization in conscious animals or people who are performing a certain task. For example, a monkey trained to use its left hand will show high glucose utilization in the right motor cortex. In some brain diseases, the pattern of glucose utilization is abnormal and can be used to detect the location of the defect. For instance, in some epilepsy patients, PET scanning (positron emission tomography) can identify the brain region where seizures are initiated. Michael Phelps of the University of California at Los Angeles and his colleagues developed the technique, which relies on fluorodeoxyglucose as a marker. PET scanning is also used to diagnose gliomas, brain tumors with a level of metabolic activity that increases with their level of malignancy.

Molecular Events Guide Embryonic Development

A pervasive mystery in developmental biology is why genes—present in every cell of the embryo—are expressed only in some cells and not others. Donald Brown of the Carnegie Institution of Washington in Baltimore and his colleagues study the developmental control of two closely related genes in *Xenopus,* the African clawed frog. Both code for the 5*S* ribosomal RNA that cells need to form functional ribosomes and carry on protein synthesis. "But by the time a fertilized egg has reached the blastula stage, the embryo is synthesizing 5*S* RNA only from the somatic 5*S* gene; the oocyte 5*S* gene is nearly shut off," says Brown.

Two mouse embryos

At 14 ½ days of gestation they have normal (left) and abnormal (right) development of the lens in the eye (arrowhead). The embryo on the right carries a transgene that causes tumor formation during lens development.



K. Mahon, NICHD

Brown thinks that the differential regulation of these two genes is due, at least in part, to the ability of specific factors to bind to an internal control region of the gene. The control regions of the two genes differ by three nucleotide units, and regulating factors—particularly transcriptional factor IIIA—bind more tightly to the control region of the somatic gene.

This more stable interaction with the somatic 5*S* gene may allow it to stay turned on during embryogenesis. In contrast, the oocyte 5*S* gene of embryos has two impediments to transcription. First, because its interaction with transcription factors is less stable, it lacks the signal for transcription; and second, the gene instead forms a complex with nucleosomes that generally suppress its expression.

The basis of creating a transgenic animal is simple, says Heiner Westphal of the National Institute of Child Health and Human Development (NICHD). "You take a piece of DNA and inject it into the early embryo of anything—from a *Drosophila* to a cow—and the DNA may become part of every cell, including the germline." Transgenes consist of two key components—a promoter that signals what tissue will express the gene, and a coding element that dictates what protein will be made.

Westphal and his colleagues study how certain transgenes affect the development of the lens of the eye, a highly organized structure. By looking in an animal's eye, researchers can see if there is anything wrong with the lens, an indication that the normal mechanisms of gene regulation have gone astray. The NICHD researchers find that two transgenes, both capable of transforming cells in vitro, have surprisingly different effects during development in vivo.

One transgene—a combination of the promoter for a normal lens protein fused to the T (for tumor) antigen of the SV40 virus—disrupts the normal orderly outgrowth of lens fibers from the developing lens epithelium. It also transforms the cells to produce a tumor in the eye. But another transgene—a hybrid of the promoter from a mouse cancer virus fused to a normal mouse gene—only causes a secondary developmental defect in vivo. Its net effect is to produce an abnormality in the posterior capsule of the eye so that the lens tissue breaks apart and fills the eye. These mice are blind, but otherwise healthy, says Westphal.

The next time someone at a cocktail party asks what jellyfish, squid, and humans have in common—basic eye structure can be offered with breezy confidence as a response. Joram Piatigorsky of the National Eye Institute notes, as others have, that mammals, squid, and jellyfish have eyes that are remarkably similar in structure.

It is not surprising that the lenses of these animals contain granules of proteins called crystallins. What is surprising, however, is that many lens crystallins are strikingly similar to enzymes from completely unrelated animals. For example, the ε-crystallin of ducks and crocodiles has the same structure and activity as lactate dehydrogenase, a sugar-metabolizing enzyme in bacteria. And the human lens proteins, β- and γ-crystallin, are related to the superfamily of bacterial spore coat proteins.

Piatigorsky speculates why the unusual evolutionary links exist. "Nature has used

what was around [enzymatically speaking] to fill a structural role," he says. Whether the lens crystallins have any enzymatic activity in the eye is doubtful, because the eye appears to lack the appropriate substrates. The new information blurs the once clear-cut distinction between structural and functional proteins.

Another puzzle for developmental biologists is understanding the processes that determines cell identity. Michael Levine of Columbia University and his co-workers attribute these events partly to the coordinated effort of homeo box genes. "Many, but not all, of the genes in *Drosophila* that specify spatial information are homeo box genes," he says. Fruit flies sport a total of about 30 such homeo box genes and 18 of them have been sequenced. But homeo box genes themselves must be regulated precisely, and Levine proposes that different mechanisms account for their expression at different times in development.

For example, homeo box genes called *eve* and *ftz* (pronounced 'futz') regulate the position of even-numbered and odd-numbered body segments, respectively, in fruit-fly embryos. Early in development, says Levine, so-called *gap* genes control their expression; later other regulatory genes take over. *Zen* genes, however, differ in several respects. These homeo box genes regulate the pattern of development in the other direction—along the top-bottom axis of the fly. Unlike *eve* and *ftz*, *zen* genes are active very early in development—as soon as 1½ hours after fertilization. At this time the *zen* gene is on in the top part of the animal and off in the bottom region, owing to an inhibitory factor present in the fertilized egg. Later in the development of the embryo, other mechanisms refine the pattern of *zen* gene expression, Levine says.

Corey Goodman of Stanford University and his collaborators find another role for homeo box genes later in development as the insect's nervous system is forming. Goodman reports that *ftz* gene expression, which helps regulate segmentation stripes early in development, reappears during the genesis of the nervous system at the time that a precursor cell's fate is determined.

Many scattered neuronal precursors appear to express the *ftz* protein transiently until they begin to mature and make neurotransmitters and synapses with other cells. And if *ftz* gene expression is selectively eliminated during neurogenesis, then the identities of some neurons are switched from one type to another. Goodman also says that "the control elements for *ftz* expression are different in segmentation versus neurogenesis." ■ **DEBORAH M. BARNES**

The Large-Scale Structure of the Universe Gets Larger—Maybe

"A decade ago, we'd have thought that as we went to larger scales we'd see more homogeneity in the universe," says R. Brent Tully of the University of Hawaii. "In fact, we see more *in*homogeneity."

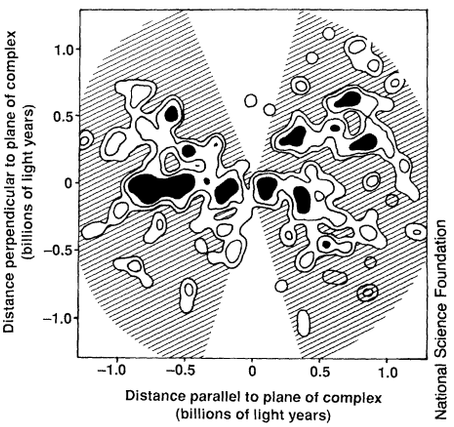
Indeed. During the past decade or so, observers have been finding evidence for bubbles, filaments, and sheets of galaxies on a gargantuan scale. Our own Milky Way galaxy lies near the edge of a huge flattened complex of galaxies known as the Local Supercluster, which is roughly 100 million light-years across. Other superclusters are considerably larger than that. Now, however, Tully has postulated a structure to dwarf them all. If real, his "Pisces-Cetus complex" includes our Local Supercluster as well as all its neighbors, and extends for more than 1 billion light-years. Moreover, says Tully, "the structure is bizarre in that it's defined by a plane—which also happens to be the plane of the Local Supercluster."

In fact, it was Tully's earlier work in mapping the Local Supercluster that first led him to Pisces-Cetus. "I kept trying to find the edge," he says. Eventually he went to the published literature, where he collected the redshifts that others had obtained for clusters in the classic catalog compiled by the late George Abell in the 1950's. Confining himself to clusters within about a billion light-years, where the Abell catalog is reasonably complete, and using the Hubble relation to convert redshifts to distances, Tully then mapped the clusters in three dimensions. The flattened Pisces-Cetus structure was the result.

Tully is the first to admit that his map is hardly conclusive. "The Pisces-Cetus plane has all kinds of holes in it," he says. "We're over to one edge in the Local Supercluster, and there happens to be a deficit of nearby galaxies between us and the center. So I'm talking about an alignment of planes here, not an actual connection."

On the other hand, says Tully, a chance alignment this good would be an incredible fluke. Moreover, the center of the Pisces-Cetus complex happens to lie in the south celestial hemisphere, where the data on galaxy clusters is still relatively poor. "My guess is that when we look, we're going to find the connection," he says.

Among other astronomers, however, the universal comment is "I'm skeptical." The theorists know of no way such a monster could have condensed in the time available since the Big Bang, especially considering that the 2.7 K background radiation reveals a universe that was very homogeneous in the beginning. "If this is more than just join-the-dots then it's very difficult to understand," says Simon White of the University of Arizona.



The Pisces-Cetus complex. *Here the density of galaxy clusters is plotted over a billion-light-year span. The band across the center is Tully's Pisces-Cetus complex. The light sectors perpendicular to it represent the "zone of obscuration" in our own galaxy.*

The observers likewise point to well-known deficiencies in the Abell catalog. In particular, it was all too easy for Abell (or anyone else) to miss distant clusters near the plane of our own galaxy, where they are obscured by interstellar gas and dust. Is it pure coincidence that Tully's hypothetical complex happens to lie almost perpendicular to that plane? And in any case, says Alan Dressler of the Mount Wilson and Las Campanas Observatories in Pasadena, "we don't know enough about the very large scale structure of the universe even to compare this structure with what you would expect from statistical effects."

On the other hand, few researchers seem willing to dismiss the finding out of hand. Tully is not the only one finding evidence for very large structures these days. Dressler, for one, has recently been advocating that our Local Supercluster is under the gravitational influence of a "Great Attractor," a mass concentration some 150 million light-years away. (The Great Attractor would also be a part of Pisces-Cetus.) Even this structure is hard for the theorists to accommodate, says Dressler, although it is only one-fourth the size of Tully's. But then, he adds, it is only when our theories fail that we begin to learn something. ■

M. MITCHELL WALDROP