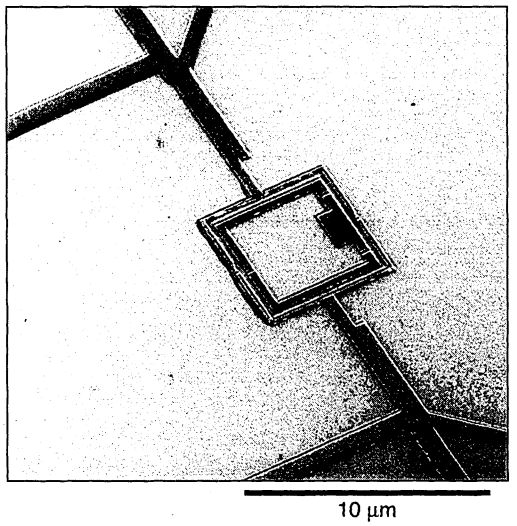

Toward quantum computation. Electron micrograph of the apparatus used by van der Wal *et al.* (*1*) to observe macroscopic quantum phenomena.

vides the necessary computational tools for the design and evaluation of the components.

Despite these impressive advances, however, the fundamental question of the inevitability of a quantum description of macroscopic phenomena has not yet been resolved. One outstanding issue is that of "quantum entanglement." The implications of this extraordinary phenomenon were first explored in a gedanken experiment in 1935 (*3*). Quantum entanglement occurs when two systems share a common quantum mechanical state. Such systems also share a common fate, even if they become physically quite separated. The results of a measurement performed on one will determine the results of future measurements on the second.

The development in time of an entangled state, or in fact any quantum state, occurs without substantial distortion only if the system is isolated from any measurement apparatus or from dissipative elements in the environment. Although van der Wal *et al.* do not address quantum entanglement as such, their careful evaluation of the effect of the measurement SQUID on the macroscopic quantum system is an important first step toward the realization of a macroscopic entangled state. Quantum entanglement lies at the heart of entanglement-based schemes for quantum cryptography (*4*) and quantum teleportation (*5*). It seems likely that the paradoxes of the past are about to become the technology of the future.

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PERSPECTIVES: BIOMEDICINE

Combating Parkinson's Disease—Step Three

Lars Olson

About one million Americans suffer from Parkinson's disease (PD), and each year 50,000 individuals are diagnosed with this neurodegenerative disorder. Because early symptoms of PD may go unrecognized, perhaps as many as 5 to 10% of individuals over 60 years of age may have the illness. It has been known since the 1960s that loss of dopamine neurons in the nigrostriatal pathway of the brain results in the motor abnormalities characteristic of PD. The quest for improved PD treatments continues in incremental steps. Replacing dopamine neurotransmitter that is lost as the dopamine neurons degenerate (step 1) is the mainstay treatment for PD patients. The next steps—transplanting fetal nerve tissue to replace dopamine neurons that have been lost (step 2) and halting neuronal loss altogether with trophic factors (step 3)—are still in the early stages of clinical testing. A breakthrough for step 3 is now at hand with the report by Kordower *et al.* on page 767 of this issue (*1*). These investigators show that gene therapy with glial cell line–derived neurotrophic factor (GDNF) results in the rescue of dopamine neurons and reversal of motor deficits in a primate model of PD.

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Although known since ancient times, PD was not formally defined until 1817 when shaking palsy (paralysis agitans) was described by James Parkinson (*2*). For this the "saddest of diseases," Parkinson realized that "until we are better informed respecting the nature of this disease the employment of internal medicines is scarcely warrantable." Indeed, it was not until the 1950s that the first major breakthrough came with the discovery by Arvid Carlsson that dopamine is a neurotransmitter in its own right (for which he has won this year's Nobel Prize in Physiology or Medicine). Treatment with L-dopa (levodopa), the immediate precursor of dopamine, was shown to replenish brain dopamine levels and to counteract parkinson-like states induced in experimental animals (*3*). This led to the realization that patients with PD had severe dopamine loss in the basal ganglia (putamen and caudate) and to the development of L-dopa medication, still the mainstay treatment for PD.

Replacing lost dopamine neurons by grafting fetal nerve tissue (*4*, *5*) into the brains of PD patients has been efficacious in some patients (*6*) and remains an option for those with late-stage disease. The ultimate goal, however, is to reduce the need for cellular replacement strategies by halting the continuing loss of midbrain dopamine neurons and inducing sprouting of additional nerve fibers in those neurons that remain. Now known to be part of a family of four neurotrophic factors and to exert effects through a dual-receptor complex, GDNF is a potent stimulator of dopamine neuron growth in vitro (*7*). This trophic factor has also proven effective in a variety of PD animal models, including primates (*8*), thus raising hopes that it may be valuable clinically. However, delivery of GDNF to the correct target areas of the brain has proven problematic and was perhaps the reason for termination of a clinical trial in which GDNF was injected into the cerebrospinal fluid (thus reaching much of the central nervous system) of PD patients.

A principal difficulty with providing trophic support for neurons in the brain is that neurotrophic factors are proteins that do not easily cross the blood-brain barrier. Moreover, they typically exert a multitude of effects, often both inside and outside the nervous system, depending on the distribution of their receptors in different cells and tissues. Unlike hormones that are released into the circulation and reach receptors in remote target tissues, neurotrophic factors operate locally—they are taken up by nerve terminals and then are transported along axons to nerve cell bodies (see the figure). To benefit from the therapeutic effect of a neurotrophic factor while avoiding unwanted side effects, local delivery schemes—such as direct injection into brain target areas rather than systemic administration—work best.

To overcome the local delivery problem, Kordower and co-workers injected the GDNF gene carried in a lentiviral vector directly into the basal ganglia and substantia nigra of rhesus monkeys. The authors capitalized on the effectiveness of a new

generation of vectors adapted from lentiviruses that readily deliver the therapeutic gene into nondividing cells including nerve cells (*9*, *10*). To better model human PD, the investigators chose to work with rhesus monkeys whose nigrostriatal dopamine system is organized much like our own. Unlike most rodents but like humans, primates are very sensitive to MPTP, a nerve toxin known to specifically damage dopamine neurons. The authors worked with aged rhesus monkeys (in which markers of the dopamine system have decreased, a feature of normal aging in both monkeys and humans) and with young adult animals rendered parkinsonian on one side by unilateral injection of the MPTP neurotoxin. They injected a lentiviral vector carrying the human GDNF gene or a control (reporter) gene into target brain areas (the putamen, caudate nucleus, and substantia nigra) of aged and toxin-treated monkeys (see the figure). In animals given the human GDNF gene, the trophic factor was produced in brain parenchyma for as long as 8 months.

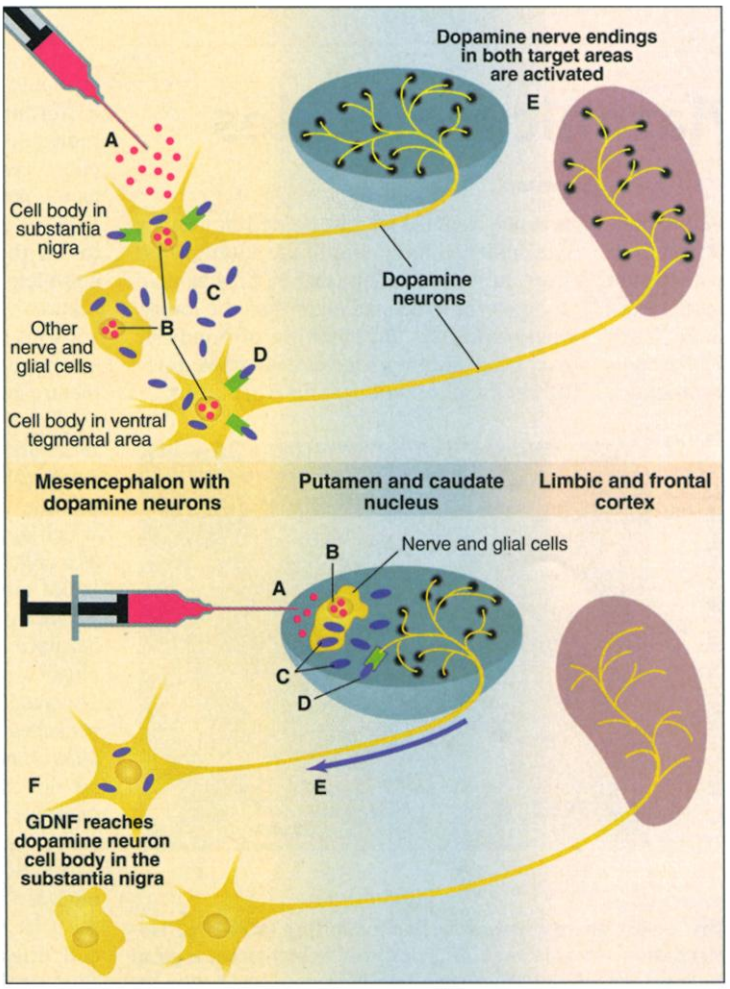
Brain imaging of fluorodopa uptake with positron emission tomography (which provides a measure of dopamine neuron activity) yielded good evidence that GDNF stimulated the dopamine system in living animals. Importantly, the unilateral parkinsonian symptoms induced in the MPTP-treated group—monitored using a clinical rating scale, and a hand-reach test that provided a measure of motor performance—began to decrease 1 to 2 months after GDNF gene delivery.

Kordower *et al.* also examined the biochemical and cellular effects of GDNF gene therapy. They looked at expression of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, and measured levels of dopamine and its metabolite homovanillic acid (HVA) to confirm robust stimulation of dopamine neurons by GDNF. In GDNF-treated monkeys (but not untreated animals), they observed an increase in the amount of tyrosine hydroxylase per neuron, a greater number of dopamine nerve cell bodies expressing tyrosine hydroxylase, and increased expression of tyrosine hydroxylase in the nerve terminal networks of brain target areas.

The Kordower study provides an optimistic outlook for treating not only PD but also other fatal neurodegenerative diseases such as amyotrophic lateral sclerosis. GDNF is a trophic factor for motor neurons (the neurons that degenerate in amyotrophic lateral sclerosis), and a similar lentiviral gene delivery scheme completely rescued injured motor neurons in mice (*11*).

Before GDNF gene therapy can enter the clinical arena, however, there are a number of hurdles that must be overcome. These include controlling the dose of the GDNF gene and protein, and circumventing the inherent risks associated with gene delivery by viruses. Kordower and colleagues noted that certain measures of dopamine levels in nigrostriatal neurons were abnormally high in GDNF-treated monkeys. Given that antipsychotic drugs work by counteracting the effects of dopamine, patients could theoretically be driven from a parkinsonian state (dopamine underactivity) toward a psychotic illness (dopamine overactivity). Local application of GDNF to the basal ganglia (containing dopamine nerve endings) rather than to the mesencephalon (containing dopamine nerve cell bodies) should minimize this risk (see the figure). A lentiviral gene delivery system (*12*) that can be switched on and off may allow the dose of trophic factor to be regulated, much as patients today titrate their L-dopa intake to avoid the side effects of too much or too little dopamine.

The fourth step in the fight against PD is to detect neuronal loss before it becomes too severe and to prevent such loss altogether. But to achieve step 4, we will have to discover what causes dopamine neurons to die in the first place. Neuropathology makes it clear that PD affects not only the nigrostriatal dopamine neurons but also many other nerve pathways including those of the peripheral and enteric nervous systems (*13*). Recent genetic research underscores the multifactorial nature of PD. Although it is well established that the rare early-onset form of PD is an inherited autosomal dominant disease, it is now apparent



Hitting the right target. Injection of a lentiviral vector (red dots) carrying a GDNF therapeutic gene into brain target areas results in insertion of the gene into the DNA of different cell types. (**Top**) When injected into the brain mesencephalon, the lentiviral vector reaches glial cells, nerve cells, and the cell bodies of dopamine neurons that project to the basal ganglia (caudate and putamen) and to the frontal and limbic cortex (**A**). These cells then begin to make GDNF protein (purple ovals), which is then secreted (**B**, **C**). Both types of dopamine neurons have GDNF receptors (green rectangles) (**D**); when the receptors bind secreted GDNF, the dopamine neurons are stimulated, leading to increased dopamine release in the caudate and putamen, and in the limbic and frontal cortex. (**Bottom**) Injection of the lentiviral vector containing the GDNF gene directly into the basal ganglia (**A**) results in the incorporation of the gene into the DNA of neurons and glial cells (**B**), which then make and secrete GDNF protein (**C**). The nerve terminals of dopamine neurons have GDNF receptors and are able to internalize secreted GDNF (**D**). This trophic factor is then carried by retrograde transport along axons to the cell bodies of dopamine neurons in the substantia nigra (**E**). In this way, GDNF selectively reaches only the dopamine neurons of the substantia nigra in the mesencephalon and does not activate dopamine nerve endings in the limbic and frontal cortex, thus avoiding possible side effects caused by overactivation of limbic and cortical dopamine pathways.

that there are also genetic components to the common late-onset forms of PD (*14*, *15*). Until we attain a full understanding of the causes of PD—a prerequisite for preventing the disease altogether—L-dopa and cell therapies supplemented with GDNF gene therapy may become the treatments of choice for PD patients. Unlike the first two treatments, GDNF gene therapy has the added promise of maintaining the wiring diagram of dopamine neurons in the brain's nigrostriatal pathway. Cocktails of genes encoding a medley of neurotrophic factors, delivered by safe inducible vectors to target areas in the brain and spinal cord, hold promise as a treatment not only for PD but also for an entire spectrum of other central nervous system disorders.

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PERSPECTIVES: CHEMISTRY

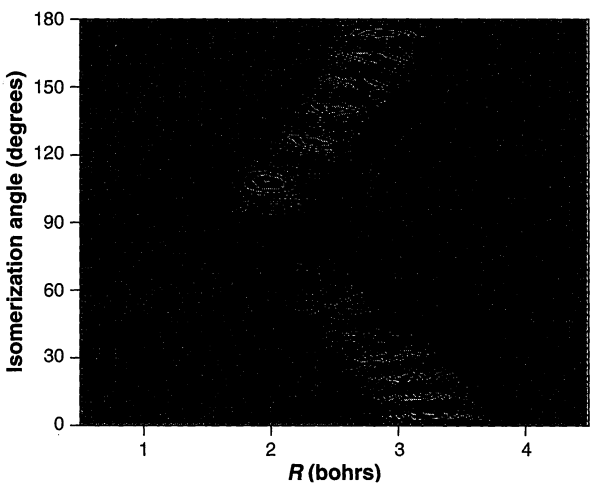
Beyond Platonic Molecules

Joel M. Bowman

Nearly every issue of *Science* contains beautiful depictions of molecules. But these depictions are "platonic," showing idealized, static structures. Real molecules are not static: They move and may do so in a regular or a complex, almost chaotic fashion. A well-known example is the cis-trans isomerization in retinal, which is of central importance in vision. Chemical reactions are a more general example of atoms on the move.

Recent studies suggest that it may be possible to control this motion with lasers, thus influencing the reaction outcome. To understand and manipulate molecular motions, however, we have to first find a way to describe (and computationally model) them. To do so rigorously requires the use of quantum mechanics, especially in the case of the ubiquitous, light hydrogen atoms, which can, in many situations, tunnel over large distances with significant probability. The correct quantum mechanical and the idealized platonic picture of a molecule can sometimes be reconciled in a structure depicting the expectation values of the bond lengths and bond angles at 0 K or in a perfect crystalline state. In most other instances, however, the platonic and quantum mechanical pictures cannot be reconciled, and molecular vibrations cannot be ignored. The resulting theoretical and computational challenge is formidable.

Two recent workshops (*1*, *2*) focused on how molecules behave when they are vibrationally excited. Highly excited molecules can undergo large-amplitude motion that leads to isomerization (that is, they sample a variety of structures other than the lowest energy one) or the breaking of bonds. It may even have dramatic effects on the rates of chemical reactions—the very heart of chemical dynamics. Several talks highlighted new theoretical connections between the quantum and classical pictures of highly excited molecular motion. Others described new experimental methods that allow isomerization to be probed directly and even the weak interaction of particle physics to be detected in chiral molecules.



The lowest energy wave function exhibiting HCN-HNC isomerization. Linear HCN corresponds to an isomerization angle of 0°, linear HNC is at 180°, and the transition state (the barrier between the two minimum energy structures) is at 67°. *R* is the distance of the H atom to the center of mass of the CN fragment.

The accepted quantum treatment of molecular vibrational motion is based on the Born-Oppenheimer approximation, which rests on the fact that atomic nuclei are much more massive than electrons and thus nearly fixed with respect to electron motion. First, the Schrödinger equation for the electronic energy and the nuclear-nuclear repulsion is solved many times for many different positions of the nuclei in the molecule. The variation of this electronic energy with nuclear geometry, referred to as the potential energy surface (PES), then determines the quantum mechanical behavior of the nuclear motion and gives the complete description of the molecular vibrational dynamics. (The molecular geometry with the minimum electronic energy is often what is represented by the platonic picture mentioned above.)

This dual procedure is extremely computationally demanding. For many years, it was greatly simplified by doing a small-amplitude normal mode analysis (NMA), which approximates the vibrational motion as a collection of uncoupled harmonic oscillators vibrating around a single reference geometry. It is used in such diverse fields as acoustics, structural engineering, geophysics, solid state physics, and chemical physics. The most widely used software packages in quantum chemistry rely on this kind of analysis. But the results of an NMA are not exact and may give a totally inadequate picture of molecular vibrations, because the nonlinear couplings and the anharmonic nature of true molecular vibrations are ignored. Over the past 15 years or so, it has been demonstrated (*3*) that these couplings are crucial in molecular vibrations, especially for excited states.

A striking departure from the NMA occurs for example in the isomerization between HCN and HNC. The three-dimensional wave function shown in the figure samples both the HCN and HNC structures and the transition state. This type of wave function cannot even be approximated by the harmonic oscillator assumption of the NMA, which is limited to the description of either HCN or HNC, but not both. (Another example of the breakdown of NMA is the catastrophic failure of the Tacoma Narrows bridge in 1940.)

Highly accurate ab initio calculations of molecular forces and dynamics have until recently been limited by computational power to three-atom molecules, because the coupling of the vibrational modes enormously expands the scope of the calculation. Increas-

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