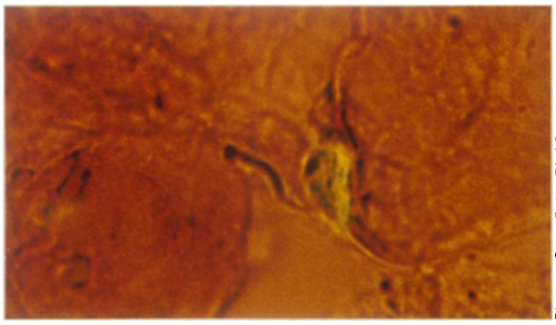
Cells of Babel

An individual nerve cell can manufacture different transmitter chemicals and thereby speak simultaneously various languages of the brain



Photos: Chan-Palay, PNAS

By JULIE ANN MILLER

Transmitter chemicals are the languages of the brain. They are the means, along with electrical signals, by which each nerve cell communicates with others to process information and send messages to the rest of the body.

When the oldest of today's neuroscientists were in medical school, there was only one known chemical signal between nerve cells — acetylcholine as the excitatory neurotransmitter. When the mid-age group was trained, there were two: one excitatory and one inhibitory. By the time the younger scientists were instructed, in the early 1970s, three to six neurotransmitters were taught. But in the last 10 years the transmitter identification business has boomed, and about 30 are now recognized and more are expected to be discovered.

While there is plenty of room among the more than 10 billion neurons of the brain for all the neurotransmitters to be assigned to separate groups of cells, the situation appears to be more complex. Findings of the last few years (SN: 11/29/80, p. 342) have overthrown what had been the basic rule that each cell makes only one transmitter chemical. At the recent meeting of the Society for Neuroscience in Minneapolis, instead of arguing whether any cell releases more than one transmitter, some scientists were now asking, "Does any nerve cell secrete only one?"

Demonstration of multiple transmitters comes from both examining nerve cells grown in isolation and visualizing, in brain slices and peripheral tissue, transmitters and the enzymes that make and destroy them. The potential neurotransmitters are categorized as: the "traditional" small transmitter molecules, such as acetylcholine, norepinephrine, dopamine and GABA (*gamma*-amino butyric acid); amino acids; peptides, which are strings of amino acids; and purines, which are ringed structures cells also employ both in energy transfer and in genetic material.

Above, sensitive visualization methods reveal human nerve-muscle junction with brown label marking enzyme that makes the transmitter acetylcholine in nerve fiber (arrow) and green-yellow marking enzyme that breaks it down.

Co-transmitters from a cell may work in a variety of patterns. They may act upon the same cells or on different cells; they may have additive effects or modulate or interfere with one another. The relative amounts of two or more neurotransmitters released may even fluctuate during a cell's lifetime. The coexistence of transmitters calls for careful investigation of the cell mechanisms "that allow for orderly synthesis, transport and release of the compounds, and, of course, [investigation of] its significance in the complexity of nerve circuits," says Victoria Chan-Palay of Harvard Medical School.

These new ideas on multiple neurotransmitters lead to an image of the brain as the lobby of the United Nations, full of people speaking different languages. Many speakers are conversant in more than one language and pepper their talk with phrases of second or third languages. They may even change among their languages for just the right nuance, perhaps speaking some languages more loudly than others. People talking in the same language would tend to cluster near those who could understand it. The overall effect from a balcony above would be babble. But by circulating among the crowd, an observer armed with the proper linguistic skills would find valuable information being effectively exchanged.

One challenge to the previous belief that a nerve cell releases only one transmitter came from work on cells grown in laboratory dishes. Over the last 10 years, Edwin J. Furshpan, David D. Potter and colleagues at Harvard Medical School have studied rat neurons that release norepinephrine to increase heart rate. They find that when the same type of nerve cells are grown under certain conditions, most of the neurons release acetylcholine, which depresses, rather than boosts, heart rate.

"We were surprised. We had thought of the transmitter as a fundamental aspect of a nerve cell," Furshpan says. "But we saw it is labile under special conditions." They are now studying the crucial factor, a protein that diffuses from surrounding nonneuronal cells through the medium on which the cells are grown. "It seems like a signal, not a nutritional factor," Furshpan says.

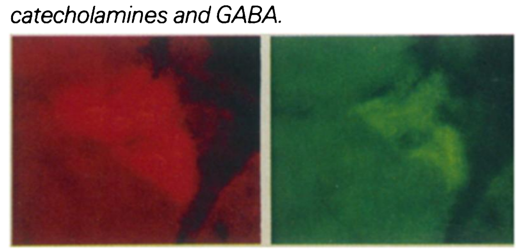
To be certain they are not looking at transmitters produced by different cells in a heterogeneous population, the scientists are studying single nerve cells grown in isolation. In these technically difficult experiments, they have observed one cell change over a period of several weeks from releasing one transmitter to releasing the other; and in other instances they have seen cells undergo a partial transition. "Neurons while they are changing could release both transmitters simultaneously," Furshpan says. "We don't know if they ever release both in their natural setting."

In addition to these dual effects, the researchers have recognized evidence for yet a third transmitter acting at the same location. It is inhibitory, like acetylcholine, but is not blocked by the same drugs. They think it is a purine but have not conclusively identified it. Furshpan says he wouldn't be surprised if these nerve cells also were to release a peptide neurotransmitter.

Evidence for cells having the capacity to produce more than one "traditional" neurotransmitter comes also from studies of cells in their normal settings. In samples of tissue prepared for microscopy, biologists identify a transmitter or enzyme with antibodies and then tag the antibodies with dyes to visualize them. Chan-Palay and collaborators have demonstrated that single nerve cells at the well-studied junctions with muscle contain enzymes for producing at least four neurotransmitters. Acetylcholine is known to be released when the motor nerve is stimulated, and it evokes a contraction of the muscle. Chan-Palay finds, however, in addition to the enzymes that synthesize acetylcholine, those that synthesize GABA; catecholamines, which include norepinephrine and dopamine; and taurine, an amino acid used as a transmitter.

"The fact that transmitter-synthesizing enzymes occur at a given locus implies, but does not prove, that the neuroactive substances are produced in amounts suitable for neural communication," Chan-Palay and collaborators say in the November PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES. "Nevertheless, the coexistence of several transmitter specific enzymes ... in motor end plates raises two obvious questions: What role might they

In human nerve-muscle junction, red and green fluorescence marks enzymes that make different neurotransmitters — catecholamines and GABA.



play in neuromuscular transmission? Do the substances synthesized — taurine, GABA, and catecholamines — participate in or modulate nerve-muscle interaction?"

Cells that produce both a traditional and a peptide neurotransmitter were among the first multiple transmitter cells to be discovered. Both chemicals appear to be made simultaneously. In the late 1970s Tomas Hokfelt of the Karolinska Institute in Sweden and Chan-Palay and colleagues at Harvard both demonstrated a traditional transmitter called serotonin and the peptide called substance P coexisting in individual nerve cells. From her findings, Chan-Palay suggests that a cell might contain varying amounts of these two substances at different times.

Keys to the function of multiple transmitters may be found in the organization of cells. In recent work Chan-Palay has looked at nerve cells in the cerebellum area of the brain. These cells, called Purkinje cells, differ among themselves in the transmitters they produce. They can contain the traditional neurotransmitter GABA, the peptide transmitter called motilin or the amino acid neurotransmitter taurine. Regions containing neurons with each transmitter are precise zones that interdigitate and overlap; some nerve cells have two of the transmitters and those in one central band have all three.

Chan-Palay, Sanford L. Palay and Jang-Yen Wu conclude in the July PNAS, "The ordered distribution of Purkinje cells with different chemical signatures throughout the cerebellar cortex indicates that they, and probably other cerebellar cells, are arranged in a mosaic of interlocking specificities, reflecting their topographical and functional roles."

The Purkinje cells, the most picturesque of neurons with their elaborate branches of dendrites and long axons, inhibit their target neurons, apparently by several means. Chan-Palay and co-workers have shown that each of the neurotransmitters the cells may contain — enkephalins and somatostatin as well as GABA, motilin and taurine — depress firing of the target neurons. The effects of some were additive, interactions of others were more complex.

"It remains for future investigations to reveal the significance of these various agents and combination of agents and to explore the nuances encoded in these multimodal messages," the scientists say.

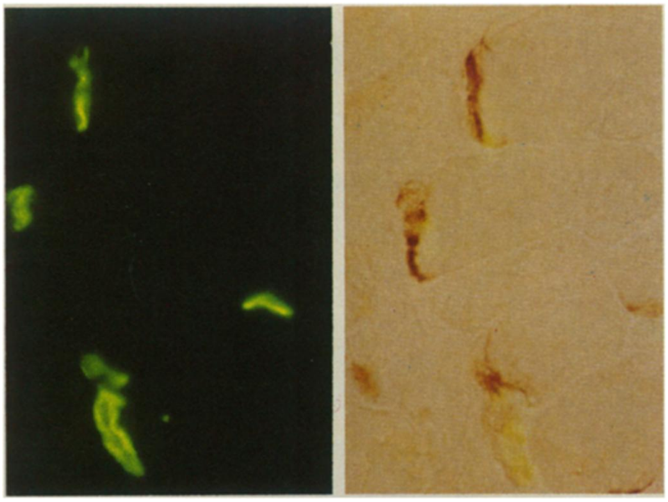
With the wide array of signal chemicals now recognized, the distinction between neurotransmitter and hormone-releasing cells is breaking down. Neurotransmitters are considered chemicals that act only on cells across a classical nerve-nerve or nerve-muscle junction, called a synapse. Hormones travel longer distances to carry slower signals to receptive cells. But now it appears that single cells may release chemicals that act in each capacity, and some chemicals may even have both actions.

"The classical synapse doesn't tell the whole story," says Yuh Nung Jan of the University of California at San Francisco. In certain nerve cells of frogs, for example, along with acetylcholine a peptide may be released. The peptide resembles a hormone mammals rely on for reproductive function, LHRH (luteinizing hormone-releasing hormone). Whereas the acetylcholine acts only on the cells directly across the synapse, the peptide seems able to diffuse to act on other cells microns away. Jan proposes that the peptide mediates the action of acetylcholine.

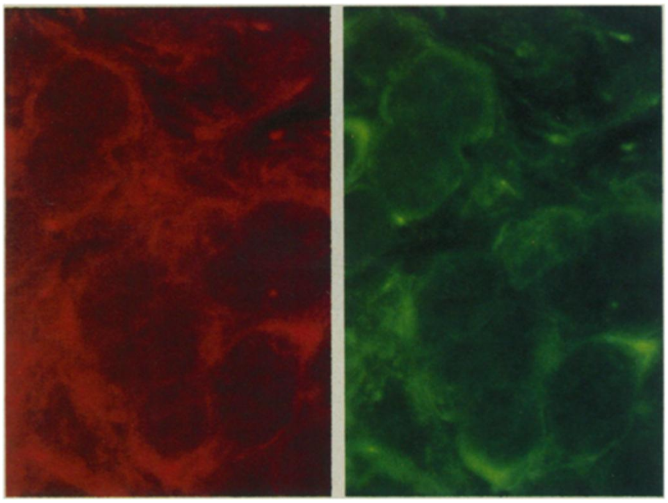
Only recently has there been evidence that some co-transmitters modulate the action of their partners. Peptides as co-transmitters can have two types of effects in the autonomic nervous system, says Jan M. Lundberg of Karolinska Institute. For example, VIP (vasoactive intestinal peptide) acts as a neurotransmitter when released from nerve cells that control blood vessel dilation. But in cells innervating secretory glands, VIP causes no response administered by itself, but it may enhance the cells' response to co-transmitter acetylcholine, Lundberg says.

Another example where the distinction between hormones and neurotransmitters is breaking down is egg-laying hormone of the slug, *Aplysia.* Egg laying is a stereotyped behavior lasting several hours (SN: 9/29/79, p. 219). Earl Mayeri of UCSF reports that the egg-laying hormone, a peptide, both acts locally as a transmitter and diffuses between the cells to serve as a hormone in the reproductive system.

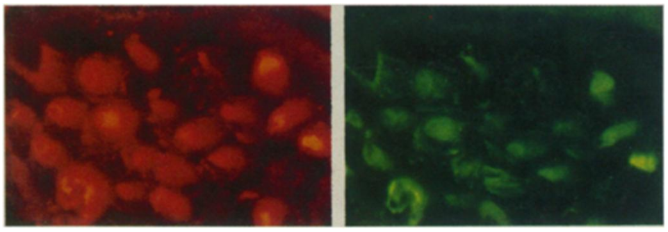
Whether they act as classical transmitters, as hormones or as something in between, the coexistence of several chemical signals opens possibilities for single cells to exert a spectrum of influences rather than simply a discrete effect. This new perspective on nerve cell interaction highlights the complexity of subtle communication possible within the brain. □



Staining techniques reveal the location of specific enzymes. These micrographs show two images of a group of human nerve-muscle junctions where the enzyme that breaks down the transmitter acetylcholine appears green and the key enzyme in the production of catecholamine neurotransmitters appears brown.



Another set of nerve-muscle junctions is stained to reveal the enzyme that breaks down acetylcholine (red) and the enzyme that catalyzes formation of another transmitter, taurine (green). Taken together, the double-staining experiments indicate all four enzymes are in a single junction.



Enzymes for catecholamines (red) and GABA (green) are revealed in nerve fiber bundle in human muscle.

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This Week

Research Notes

Articles

Departments

Science on the Air

Science News prints the latest written word of scientific development and noteworthy news. We've set this space aside to inform our readers of programs of scientific interest that are scheduled on television and radio. Check your local listings for exact times. (R) indicates a repeat broadcast.

- **Jan. 10** (PBS) **"Right From the Start"** investigates the importance of infant/parent relationships during the critical days following birth.

- **Jan. 12** (PBS) National Geographic Society—**"Rain Forest"** explores the varieties of plant and animal life that exist in the rain forests of Costa Rica.

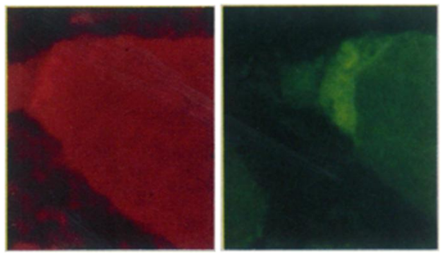
Series

- (PBS) **"Nature": Jan. 2 "Living Together"** is an exploration of the relationship between the behavior animals exhibit and the kinds of communities in which they live and function.

- (PBS) **"NOVA": Jan. 4 "The Making of a Natural History Film"** (R) gives a behind-the-scenes look at the making of a film about the stickleback fish; **Jan. 11 "Salmon on the Run"** (R) captures the power and determination of this threatened fish; **Jan. 18 "Hawaii: Crucible of Life"** reveals more than any tourist ever saw of this popular vacation spot; **Jan. 25 "The Pleasure of Finding Things Out"** is a candid portrait of Nobel Prize-winning physicist Richard Feynman.

- (PBS) **"Odyssey": Jan. 6 "Some Women of Marrakech"** (R) provides an unusually intimate glimpse into the lives of several Moroccan women.

- (PBS) **"Life on Earth"** (R): **Jan. 16 "The Infinite Variety"** investigates the factors behind the 4 million different forms of life; **Jan. 23 "Building Bodies"** examines three groups of invertebrates that have endured; **Jan. 30 "The First Forests"** explores the fight for survival of the first inhabitants of the land.



Nerve multimodal messages

Holiday colors reveal that nerve cells may be multilingual, producing different signal chemicals perhaps to achieve nuances of communication. Current microscopy techniques allow scientists to label two chemicals with different fluorescent dyes in the same preparation and then observe the location of each. The micrographs above show that the nerve cell of a single human nerve-muscle junction contains the enzymes required to produce at least two transmitters, the enzyme for catecholamines (red) and for GABA (green). See page 394