ber of brown dwarfs suggests that the distribution of mass of brown dwarfs does not extend to masses as small as giant planets. The new measurements indicate that brown dwarfs orbiting solar-type stars are very rare. The explanation for this rarity, although unknown at present, is probably related to the different formation mechanisms for massive planets and brown dwarfs.

Another remarkable aspect of the data is the discontinuity of orbital eccentricities of companions less massive than about 0.005 $M_\odot$ as compared with companions in the stellar domain of masses. This behavior is in good agreement with the standard model of planetary formation. Planets are thought to originate in a protoplanetary disk of gas and dust from the collisional accumulation of successively larger planetesimals, which move in nearly circular orbits. On the other hand, initially eccentric orbits are natural in double-star systems because they result from the collapse and fragmentation processes in a mass of gas and dust.

The discovery of giant planets orbiting solar-type stars with small orbital radii raises the question of how they formed. One mechanism that has been proposed is core accretion leading to the formation of rocky cores of about 10 Earth masses, which are then massive enough to accrete gas from the protoplanetary disk. This process requires about 10 to 20 million years to form Jupiter-mass planets. The other mechanism is gravitational instability and proceeds much more quickly, in about 100,000 years. In this process, an unstable disk breaks up into giant gaseous protoplanets where dust grains settle down. Boss proposed that the observation of optically visible young stellar objects, over a period of decades, should allow determination of which of the two mechanisms is responsible for the formation of giant planets (*4*). The observation of astrometric wobbles caused by Jupiter-mass protoplanets in young stellar objects with an age in the range of 0.1 to 1 million years would rule out the core accretion mechanism. However, if gravitational wobbles are found only in the older young stellar objects, the core accretion would be the favored mechanism of giant-planet formation. According to Boss, a sample on the order of 100 young stellar objects of different ages would be necessary to identify unambiguously the formation mechanism.

In both proposed mechanisms, giant planets should only form in the relatively cool outer regions of protoplanetary disks. The discovery of Jupiter-mass planets with orbits very close to their stars causes a considerable problem because it is difficult to understand how such planets could form in place. Five Jupiter-mass planets found orbiting solar-type stars have orbital radii smaller than the distance from Mercury to the sun. The suggested explanation is that Jupiter-mass planets can form at an orbital radius of a few astronomical units and then migrate inward (*5*). Various migration mechanisms have been recently proposed, but it is still not possible to distinguish them observationally. Further searches with improved and diversified means of observation are strongly needed. Clearly, the discovery of planetary systems outside our solar system has opened a Pandora's box of startling phenomena and new questions.

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PERSPECTIVES: NEUROSCIENCE

# A Tale of Two Transmitters

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Scientists are crazy people. How else would you describe an individual who works late into the night in order to destroy or falsify another scientist's hypothesis, or even more bizarre, to destroy his or her own hypothesis? Yet, as clearly enunciated by the philosopher Karl Popper, this is the very essence of scientific inquiry. On the basis of a few bits of data, we form a hypothesis that goes far beyond the data. The hypothesis provides a framework upon which experiments are designed to verify—or refute—the hypothesis. The longer the hypothesis can withstand these potshots, the more likely it is to be "true." More often than not, hypotheses do not withstand the onslaught of experiments and have either to be abandoned altogether or to undergo major overhauls. As cumbersome as it may seem, this is the way science advances. The history of Dale's principle, which receives a direct hit from a series of elegant experiments reported in this issue of *Science* on page 419 (*1*), is a beautiful example of this process.

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In the early 1930s Sir Henry Dale was struck by the strict separation of neurons in the peripheral nervous system that used the transmitter acetylcholine from those that used adrenaline (later shown to be noradrenaline). To reflect his notion that each neuron was a single biochemical unit, he proposed the terms cholinergic and adrenergic to characterize the two classes. In his 1935 Dixon Lecture (*2*) he expanded on this theme and developed what would later become known as Dale's principle, a modern version of which states that a neuron releases a single transmitter from all of its terminals. He suggested that the daunting task of identifying the transmitters used in the central nervous system could be eased by taking advantage of this notion—by assuming that the same transmitter is released from all of a neuron's terminals. Using the spinal primary afferents as an example, he proposed that the substance that caused cutaneous vasodilation when released by stimulated primary afferents would likely also serve as a transmitter at the afferent synapses in the spinal cord. Identification of the transmitter at one site would predict the transmitter at the other. Indeed this approach bore fruit when substance P was found to be released by these neurons (*3*). By this same reasoning, Eccles successfully identified the first transmitter in the central nervous system by showing that motoneuron axons, which release acetylcholine onto muscle, also release acetylcholine from their collaterals onto Renshaw cells in the spinal cord (*4*). Basking in the resounding success of this approach—and possibly feeling a little guilt for the heated arguments he had with Dale over the years as to whether neurons communicated electrically (Eccles) or chemically (Dale)—Eccles immediately elevated Dale's ruminations to the rarefied level of a "principle." (Although Dale always used the singular when discussing the transmitter content of a cell, he never explicitly addressed the issue of multiple transmitters in one cell; only later interpretations linked this idea to Dale's principle.)

Since its original conception, Dale's principle has undergone considerable revision. We now know that more than one transmitter can be released from a single

neuron (although likely from all of its branches), with the most dramatic example being neurons in which a classic transmitter and a neuropeptide are colocalized (*5*). In this case, it is as if two distinct neurons were fused into one, because the synthesis, packaging, and release of the two substances appear in most cases to be independent processes.

The report (*1*) demonstrates the corelease of the two small-molecule transmitters GABA and glycine from single presynaptic terminals. What makes this finding so interesting? First, while the presence of peptides in neurons is extremely widespread, it has, with a few notable exceptions, been impossible to demonstrate electrophysiologically that these peptides actually function as transmitters (*6*). Second, the distribution of glycine (the second identified transmitter in the central nervous system) and GABA (the third) had provided strong evidence for the segregation of transmitters into distinct classes of neurons. Dogma held that glycine was the inhibitory transmitter in spinal interneurons, and GABA served the same role in supraspinal interneurons. What greater separation could there be?

As the years went by, it became clear that GABA was also a presynaptic inhibitory transmitter in the spinal cord (*7*), and that recurrent postsynaptic inhibition of motoneurons, which dogma had held to be purely glycinergic, also had a GABA component—interpreted at the time to involve distinct classes of GABA and glycine interneurons (*8*). Serious doubts about the validity of this two-class notion were raised by immunohistochemical studies that demonstrated unambiguously that many terminals in the spinal cord contained very high levels of both GABA and glycine (*9*). Furthermore, glycine receptors could be found across from terminals labeled for the GABA synthetic enzyme glutamic acid decarboxylase (GAD) (*10*). Now the new report by Jonas *et al.* (*1*) shows not only that the same neuron releases both amino acids, but that the two transmitters are released from the very same vesicle. Such provocative claims require squeaky clean data, and this report provides just that support. Using the technique of paired recording and selective receptor antagonists, the authors show that a single interneuron can generate a fast glycinergic component and a slow GABAergic component. They then show that individual quanta, which represent the release of transmitter from a single vesicle, also have both components. The figure shows the three types of inhibitory synapses. The GABA-only synapses, abundant at supraspinal sites, are only a small (~15%) fraction of the input to spinal motoneurons; the other two classes of synapse contribute equally.

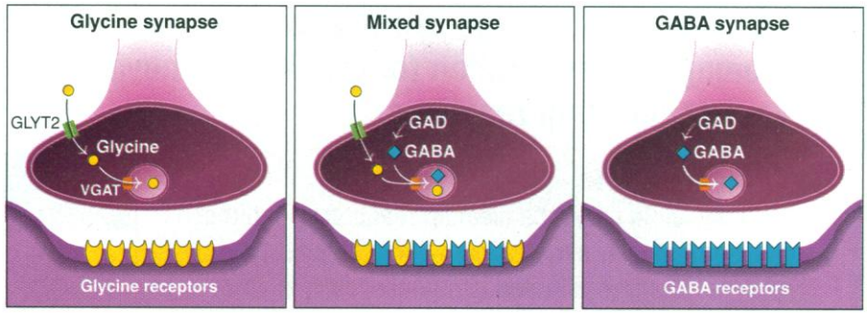
These results raise a large number of questions. We will consider just a few: What is it that determines which transmitter is released by an individual terminal? For GABA the answer is easy—the presence of the GABA synthetic enzyme GAD. For glycine the answer is more difficult, because glycine has many roles other than as a transmitter. However, the cytoplasmic glycine concentration is estimated to be a factor of 10 to 100 times higher in glycinergic neurons than in other cells (*11*). Associated with these neurons is the glycine transporter GLYT2 (*12*); therefore, the simplest explanation is that the presence of GLYT2 in the cell membrane is critical.

How does a single vesicle package both amino acids? There is now considerable evidence that the molecule that loads the vesicles, the GABA vesicular transporter, can also transport glycine (*13*) and, in fact, is present in terminals containing only glycine (*14*).

How does the correct complement of receptors get inserted into the postsynaptic membrane? Glycine receptor activation is essential for the clustering of glycine receptors (*15*). GABA receptors, however, have an identical ionic conductance. So, how on earth can the synapse know which receptor was responsible for the conductance change? Perhaps both receptors cluster at all inhibitory synapses on motoneurons. However, the fact that outside-out patches of membrane isolated on a pipet can respond one of three ways—to only glycine, to only GABA, or to both (*16*)—supports the possibility that the clustering of the two receptors is controlled independently.

Finally, why would the brain go to such efforts to have two transmitters that activate an identical conductance mechanism released from the same synapse? The only difference between the action of the two is that GABA has a more prolonged action than glycine. It seems reasonable to presume that these kinetic differences are critical for motor coordination, which depends on precise timing, but their actual purpose must await more precise knowledge about the reflexes in which these three types of synapse are involved.

Should we mourn the fact that Dale's principle is in trouble? In a limited sense the principle may still stand, because none of the new results show that different processes of the same neuron release a different complement of transmitters. Our new understanding of transmission is, however, much richer and we should celebrate it, as Dale would have done. These recent developments open up a whole host of fascinating issues and new ways to look at old problems about how neurons communicate with one another.




**GABA and glycine: Partners for synaptic inhibition.** **(Left)** The glycine-only synapse is proposed to possess the GLYT2 glycine transporter, which increases cytoplasmic glycine concentrations, and the vesicular GABA transporter (VGAT), which transports glycine into the vesicle. **(Middle)** The mixed synapse contains all of the components of the glycine synapse but in addition contains GAD, which synthesizes GABA. **(Right)** The GABA-only synapse, most prevalent at supraspinal sites, lacks the GLYT2 glycine transporter.

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