Annu. Rev. Pharmacol. Toxicol. 2003. 43:521–44
doi: 10.1146/annurev.pharmtox.43.100901.135934

First published online as a Review in Advance on October 4, 2002

THE *CAENORHABDITIS ELEGANS* DOPAMINERGIC SYSTEM: Opportunities for Insights into Dopamine Transport and Neurodegeneration

Richard Nass and Randy D. Blakely
Departments of Pharmacology and Center for Molecular Neuroscience, Vanderbilt University Medical Center, Nashville, Tennessee 37232-6420; email: richard.nass@vanderbilt.edu, randy.blakely@vanderbilt.edu

Key Words nematode, genetics, Parkinson's disease, apoptosis, 6-OHDA

■ **Abstract** The neurotransmitter dopamine (DA) plays a central role in the coordination of movement, attention, and the recognition of reward. Loss of DA from the basal ganglia, as a consequence of degeneration of neurons in the substantia nigra, triggers postural instability and Parkinson's disease (PD). DA transporters (DATs) regulate synaptic DA availability and provide a conduit for the uptake of DA mimetic neurotoxins, which can be used to evoke neuronal death and Parkinson-like syndrome. Recently, we have explored the sensitivity of DA neurons in the nematode *Caenorhabditis elegans* to the Parkinsonian-inducing neurotoxin 6-hydroxydopamine (6-OHDA) and found striking similarities, including DAT dependence, to neurodegeneration observed in mammalian models. In this review, we present our findings in the context of molecular and behavioral dimensions of DA signaling in *C. elegans* with an eye toward opportunities for uncovering DAT mutants, DAT regulators, and components of toxin-mediated cell death.

INTRODUCTION

In the year 1817, the English neurologist James Parkinson published his "Essay on the Shaking Palsy," which described a devastating neurological disease that deteriorates into uncontrollable tremors, bradykinesia, and loss of motor skills. We now recognize this neurodegenerative disorder as Parkinson's disease (PD). It was not until almost a century and a half later that the biochemical basis for PD was clarified by Arvid Carlsson as a depletion of dopamine (DA) from the basal ganglia, studies for which he was jointly awarded the 2000 Nobel Prize in Physiology or Medicine. His investigations also led to the identification of the DA precursor L-DOPA, which thirty years later is still the principle medication used to alleviate the clinical manifestations of PD. Since these early studies, DA has been found to play a key role in a number of other physiological processes, including cognition, emotion, reward, memory, and endocrine function. Moreover, DA neuronal

dysfunction has been associated with several prevalent neurobehavioral disorders, including drug addiction, schizophrenia, and attention-deficit hyperactivity disorder (ADHD). But despite almost a half-century of intense investigations into DA neurotransmission and its clear role in neurological disorders, much remains to be understood regarding the regulation of DA signaling during normal physiological states as well as what confers the unique sensitivity of DA neurons to damage in PD. The complexity of the human brain, which contains over 100 billion neurons and tens of thousands of DA-containing cells (1), each capable of forming many thousands of synaptic connections, has greatly limited our ability to dissect the regulatory machinery involved in DA neurotransmission. Moreover, the relative inaccessability of DA neurons in vertebrates, and our inability to directly visualize DA neurons and their synaptic connections in vivo, significantly restricts the pace of progress in elucidating the molecular mechanisms involved in DA neurodegeneration. Recently, we and others have taken advantage of the high conservation that exists at a molecular level in DAergic signaling across phylogeny to propose new models for the study of DA neurotransmission in normal and pathogenic states (2–5). In the present review, we discuss how the opportunities presented by the nematode *Caenorhabditis elegans* can increase our understanding of DA signaling, dopamine transporter (DAT) regulation, and DA neuron pathogenisis. We also describe how this system can be utilized to identify new molecular components of DA signaling as well as evaluate pharmaceutics that may be protective in PD.

C. ELEGANS AS A MODEL SYSTEM: GENERAL FEATURES

C. elegans has been well recognized as a powerful model system for dissecting the components involved in neurotransmission and disease (2, 6, 7). Its genome, biosynthetic, and metabolic pathways are highly conserved with mammalian systems, yet their small size (adults are approximately 1-mm long), ease of maintenance in the laboratory (they grow on agar plates coated with bacteria), quick generation time (3 days), and large brood sizes (over 300 progeny per hermaphrodite) allow for the rapid growth of many animals for a variety of cellular, molecular, and genetic analysis (8–10). The transparency of the animal and the ease of making reporter gene fusions allow for examination of neuronal morphology and direct viewing of protein expression patterns within the living nervous system (11–14). Moreover, the availability of a complete three-dimensional map of the 302-cell nervous system allows for the identification of most synapses between neurons (15, 16). The self-fertilizing hermaphrodite permits quick and easy homozygosity of mutations, and males can be used for mating to generate lines with multiple mutations. The completed sequence of the genome, the ability to perform whole-animal PCR (“single-worm PCR”), and the existence of a high-density polymorphism map of a related strain of the wild-type (WT) *C. elegans* allows for quick and easy mapping of mutations within practically any gene (8, 17). Gene knockouts can also be generated (minimum of about one week),

and loss-of-function mutant phenotypes can be evaluated within most cell types with the use of RNA-mediated interference (RNAi) technology (18, 19). With the recent advances in primary *C. elegans* cultures, reliable knockout phenotypes can now be generated in vitro within the nervous system (20), and compounds that are impermeable to the whole animal due to the barrier of the cuticle or that could not be solubilized to sufficiently high concentrations to permit the drug to penetrate the cuticle can now be effectively applied to cultured cells. Also, electrophysiological methods have been developed to examine the electrical properties of a variety of neurons and other cell types both in vivo and in vitro (16, 20).

The *C. elegans* nervous system contains many of the known signaling components and neurotransmitter systems found in the mammalian nervous system. The worm contains acetylcholine, glutamate, γ-aminobutyric acid (GABA), serotonin (5-HT), DA, and neuropeptides (21), among other chemical messengers. Neurotransmitter-specific transporters (membrane and vesicular) and receptors (including G-protein-coupled) are also highly conserved with their mammalian counterparts (21). Most ligand-gated and voltage-gated ion channels are present within the worm, with the notable exception of the voltage-gated Na^+ channel (21); however, the voltage-gated Ca^+ channels are conserved and, as in mammals, could generate the necessary action potentials for neurotransmission (16). Finally, all the known synaptic components involved in synaptic vesicle plasma membrane interactions and exocytosis, including syntaxins, synaptotagmin, synaptobrevin, and SNAP-25, are highly conserved between the worm and mammals (21). This remarkable similarity between the mammalian and worm nervous systems has expedited the identification of novel proteins required for normal synaptic transmission in humans (22).

NEURONS AND GENES SUPPORTING DOPAMINE SIGNALING IN *C. ELEGANS*

The *C. elegans* hermaphrodite contains eight DA neurons, four symetrically arranged cephalic cells (CEPs) and two bilateral anterior deirids (ADEs) in the head, and two bilateral posterior deirids (PDEs), which contain processes that run through the main part of the body and tail (Figure 1) (23). These neurons are believed to be mechanosensory neurons because the microtubule-containing cilium at the end of the dendrites are embedded in the subcuticle and may detect movement or food (see below) (24–26). The CEPs (two dorsal and two ventral) contain a single long dendrite that extends from the cell body near the nerve ring, or "brain," through the length of the head where the ciliated endings enter the cuticle near the nose of the animal (15, 24). The ADE cell bodies are located behind the second bulb of the pharynx, and the dendrites contain ciliated endings that travel into the deirid sensilla (15). The axons of both the CEPs and ADEs are directed into the nerve ring, and the dorsal pair of CEPs may receive some synaptic input from the ADEs (15). The cell bodies of the PDEs are located posterior

to the vulva, and as with other DA neurons, the dendrites contain ciliated endings that enter the sensillum (15). The PDE axons enter the nerve chord, where the anterior process extends to near the nerve ring, and receive en passant synaptic connections from other neurons (15, 27). The *C. elegans* male contains another three pairs of DA neurons that are located within the tail, as well as four DA-containing male-specific spicule socket cells (28, 29).

The DA neurons were initially identified by Sulston and coworkers, who used the catecholamine-specific technique of formaldehyde-induced fluorescence (FIF) (23). DA cell bodies and processes were visualized using fluorescence microscopy, confirmed as DA by alumina absorption and thin-layer chromatography (TLC). The precursor L-DOPA was also identified but not the catecholamines norepinephrine and epinephrine. Based on FIF micrographs and worm DA content as well as the estimated volume of the cell bodies and processes, the concentration of DA in the nerve endings is predicted to be very similar to the concentration within mammalian varicosities (23). We and others [(6); R. Nass, unpublished data] have confirmed via high-perfomance liquid chromatography (HPLC) that DA and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) are present in the animal.

Sulston and coworkers isolated several mutant lines deficient in catecholamine fluorescence. Once cloned and characterized, these mutants reveal that the *C. elegans* DAergic system is at least fundamentally very similar to vertebrates (Figure 2). *cat-1* animals (catecholamine abnormal) have a dramatic reduction in DA content yet over a threefold increase in L-DOPA (23). FIF in this animal was also only seen in the cell bodies and not the processes. The animals were also similar to WT worms treated with the drug reserpine, which depletes vertebrate secretory vesicles of catecholamines (23). Taken together, these results suggest that these mutants are unable to load DA into synaptic vesicles (23). Duerr and coworkers confirmed these results when they identified the mutation in the *C. elegans* orthologue of mammalian vesicular monoamine transporters (VMATs) (30). And like the neuronal VMAT, VMAT2, *cat-1* is associated with synaptic vesicles that are localized to both DA and 5-HT neurons and has a high affinity for DA, serotonin, norepinephrine, and histamine. Furthermore, the *cat-1* behavioral phenotype (see below) can be rescued by the human VMAT2 transgene, which strongly supports the role of *cat-1* as a synaptic vesicle neurotransmitter transporter (30). The VMATs also transport octopamine. Octopamine is present within the worm (31) and is believed to be the norepinephrine analogue found in many invertebrate systems.

Sulston identified two other mutants that have altered DA production, and FIF patterns—*cat-2* and *cat-4*. *cat-2* mutants have a complete loss of DA, as detected by FIF and TLC (23). Lints & Emmons subsequently identified the mutant protein via BLAST and gene sequencing to encode a polypeptide with 50% amino acid identity to tyrosine hydroxylase (TH) (32). TH is the rate-limiting enzyme in DA biosynthesis and is expressed in mammalian DA neurons, and their GFP-reporter expression pattern using *cat-2* promotor fusions agreed with this functional

assignment, revealing expression limited to DA neurons. Based on reductions in 5-HT levels and cuticle defects in the *cat-4* mutants, it has been suggested that this gene encodes a pterin cofactor, GTP cyclohydrolase (GTPCH). The pterin cofactor is used in the biosynthetic pathway of both DA and 5-HT and is involved in worm cuticle formation (23, 33–35). Notably, dopa-responsive dystonia, a disease causing parkinsonian-like phenotypes, is due to mutations within the GTPCH gene (36).

Metabolic pathways for catecholamines also appear to be conserved from man to worm. Sulston identified aromatic amino acid decarboxylase (AAAD) activity in his *C. elegans* preparations (23). AAAD is an enzyme required for the conversion of L-DOPA to DA and for the conversion of 5-hydroxytryptophan (5-HTP) to 5-HT in mammals. Loer & Kenyon loaded 5-HTP in DA neurons and found that they became 5-HT immunoreactive, implying AAAD activity similar to what is found in mammalian systems (34). Mutants containing the *bas-1* (biogenic amine synthesis-defective) allele, which do not produce DA or 5-HT and cannot convert exogenous 5-HTP to 5-HT, maps to the putative AAAD gene (23, 34). *C. elegans* also contains homologues of mammalian DA metabolizing enzymes, including monoamine oxidase (MAO) and catechol-o-methyltransferase (COMT) (6, 37), enzymes involved in the pathways for the conversion of DA to DOPAC or to 3-methoxytyramine (3-MT) and HVA, and we and others have identified these metabolites in whole-animal worm preparations via HPLC [(6); R. Nass, unpublished data].

DA and mammalian DA receptor antagonists alter behaviors in *C. elegans* consistent with the presence of DA receptors in the worm [(38) and references therein]. Exposure of animals to DA results in a decrease of movement, grazing, and egg-laying; however, addition of the mammalian D2 antagonist haloperidol reverses this effect (38, 39). Chlorpromazine, another D2-antagonist, can also blunt the DA contribution to egg-laying (40). We have found that the DA transporter substrate amphetamine, which causes an increase in synaptic DA by eliciting efflux of DA from DAergic terminals, also causes a decrease in egg-laying and larval development (R. Nass, unpublished data) (41). This effect is also blunted in the ΔVMAT *cat-1* line, which cannot package DA for release and also exhibits lower whole-animal DA levels (R. Nass, unpublished data) (41). Finally, gain-of-function *egl-2* mutants contain a mutation in a voltage-gated eag-like K^+ channel that causes a defect in egg-laying and defecation; this phenotype can be suppressed in the presence of different D2 family DA antagonists, including chlorpromazine, haloperidol, butachlamol, droperido, and pimozide (38), suggesting that EGL-2 may participate in circuits modulated by DA. Recently, the first DA receptor was identified in *C. elegans*. CeDOP1 has high sequence similiarity (43%) to the human G-protein-coupled D1-like receptors (D1 and D5) (42). In vitro DA and lysergic acid diethylamide (LSD) binding assays suggest that DA is likely the natural ligand and that CeDOP1 is a DA receptor in vivo. At least nine other cDNAs encoding DA receptor homologs have been cloned from the worm, though the functional identity of these receptors has yet to be determined (6).

The behavior of *cat* mutants, as well as the results of DA neuron cell-ablation studies, support the role that DA is involved in mechanosensation (26). Worms normally move slowly on bacteria relative to when they are off their food source (26). Laser ablations of all the DA neurons [the DA neurons are not required for survival (an advantage in genetic screens, see below)] or mutations that affect DA synthesis or storage (*cat-1*, *cat-2*, *cat-4*, and *bas-1*), cause the animals to move at similar rates on or off bacteria; however, the addition of DA to *cat-2*, *cat-4*, and *bas-1* selectively restores the slower movement on the bacterial lawn, suggesting that DA plays a role in context-dependent locomotion (26). Furthermore, male mating is a complex behavior that requires the normal function of the DA neurons in the male tail; laser ablation of the tail DA ray neurons results in inefficient attempts to locate the vulva for copulation (43). Similarly, well-fed *cat-2* males, unlike WT males, often appear disinterested when encountering hermaphrodites and do not attempt to mate (34). Abnormal DA signaling in *cat-1*, *cat-4*, and *bas-1* males may also contribute to their disinterest in mating with the hermaphrodites (34). Finally, worms exposed to DA become paralyzed and do not lay eggs, but after a few hours are able to move normally (39). *unc-2* mutants do not adapt to DA. *unc-2* encodes for the α-1 subunit of a voltage-sensitive calcium-channel, and calcium-dependent transcription modulation has been implicated in DA reward pathways (39, 44). It will be interesting to determine if the ability of *C. elegans* to adapt to DA exposure shares features common to adaptations found in DA signaling pathways following chronic psychostimulant exposure (44).

MAMMALIAN AND *C. ELEGANS* DOPAMINE TRANSPORTERS

A unique and identifying characteristic of mammalian DA neurons is the presence of the DAT, which is responsible for the reuptake of DA in the presynaptic terminal following release into the synaptic cleft (45–47). DAT localizes to axons and dendrites of mesencephalic and hypothalamic DA neurons, innervating the striatum and frontal cortex, and directly or indirectly regulates locomotor activity, cognition, emotion, reward, and neuroendocrine function (48–50). The transporter is the target for many psychoactive drugs, including cocaine, amphetamine, and methylphenidate. These agents confer euphoria and can lead to addiction. DATs also provide the molecular gateway for the accumulation of neurotoxins that can evoke neuronal death (see below) (47, 51, 52).

Studies in knockout mice indicate that DAT significantly modulates many aspects of DA neuronal function and neurotransmission. DAT knockout mice display a 75% decrease in DA release into the synaptic cleft and a 300-fold decrease in extracellular DA clearance (clearance approximately correlates with normal diffusion) (53, 54). TH levels, as measured by immunohistochemistry, are also dramatically decreased to 90% of WT, although TH activity is increased 200-fold; this concomitant increase in TH activity is not sufficient to account for the reduction in

TH, and cellular DA levels are reduced 20-fold (53, 55). Furthermore, D1 and D2 DA receptors are reduced approximately twofold, and DA autoreceptor function is significantly reduced or completely lost (54, 56). Because of the importance of DAT in DA neurotransmission, it is likely that DAT is under strict regulatory control, but the mechanism of this regulation is poorly understood (51). DATs are now believed to be regulated by posttranslational modification and altered trafficking. Mammalian DATs contain multiple phosphorylation sites, including consensus sites for protein kinase A (PKA), protein kinase C (PKC), calcium calmodulin kinase, and cAMP-dependent protein kinase (57). PKC activation via phorbol esters causes DAT phosphorylation and leads to DAT redistribution from the plasma membrane to intracellular compartments, suggesting that phosphorylation may play a role in DAT endocytosis and localization (58–65). It is not clear though whether the direct phosphorylation of DAT is involved in the internalization or whether another protein mediating cell surface expression of DAT is responsible (66).

Evidence exists that DATs are regulated by multple cell surface receptors. D2 receptor (D_2R) antagonists decrease DA transport in vivo and in vitro, but not in all cell types (67–69). Recently, Mayfield & Zahniser showed that D_2R activation increases DAT expression on the cell surface in *Xenopus* oocytes, indicating that the presynaptic autoreceptor can play a significant role in DAT regulation. Muscarinic agonists also cause an increase in extracellular DA, which could be due to a reduction in DAT activity (70), and $\sigma 2$-receptors may regulate DAT activity via a PKC dependent pathway (71).

Recently, DAT has been shown to have direct interaction, both in vivo and in vitro, with two proteins that influence the density of DAT on the plasma membrane. PICK1, a PDZ domain-containing protein and a PKC binding partner, binds to the PDZ domain recognition sequence at the C-terminas of DAT (72). This association appears to enhance DA uptake by increasing the level of, and possibly stabilizing, DATs on the cell surface. Likewise, α-synuclein, a presynaptic protein that associates with synaptic vesicles and participates in excitation-secretion coupling, has been reported to form complexes with human DAT (hDAT) at the C-terminus that also increases DAT clustering at the cell surface and DA transport (73). Interestingly, the degree to which PICK1 and α-synuclein interact is unknown. Importantly, familial PD has been associated with two independent autosomal dominant missense mutations in α-synuclein (74, 75). Although α-synuclein is a major component of Lewey bodies, the pathological hallmark of PD, it is possible that certain facets of increased susceptibility to DA neurodegeneration in individuals carrying the mutant gene is due to altered DAT-α-synuclein interactions (see below).

We have previously cloned the *C. elegans* DAT, DAT-1 (76). It is a 615-residue polypeptide that is highly homologous to the mammalian DATs (45% amino acid identity), with 12 predicted transmembrane domains and multiple predicted N-glycosylation sites (77). We have expressed DAT-1 in mammalian cells, and similar to its mammalian ortholog, DAT-1 exhibits saturable and high-affinity, Na^+- and Cl^--dependent DA transport ($Km = 1.2\ \mu M$) (76, 77). Transport of DA by

transfected DAT-1 is also potently inhibited by the mammalian DAT antagonist cocaine, nomifensine, and GBR 12909, and the agonist amphetamine (76–78). DAT-1 also exhibits high affinity for tricyclic antidepressants such as imipramine and the more norepinephrine transporter (NET)-selective antagonists, such as nisoxetine. Like its mammalian counterpart, DAT-1 is also expressed exclusively in DA neurons (2, 3). We developed transgenic lines that contain 0.7 Kb of the sequence immediately upstream of DAT-1 fused to coding sequences of the green fluorescent protein (GFP) (2). This construct results in the specific expression of GFP in all eight DA neurons (Figure 1) in the hermaphrodite as well as the three pairs of the DA neurons in the male tail (3). The GFP expression level is intense, allowing for direct visualization of the neurons in live animals as they move underneath the objective of a fluorescent dissecting microscope. We have also generated a translational GFP fusion ($P_{dat\text{-}1}$::DAT-1::GFP) that results in a similar expression pattern, though the coincidence of this expression pattern with endogenous DAT-1 remains to be validated (3). Studies are currently underway to compare alternative GFP fusions and to identify native patterns of DAT localization using site-specific DAT-1 antibodies.

DOPAMINE, DOPAMINE TRANSPORTER, AND PARKINSON'S DISEASE

We have recently considered how the presence of DA neurons in *C. elegans* may be useful in modeling DA and DAT regulation and provide opportunities for PD research (2). PD results from the loss of greater than 80% of the DA neurons within the substantia nigra pars compacta (SNpc). It is the second most prevalent neurodegenerative disorder, affecting 1% of the population over 55 years (79). Although the specific etiology of PD is unknown, abundant pathological data suggests that oxidative stress and mitochondrial dysfunction play a role in the DA neuron degeneration (80, 81). DA itself may be an endogenous neurotoxin, clarifying the specificity of DA neuron vulnerablility seen in PD. Several studies have shown that the highly reactive DA molecule can cause striatal neuronal death both in vitro and in vivo in a variety of organisms (82, 83). DA can be oxidized by at least two independent pathways. It can be oxidized enzymatically by monoamine oxidase to produce the relatively inert metabolites HVA and DOPAC, and it can be nonenzymatically auto-oxidized to produce highly reactive quinones and the superoxide radical. Both pathways also produce hydrogen peroxide, which in the presence of transition metals, such as iron (which is found in higher abundance in PD substantial nigra, possibly due to abnormal ferritin metabolism), can further be oxidized to the highly reactive hydroxyl radical (82, 83). Several studies suggest these reactive oxygen species (ROS) and quinones could be major contributors to the DA neuron cell death because of their ability to cause protein denaturation, lipid peroxidation, and DNA damage (82, 83). DA has also been proposed to cause cellular death via calcium channel activation in a DA receptor-dependent

mechanism (82, 84). Finally, DA can confer toxicity to the DA neurons via catechol hydroxylation to form 6-OHDA (see below) (83).

Exposing the SNpc in rodents and mammals to neurotoxins that cause DA neurodegeneration is the most commonly utilized animal model for PD. Vertebrate exposure to the neurotoxins 6-OHDA, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridinium ion [MPP+ (the active metabolite of MPTP)], or the insecticide rotenone causes SNpc DA neuronal death within several weeks, and the animals exhibit many of the same symptoms as PD (81, 85, 86). 6-OHDA lesioning of nigrostriatal neurons is a particularly intriguing model for PD, because 6-OHDA may be an endogenous neurotoxic metabolite of DA causing or contributing to the disease. The toxin has been identified in untreated rat brains, as well as in brains and urine of PD patients (87–90). It has been proposed that the in vivo production of 6-OHDA occurs via a nonenzymatic reaction between DA, hydrogen peroxide, and free iron at physiological concentrations and that this reaction may be more likely to occur in the highly oxidizing environment of DA neurons (90, 91). The substantia nigra, with its large stores of iron, may provide the catalyst for 6-OHDA production (83, 92). Furthermore, 6-OHDA could be generated in this environment in the presence of DA by either free nitrite ions or manganese (93, 94). 6-OHDA generates an increase in the production of hydrogen peroxide and free radicals, including the superoxide ion and hydroxyl radical (95, 96). These ROS are likely generated through the nonenzymatic breakdown of 6-OHDA and/or the direct inhibition of complex I and IV of the mitochondrial electron transport chain (87, 95, 97). The resulting ROS production leads to lipid peroxidation, protein denaturation, and a decrease in reduced glutathione, which are similar hallmarks found in postmortem PD patients (80, 81).

The specificity of 6-OHDA for the DA neurons lies in its affinity for DAT (54, 55, 87, 98–101). DAT antagonists can block cellular uptake of the neurotoxins both in vivo and in vitro (87, 99–101; R. Nass, unpublished data). Whether DAT expression is important for human PD is unknown; however, DA neurons of the substantia nigra that express the highest levels of DAT in vivo are most affected in PD, whereas the mesencephalic and hypothalamic DA systems that express lower levels of DAT are less severely compromised (52, 102). Because the *C. elegans* DAT-1 is both structurally and functionally similar to mammalian DATs, we asked whether DAergic neurotoxins could gain access to the worm DA neurons and effect their degeneration. Indeed, we found that brief (1 h) exposure of worms to 6-OHDA results in a time- and concentration-dependent loss of DA neuron GFP fluorescence (Figure 3) (R. Nass, unpublished data) (3). Moreover, coincubation of worms with either of two inhibitors of DAT-1, imipramine or D-amphetamine, completely blocked the 6-OHDA effects (3). Using a *dat-1* loss-of-function strain generated by J. Rand and J. Duerr [Oklahoma Medical Research Foundation (OMRF), Oklahoma City, Oklahoma], we found that DAT-1 is required for the 6-OHDA effects (Figure 3) (3). Furthermore, using other lines exhibiting GFP labeling in other neurons (i.e., lacking DAT), we found that the effects are selective for the DA neurons (3). The establishment

and initial characterization of our *C. elegans* model of DA neurodegeneration provides an opportunity to explore fundamental questions concerning the regulation of DAT-1. The 6-OHDA sensitivity of the DA neurons also provide an opportunity to examine the role various endogenous and exogenous compounds, as well as proteins involved in the biosynthetic pathways of DA neurotransmission, may play in normal DA neuron function and disease.

USING *C. ELEGANS* TO EXAMINE THE REGULATION OF DAT-1

The ability to observe live DA neurons in the living worm, to utilize a translational DAT-1::GFP (or CFP or YFP) fusion protein to follow DAT expression levels and localization, and the DAT-1 dependence of the 6-OHDA sensitivity, provide a unique opportunity to explore the structure, function, and regulation of the DAT. Trafficking-based regulation of DAT and the regulation of DA synaptic transmission are poorly understood, but they are under increasing scrutiny as modes by which to localize and control DA clearance (51). DATs also appear to reside, both at the plasma memebrane and intracellularly, in tubulovesicular structures that may represent intermediate trafficking compartments (50). Whether this compartment shares synaptic vesicle components is unknown. Transporters related to DATs appear to reside at steady-state, largely in synaptic vesicle-like structures (103, 104). All known proteins required for neurotransmission in *C. elegans* have homologs in mammalian systems, and well over two dozen mutant proteins that localize to the synapse have been identified (22). Fluorescently-tagged DAT-1-expressing animals (e.g., $P_{dat\text{-}1}$::DAT-1::GFP) can be mated with animals that contain mutations within specific synaptic-targeted proteins to determine in vivo whether these genes may play a role in DAT localization and function. For example, the DAT-1 translational fusion can be crossed into the *unc-104* or *unc-116* lines, which contain mutations within synaptic vesicle motor proteins (105, 106). These kinesin-like molecules are responsible for the transport of a number of synaptic vesicle–associated proteins to synapses, and mutations within these genes accumulate proteins within the neuronal somas (105, 107–109). KIF1A and KIF1B, the mammalian orthologues of UNC-104 and UNC-16, respectively, are also involved in transport of synaptic vesicles to neuronal processes (110, 111). DAT-1::GFP localization can be compared in WT and mutant backgrounds to determine if DAT-1 requires these proteins for proper localization. The sensitivity of these lines to 6-OHDA can also be utilized to determine if DAT-1 function is altered; decreased toxin sensitivity would suggest a failure of DAT-1 to reach or be retained at the plasma membrane. Furthermore, viable *C. elegans* lines exist with mutations in the soluble N-ethylmaleimide-sensitive attachment protein receptor complexes (v- and t-SNARE), *unc-18*, and *unc-64* syntaxin, which are all proteins that are highly conserved with mammals in synaptic vesicle fusion and plasma membrane protein regulation (22, 112, 113). Recently, syntaxin was also shown to directly

down-regulate the GABA transporter GAT1, a member of the DAT family of Na^+- and Cl^--dependent neurotransporters (114). Our translational DAT-1::GFP fusion strain can be crossed into these lines to explore DAT-1 plasma membrane and subcellular localization, regulation, and function.

Mammalian DATs are the target of multiple psychoactive drugs, including the DAT substrate amphetamine and antagonist cocaine. DA neuronal exposure to amphetamine has been reported to cause an internalization of DAT, whereas exposure to cocaine increases DAT on the cell surface (115, 116). The proteins involved in this trafficking modulation are not known; monitoring DAT-1 localization in various synaptic mutant backgrounds could yield insight into this phenomena. As noted, mammalian DATs have been shown to physically interact with two proteins, PICK-1 and α-synuclein, to effect DAT clustering on the plasma membrane and increase DA transport (see above) (72, 73). *C. elegans* contains a homolog to the mammalian PICK1 (Y57G11C.22, 48% AA identity); colocalization studies of PICK1 by fluorescently tagging the protein could help elucidate its regulatory role in vivo and whether sensitivity to 6-OHDA is influenced under normal and oxidizable (6-OHDA) conditions.

SCREEN FOR NOVEL GENES INVOLVED IN DAT-1 REGULATION AND CELL DEATH

Forward genetics provides an opportunity to search for novel genes involved in DAT regulation and DA neurodegeneration (2, 117). The power of a forward genetic screen is that no prior knowledge is necessary concerning the function of the gene in order to identify it as part of a particular regulatory pathway; the only requirement is that the gene is necessary for the phenotype that is assayed. Typically in this type of screen, older worm larvae (P_0) are exposed to a mutagen at a stage of development when the number of germ-line nuclei is at its highest concentration, which ensures the maximum number of mutagenized gametes (118). Following mutagenesis, the eggs from P_0 animals develop into F_1 adults that are allowed to self-fertilize to produce F_2 progeny. The F_2 progeny, in which recessive mutations can now be identified because of their homozygosity, are screened for disruption of behavioral, morphological, or biochemical phenotypes that reflect interference of normal physiological processes. The animals that contain mutant alleles can then be quickly mapped and identified as a protein necessary in the regulatory process.

We are now in the initial stages of a forward genetic screen using our DAT-1::GFP reporter line to identify molecules involved in DAT regulation and 6-OHDA-induced DA neuronal death. Second generation progeny (F_2) of mutagenized animals are being screened for their retention of GFP in the DA neurons following exposure to 6-OHDA. Animals with DA neurons that are insensitive to the neurotoxin could have mutations within DAT or DAT regulatory proteins (Figure 4), for example, which do not allow DAT to efficiently transport the toxin into the cell or localize DAT to the plasma membrane (2).

Alternatively, we envision that a mutation could exist within a cell death pathway that is involved in the toxin-induced neurodegeneration and that could inhibit the onset of neuronal death (see below) (2). Our pilot screen has yielded several dozen mutants that maintain varying degrees of DA neuronal resistance to 6-OHDA. Some of these lines, which are as much as 100% tolerant to the toxin, contain mutations within DAT. The identification of DAT mutants provides proof-of-concept that we should be able to isolate mutations within genes required for proper DAT regulation or genes involved in DA neurodegeneration. We also expect that these lines with mutations in DAT may be able to provide us with insight into critical regions and residues supporting DA transport. A recent study, such as those by Vasudevan et al. (119), with a nucleoside transporter demonstrates how forward genetic approaches can yield insights into facets of substrate translocation pathways (119). Proteins involved in DA production or breakdown may also be identified if cellular concentrations of DA play a role in DAT cell surface expression, regulation, or vulnerability to ROS-induced cell death (51, 82). To this end, DA and ROS have been reported to affect the regulation of DAT (51). In order to determine if DA may also play a role in 6-OHDA-induced DA neuron degenerations, we have crossed our DAT-1::GFP reporter line into mutant lines deficient in DA. Our preliminary results reveal that animals containing lower amounts of DA are more resistant to the toxin; whether this is directly due to a reduction in DAT expression or an overall reduction in ROS-mediated cellular damage due to the loss of DA is not yet clear. Nonetheless, these results are consistent with mammalian studies that suggest that DA itself may mediate the vulnerability of DA neurons to degeneration (82, 83).

Although this model provides significant possibilities for isolating regulators of DAT-1, the screen could miss identifying some proteins involved in proper DAT function. If the protein is required for viability, a loss-of-function mutation precludes isolation by classical means, although the existence of genetic balancers still allows for isolation of these mutations (120). Also, the loss of a regulator of DAT may be missed due to other existing orthologues or other proteins that could compensate for the functional loss of the gene in the worm. Fortunately *C. elegans* often contains only a single locus of a particular gene, allowing for the identification of many genes in biochemical pathways without the interfering aspect of genome redundancy (21).

CELL DEATH AND PARKINSON'S DISEASE

As stated above, our system should also allow for the identification of genes involved in 6-OHDA-induced DA neuron degeneration and cell death pathways reminiscent of PD. The two main types of cell death that can occur during normal development and disease are apoptosis and necrosis (121, 122). Both forms of cell death are morphologically distinct. Apoptosis results in cell shrinkage, nuclear fragmentation, and the formation of apoptotic bodies, whereas necrosis

typically features cell and mitochondrial swelling, cell rupture, and inflammation (122). There are at least two main types of apoptosis—caspase-dependent (classical apoptosis) and caspase-independent (see below). Caspase-dependent apoptosis is the most studied form of programmed cell death that has been identified in invertebrates and mammals (123). This apoptotic cell death pathway is strongly conserved between *C. elegans* and humans (124). Indeed, the molecular components in classical apoptosis were first identified in the worm (123). The BH3-domain protein EGL-1 triggers apoptosis by interacting with CED-9 (cell death abnormal), the mammalian proto-oncogene Bcl-2 homologue. CED-9 is released from the CED-3/CED-4 complex (homologous to the mammalian Caspase-9/Apaf-1 complex) at the mitochondria, and CED-4 translocates to the nuclear envelope where CED-3 is activated and initiates the breakdown of critical cellular components, resulting in cellular death (125, 126).

The mechanism of cell death in PD remains to be elucidated. Indeed, whether classical apoptosis or necrosis play a central role in DA neurodegeneration in PD remains controversial (83, 127, 128). Compared with controls, there are not significant differences in the number of apoptotic bodies, TUNEL-positive cell bodies, or the immunohistochemical patterns or expression levels of the late-stage apoptosis specific protein (ASP) c-Jun/AP-1, nor Bcl-2, or caspase-3 (127, 128). Furthermore, morphological characteristics of apotosis or necrosis, including chromatin condensation, nuclear fragmentation, or cell shrinkage or swelling are not readily apparent (127, 128). Confounding the problem in determining the mechanism of cell death in PD, however, is the apparent long, slow rate of degeneration that occurs within the nigra (at least before the idiopathic form of the disease manifests itself), the relatively quick rate of the apoptotic process (at least in experimental models), the normal background of neuronal cells dying via apoptosis, and the relative small number of cells that are dying at any given time (127–129).

In vivo and in vitro toxin-induced model studies of PD indicate either apoptosis or necrosis depending on the particular type of toxin to which the animal or culture system is exposed. Most in vivo and in vitro DA- or 6-OHDA-exposure experiments suggest an apoptotic form of cell death, whereas MPP^+/MPTP indicate necrotic cell death (130, 131). The pesticide rotenone has been implicated in both apoptotic and necrotic cell death (132–134).

Because classical apoptosis and necrosis are well-defined events in *C. elegans*, we asked whether either of these forms of cell death could be involved in the 6-OHDA-induced degeneration of DA neurons in the worm. Electron microscopy analysis of 6-OHDA-treated worms suggests that the DA neuron degeneration does not occur through a necrotic mechanism because the degenerating cells did not display any swollen organelles or swollen cell bodies or membranous whorls (a characteristic of necrotic cell death in *C. elegans*) (135). The only morphological changes we found relative to the control animals were small, dark, and rounded DA neuronal cell bodies and loss of dendritic endings (without apparent collateral cellular damage), which is most indicative of apoptosis (3, 135). We then crossed our DAT-1::GFP reporter into strains deficient for the cell death genes *ced-3* and

ced-4 and found the sensitivity to 6-OHDA to be indistinguishable from the lines that do not contain the *ced* mutations, which indicates that cell damage here does not involve the classical apoptotic pathway utilized for programmed cell death (3). These results are intriguing because they are consistent with the observations found in postmortem PD patients and could provide a model of DA neurodegeneration that occurs in vivo (127, 128).

If a *ced-3*/*ced-4* linked pathway is not involved in 6-OHDA induced neural degeneration, what molecular pathways may be involved in the 6-OHDA-induced cell death? *C. elegans* contains three other caspase-related (csp, caspase homolog) genes that could play a role in the cellular death. csp-1, csp-2, and csp-3 encode a total of seven transcripts in which a clear function in vivo has not been assigned (136). It could be that one or more of these caspases play a role in ROS-mediated cellular degeneration or, specifically, in DA neurodegeneration. *C. elegans* also contains a homologue to the mammalian caspase-independent death effector, apoptosis inducing factor (AIF) (GenBank accession number U50301) (137). Studies with mammalian AIFs show that following exposure to cell death stimuli, AIF translocates from the mitochondria to the nucleus where it initiates the breakdown of DNA, causing chromatin condensation and conferring cellular death (138, 139). The putative *C. elegans* orthologue is 56% similar (25% identical) to the human enzyme, contains a mitochondria localization domain, and like the mammalian gene, has significant homology with oxidoreductases (137). Considering that 6-OHDA can cause a dramatic increase in oxidative stress in the mitochondria and cytoplasm, the death effector could play a role in the 6-OHDA-mediated DA neuron degeneration (3).

FUTURE DIRECTIONS

C. elegans provides an opportunity to characterize potential human regulators of DAT and gain insight into the roles they may play in human disease (140). Mammalian proteins associated with diseases can compliment their endogenous function in invertebrate systems, such as *Drosophila melanogaster* or *C. elegans*, and recapitulate many aspects of their cellular function and pathology or behavior; examples include genes involved in polyglutamine disorders (Huntington's disease and Spinocerebellar Ataxias), Alzheimers, and prion disease (5, 141). Human WT and mutant α-synuclein, a major component of Lewey bodies, have also been expressed in DA neurons within the fly, and similar to its human counterpart, these animals display adult-onset DA neurodegeneration, filamentous intraneuronal inclusions, and progressive locomotor dysfunction (5, 142). Interestingly though, there does not appear to be a difference in the rate or severity of the degeneration found in the transgenic animal expressing the mutant form. Presently, it is not clear if α-synuclein inclusion contributes to or is a result of the degeneration. As α-synuclein is a proposed DAT regulator, expression studies of human WT and mutant α-synuclein in the worm could provide significant insight into the role

this protein plays both in DAT regulation and neurodegeneration (74, 75). It will be important to determine whether the mutant form of α-synuclein preferentially causes an increase in DA neurodegeneration relative to WT and whether an increase in sensitivity is due to altered interactions with DAT and/or inclusion body formation. Mutant α-synuclein has also been shown to cause an increase in DA toxicity in vitro (143); examination within DA deficient lines will assist in elucidating the role DA plays in the vulnerability of the neurons to degeneration in vivo. Moreover, it will be important to test whether mutant α-synuclein lines are more sensitive to 6-OHDA-induced DA neurodegeneration, further suggesting the role of α-synuclein in oxidative stress-induced cell death. To this end, in collaboration with G. Wong at the A.I. Virtanen Institute for Molecular Sciences in Kuopio, Finland, we are examining human α-synuclein-expressing worm lines for altered DAT expression, localization, and 6-OHDA sensitivity.

Expression of the human parkin gene, which is linked to an autosomal recessive juvenile form of PD, is also widely expressed in DA neurons, suggesting that facets of DA physiology intersect with mutant alleles to lead to DA neuron vulnerability (144, 145). Recent biochemical studies indicate that parkin may participate in the ubiquitin-proteosome pathway of protein degradation as a ubiquitin ligase (145). Interestingly, α-synuclein appears to be normally degraded by a proteosomal pathway, suggesting a link between the known causes of familial PD in the formation of α-synuclein aggregates and pathways that may be altered by oxidative stress. *C. elegans* also contains an orthologue to parkin (Wormpep, K08E3.7), with 53% similarity to the human gene (141); isolation of a worm parkin knockout line or expression of mutant human parkin genes in the worm could provide valuable insights into the role it may play in DA neurodegeneration.

As noted, the transcriptional and translational DAT-1::GFP reporter lines provide an opportunity to explore the role that drugs of abuse and environmental toxins may play in DAT regulation and vulnerability to DA neuron degeneration in vivo. Altered DAT trafficking and plasma membrane density has been proposed to play a role in cocaine and amphetamine toxicity and in neurological disorders such as PD and ADHD, although the precise mechanisms are not clear (51, 116, 146). DAT trafficking can be followed in real time following exposure to the drugs of abuse, and suppressor screens to identify proteins that alter DAT trafficking following drug exposure can be exploited to identify possible proteins involved in DA-mediated behaviors, such as reward and addiction. Furthermore, because environmental agents continue to lie at the forefront of explanations for idiopathic PD (147), exposure of WT or mutant worms with genetic backgrounds deficient in DA biosynthesis or metabolism or ROS protecting proteins (e.g., superoxide dismutase) to suspected pesticides, fungicides, or other xenobiotics will likely yield important clues to the role these compounds play in the vulnerability of DA neurons to degeneration.

Finally, we envision that the nematode model will allow the establishment of a high-throughput screen (HTS) for agents that can efficiently protect against

6-OHDA-induced neural degeneration. *C. elegans* can easily be grown in liquid medium in standard 96- and 384-well microtitre plates (148). These microcultures allow for rapid screening of animals with particular behavioral phenotypes, or optical properties. The high conservation of genes between mammals and worms suggest that the worm is a valid model for neuroprotective drug discovery (149). Indeed, the worm is already known to be sensitive to a wide range of human neuroreactive drugs, including acteylcholine receptor agonsist [e.g., levamisole and nicotine (150, 151)], anesthetics [e.g., halothane (152)], cholinesterase inhibitors [e.g., aldicarb (153)], caffeine (154), serotonin-related [e.g., imipramine and fluoxetine (38, 155)], GABA-related muscimol (156), and dopamine-related [cocaine and amphetamine (76)] compounds. Though inherently simple, the nematode offers a rich array of conserved molecular targets whose manipulation may prove of benefit to halt or perhaps prevent DA neuron degeneration.

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

LITERATURE CITED

1. Smith Y, Kieval JZ. 2000. Anatomy of the dopamine system in the basal ganglia. *Trends Neurosci.* 23:S28–33
2. Nass R, Miller DM, Blakely RD. 2001. *C. elegans*: A novel pharmacogenetic model to study parkinson's disease. *Parkinsonism Relat. Disord.* 7:185–91
3. Nass R, Hall DH, Miller DM 3rd, Blakely RD. 2002. Neurotoxin-induced degeneration of dopamine neurons in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 99:3264–69
4. Link CD. 2001. Transgenic invertebrate models of age-associated neurodegenerative diseases. *Mech. Ageing Dev.* 122: 1639–49
5. Feany MB. 2000. Studying human neurodegenerative diseases in flies and worms. *J. Neuropathol. Exp. Neurol.* 59: 847–56
6. Wintle RF, Van Tol HHM. 2001. Dopamine signaling in *Caenorhabditis elegans*-potential for parkinsonism research. *Parkinsonism Relat. Disord.* 7:177–83
7. Rand JB, Nonet ML. 1997. Synaptic transmission. See Ref. 157, pp. 611–43
8. The *C. elegans* Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282:2012–18
9. Wood WB. 1988. Introduction to *C. elegans*. In *The Nematode Caenorhabditis elegans*, ed. WB Wood, pp. 1–16. New York: Cold Spring Harbor Lab. Press
10. Riddle DL, Blumenthal T, Meyer BJ, Priess JR. 1997. Introduction to *C. elegans*. See Ref. 157, pp. 1–22
11. Mello C, Fire A. 1995. DNA transformation. *Methods Cell Biol.* 48:451–82
12. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. 1994. Green fluorescent protein as a marker for gene expression. *Science* 263:802–5
13. Rongo C, Whitfield CW, Rodal A, Kim SK, Kaplan JM. 1998. LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. *Cell* 94:751–59
14. Miller DMI, Desai NS, Hardin DC, Piston DW, Patterson GH, et al. 1999. Two-color GFP expression for *C. elegans*. *BioTechniques* 26:914–21
15. White JG, Southgate E, Thompson JN, Brenner S. 1986. The structure of the

nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. London Ser. B* 314:1–340
16. Thomas JH, Lockery SH. 1999. Neurobiology. In *C. elegans: A Practical Approach*, ed. IA Hope, pp. 143–79. New York: Oxford Univ. Press
17. Wicks SR, Yeh RT, Gish WR, Waterston RH, Plasterk RH. 2001. Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. *Nat. Genet.* 28:160–64
18. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391:806–11
19. Liu LX, Spoerke JM, Mulligan EL, Chen J, Reardon B, et al. 1999. High-throughput isolation of *Caenorhabditis elegans* deletion mutants. *Genome Res.* 9:859–67
20. Christensen M, Estevez A, Yin X, Fox R, Morrison R, et al. 2002. A primary culture system for functional analysis of *C. elegans* neurons and muscle cells. *Neuron* 33:503–14
21. Bargmann CI. 1998. Neurobiology of the *Caenorhabditis elegans* genome. *Science* 282:2028–33
22. Nonet ML. 1999. Studying mutants that affect neurotransmitter release in *C. elegans*. In *Neurotransmitter Release*, ed. HJ Bellen, pp. 265–93. New York: Oxford Univ. Press
23. Sulston J, Dew M, Brenner S. 1975. Dopaminergic neurons in the nematode *caenorhabditis elegans*. *J. Comp. Neurol.* 163: 215–26
24. Perkins LA, Hedgecock EM, Thomson JN, Culotti JG. 1986. Mutant sensory cilia in the nematode *Caenorhabditis elegans*. *Dev. Biol.* 117:456–87
25. Ward S, Thomson N, White JG, Brenner S. 1975. Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J. Comp. Neurol.* 160:313–37
26. Sawin ER, Ranganathan R, Horvitz HR. 2000. *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron* 26:619–31
27. Hedgecock EM, Culotti JM, Thomson JN, Perkins LA. 1985. Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. *Dev. Biol.* 111:158–70
28. Sulston JE, Albertson DG, Thomson JN. 1980. The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. *Dev. Biol.* 78:542–76
29. Lints R, Emmons SW. 1999. Genetic analysis of dopaminergic neurons in the nematode *Caenorhabditis elegans*. In *Development of Dopaminergic Neurons*, ed. U di Porzio, R Pernas-Alonso, C Perrone-Capano, pp. 175–90. Austin, TX: Landes
30. Duerr JS, Frisby DL, Gaskin J, Duke A, Asermely K, et al. 1999. The *cat-1* gene of *Caenorhabditis elegans* encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. *J. Neurosci.* 19:72–84
31. Horvitz HR, Chalfie M, Trent C, Sulston JE, Evans PD. 1982. Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science* 216:1012–14
32. Lints R, Emmons SW. 1999. Patterning of dopaminergic neurotransmitter identity among *Caenorhabditis elegans* ray sensory neurons by a TGFbeta family signaling pathway and a Hox gene. *Development* 126:5819–31
33. O'Donnell JM, McLean JR, Reynolds ER. 1989. Molecular and developmental genetics of the Punch locus, a pterin biosynthesis gene in Drosophila melanogaster. *Dev. Genet.* 10:273–86
34. Loer CM, Kenyon CJ. 1993. Serotonin-deficient mutants and male mating behavior in the nematode *Caenorhabditis elegans*. *J. Neurosci.* 13:5407–17
35. Kapatos G, Hirayama K, Shimoji M, Milstien S. 1999. GTP cyclohydrolase I feedback regulatory protein is expressed

in serotonin neurons and regulates tetrahydrobiopterin biosynthesis. *J. Neurochem.* 72:669–75
36. Bandmann O, Wood NW. 2002. Dopa-responsive dystonia—The story so far. *Neuropediatrics* 33:1–5
37. Sulston J, Du Z, Thomas K, Wilson R, Hillier L, et al. 1992. The *C. elegans* genome sequencing project: a beginning. *Nature* 356:37–41
38. Weinshenker D, Garriga G, Thomas JH. 1995. Genetic and pharmacological analysis of neurotransmitters controlling egg laying in *C. elegans*. *J. Neurosci.* 15(10): 6975–85
39. Schafer WR, Kenyon CJ. 1995. A calcium-channel homologue required for adaptation to dopamine and serotonin in *Caenorhabditis elegans*. *Nature* 375:73–78
40. Patel MR, Campbell WC. 1998. Inhibitory effect of chlorpromazine on nematode eggs and larvae. *J. Parasitol.* 84:191–92
41. Nass R, Duerr J, Rand J, Miller DM, Blakely RD. 1999. *Pharmacogenetic analysis of transporter regulators: studies of the dopamine transporter*. Presented at Soc. Neurosci., Miami, FL
42. Suo S, Sasagawa N, Ishiura S. 2002. Identification of a dopamine receptor from *Caenorhabditis elegans*. *Neurosci. Lett.* 319: 13–16
43. Liu KS, Sternberg PW. 1995. Sensory regulation of male mating behavior in *Caenorhabditis elegans*. *Neuron* 14:79–89
44. Hyman SE. 1996. Addiction to cocaine and amphetamine. *Neuron* 16(1):901–4
45. Rudnick G, Clark J. 1993. From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. *Biochim. Biophys. Acta* 1144:249–63
46. Amara SG, Pacholczyk T. 1991. Sodium-dependent neurotransmitter reuptake systems. *Curr. Opin. Neurobiol.* 1:84–90
47. Masson J, Sagn C, Hamon M, Mestikawy SE. 1999. Neurotransmitter transporters in the central nervous system. *Pharmacol. Rev.* 51:439–64
48. Nirenberg MJ, Chan J, Vaughan RA, Uhl GR, Kuhar MJ, Pickel VM. 1997. Immunogold localization of the dopamine transporter: an ultrastructural study of the rat ventral tegmental area. *J. Neurosci.* 17:4037–44
49. Pickel VM, Nirenberg MJ, Milner TA. 1996. Ultrastructural view of central catecholaminergic transmission: immunocytochemical localization of synthesizing enzymes, transporters and receptors. *J. Neurocytol.* 25:843–56
50. Nirenberg MJ, Vaughan RA, Uhl GR, Kuhar MJ, Pickel VM. 1996. The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. *J. Neurosci.* 16:436–47
51. Zahniser NR, Doolen S. 2001. Chronic and acute regulation of Na^+/Cl^--dependent neurotransmitter transporters: drugs, substrates, presynaptic receptors, and signaling systems. *Pharmacol. Ther.* 92:21–55
52. Miller GW, Gainetdinov RR, Levey AI, Caron MG. 1999. Dopamine transporters and neuronal injury. *Trends Pharmacol. Sci.* 20:424–29
53. Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, Caron MG. 1998. Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proc. Natl. Acad. Sci. USA* 95(7):4029
54. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. 1996. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* 379:606–12
55. Gainetdinov RR, Jones SR, Fumagalli F, Wightman RM, Caron MG. 1998. Re-evaluation of the role of the dopamine transporter in dopamine system homeostasis. *Brain Res. Brain Res. Rev.* 26: 148–53
56. Drago J, Padungchaichot P, Accili D,

Fuchs S. 1998. Dopamine receptors and dopamine transporter in brain function and addictive behaviors: insights from targeted mouse mutants. *Dev. Neurosci.* 20:188–203
57. Reith ME, Xu C, Chen NH. 1997. Pharmacology and regulation of the neuronal dopamine transporter. *Eur. J. Pharmacol.* 324:1–10
58. Kitayama S, Dohi T, Uhl G. 1994. Phorbol esters alter functions of the expressed dopamine transporter. *Eur. J. Pharmacol.* 268:115–19
59. Huff RA, Vaughan RA, Kuhar MJ, Uhl GR. 1997. Phorbol esters increase dopamine transporter phosphorylation and decrease transport V_{max}. *J. Neurochem.* 68: 225–32
60. Vaughan RA, Huff RA, Uhl GR, Kuhar MJ. 1997. Protein kinase C-mediated phosphorylation and functional regulation of dopamine transporters in striatal synaptosomes. *J. Biol. Chem.* 272:15541–46
61. Pristupa ZB, McConkey F, Liu F, Man HY, Lee FJ, et al. 1998. Protein kinase-mediated bidirectional trafficking and functional regulation of the human dopamine transporter. *Synapse* 30:79–87
62. Daniels GM, Amara SG. 1999. Regulated trafficking of the human dopamine transporter. Clathrin-mediated internalization and lysosomal degradation in response to phorbol esters. *J. Biol. Chem.* 274:35794–801
63. Zhang L, Coffey LL, Reith ME. 1997. Regulation of the functional activity of the human dopamine transporter by protein kinase C. *Biochem. Pharmacol.* 53:677–88
64. Zhang L, Reith MEA. 1996. Regulation of the functional acitivity of the human dopamine tranporter by the arachidonic acid pathway. *Eur. J. Pharmacol.* 315: 345–54
65. Melikian H, Buckley K. 1999. Membrane trafficking regulates the activity of the human dopamine transporter. *J. Neurosci.* 19:7699–710
66. Chang MY, Lee SH, Kim JH, Lee KH, Kim YS, et al. 2001. Protein kinase C-mediated functional regulation of dopamine transporter is not achieved by direct phosphorylation of the dopamine transporter protein. *J. Neurochem.* 77:754–61
67. Cass WA, Gerhardt GA. 1994. Direct in vivo evidence that D2 dopamine receptors can modulate dopamine uptake. *Neurosci. Lett.* 176:259–63
68. Meiergerd SM, Patterson TA, Schenk JO. 1993. D2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies in vitro and in vivo. *J. Neurochem.* 61:764–67
69. Pothos EN, Przedborski S, Davila V, Schmitz Y, Sulzer D. 1998. D2-Like dopamine autoreceptor activation reduces quantal size in PC12 cells. *J. Neurosci.* 18: 5575–85
70. Smolders I, Bogaert L, Ebinger G, Michotte Y. 1997. Muscarinic modulation of striatal dopamine, glutamate, and GABA release, as measured with in vivo microdialysis. *J. Neurochem.* 68:1942–48
71. Derbez AE, Mody RM, Werling LL. 2002. Sigma(2)-receptor regulation of dopamine transporter via activation of protein kinase C. *J. Pharmacol. Exp. Ther.* 301:306–14
72. Torres GE, Yao WD, Mohn AR, Quan H, Kim KM, et al. 2001. Functional interaction between monoamine plasma membrane transporters and the synaptic PDZ domain-containing protein PICK1. *Neuron* 30:121–34
73. Lee FJ, Liu F, Pristupa ZB, Niznik HB. 2001. Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. *FASEB J.* 15:916–26
74. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, et al. 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 276:2045–47

75. Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, et al. 1998. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat. Genet.* 18:106–8
76. Jayanthi LD, Apparsundaram S, Malone MD, Ward E, Miller DM, et al. 1998. The *Caenorhabditis elegans* gene T23-G5.5 encodes an antidepressant- and cocaine-sensitive dopamine transporter. *Mol. Pharmacol.* 54:601–9
77. Giros B, El Mestikawy S, Godinot N, Zheng K, Han H, et al. 1992. Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol. Pharmacol.* 42:383–90
78. Giros B, Caron MG. 1993. Molecular characterization of the dopamine transporter. *Trends Pharmacol. Sci.* 14:43–49
79. Mitchell SL, Kiely DK, Kiel DP, Lipsitz LA. 1996. The epidemiology, clinical characteristics, and natural history of older nursing home residents with a diagnosis of Parkinson's disease. *J. Am. Geriatr. Soc.* 44:394–99
80. Yahr MD, Bergman K, eds. 1986. *Advances in Neurology: Parkinsons's Disease*, pp. 1–616. New York: Raven
81. Jenner P. 1998. Oxidative mechanisms in nigral cell death in Parkinson's disease. *Mov. Disord.* 13:24–34
82. McLaughlin B. 2001. Dopamine neurotoxicity and neurodegeneration. In *Molecular Mechanisms of Neurodegenerative Disease*, ed. M Chesselet, pp. 195–231. Totowa, NJ: Humana
83. Blum D, Torch S, Lambeng N, Nissou M, Benabid AL, et al. 2001. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. *Prog. Neurobiol.* 65:135–72
84. McConkey DJ, Orrenius S. 1996. The role of calcium in the regulation of apoptosis. *J. Leukocyte Biol.* 59:775–83
85. Sauer H, Oertel WH. 1994. Progressive degeneration of nigrostriatal dopamine neurons following intrastriatal terminal lesions with 6-hydroxydopamine: a combined retrograde tracing and immunocytochemical study in the rat. *Neuroscience* 59:401–15
86. Reading RJ, Dunnett SB. 1994. 6-hydroxydopamine lesions of nigrostriatal neurons as an animal model of Parkinson's Disease. In *Toxin-Induced Models of Neurological Disorders*, ed. ML Woodruff, AJ Nonnemann, pp. 89–119. New York: Plenum
87. Glinka Y, Gassen M, Youdim MBH. 1997. Mechanism of 6-hydroxydopamine neurotoxicity. *J. Neural Transm.* 50:55–66
88. Curtius HC, Wolfensberger M, Steinmann B, Redweik U, Siegfried J. 1974. Mass fragmentography of dopamine and 6-hydroxydopamine. Application to the determination of dopamine in human brain biopsies from the caudate nucleus. *J. Chromatogr.* 99:529–40
89. Andrew R, Watson DG, Best SA, Midgley JM, Wenlong H, Petty RK. 1993. The determination of hydroxydopamines and other trace amines in the urine of parkinsonian patients and normal controls. *Neurochem. Res.* 18:1175–77
90. Jellinger K, Linert L, Kienzl E, Herlinger E, Youdim MB. 1995. Chemical evidence for 6-hydroxydopamine to be an endogenous toxic factor in the pathogenesis of Parkinson's disease. *J. Neural Transm. Suppl.* 46:297–314
91. Slivka A, Cohen G. 1985. Hydroxyl radical attack on dopamine. *J. Biol. Chem.* 260:15466–72
92. Sulzer D, Bogulavsky J, Larsen KE, Behr G, Karatekin E, et al. 2000. Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. *Proc. Natl. Acad. Sci. USA* 97:11869–74
93. Palumbo A, Napolitano A, Barone P, d'Ischia M. 1999. Nitrite- and peroxide-dependent oxidation pathways of dopamine: 6-nitrodopamine and 6-hydroxydopamine formation as potential contributory

mechanisms of oxidative stress- and nitric oxide-induced neurotoxicity in neuronal degeneration. *Chem. Res. Toxicol.* 12:1213–22

94. Garner CD, Nachtman JP. 1989. Manganese catalyzed auto-oxidation of dopamine to 6-hydroxydopamine in vitro. *Chem. Biol. Interact.* 69:345–51
95. Cohen G, Heikkila RE. 1974. The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. *J. Biol. Chem.* 249:2447–52
96. Graham DG, Tiffany SM, Bell WR Jr, Gutknecht WF. 1978. Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Mol. Pharmacol.* 14:644–53
97. Glinka YY, Youdim MB. 1995. Inhibition of mitochondrial complexes I and IV by 6-hydroxydopamine. *Eur. J. Pharmacol.* 292:329–32
98. Sachs C, Jonsson G. 1975. Mechanisms of action of 6-hydroxydopamine. *Biochem. Pharmacol.* 24:1–8
99. Kitayama S, Shimada S, Uhl GR. 1992. Parkinsonism-inducing neurotoxin MPP^+: uptake and toxicity in nonneuronal COS cells expressing dopamine transporter cDNA. *Annu. Neurol.* 32:109–11
100. Pifl C, Giros B, Caron MG. 1993. Dopamine transporter expression confers cytotoxicity to low doses of the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium. *J. Neurosci.* 13(10): 4246–53
101. Javitch JA, D'Amato RJ, Strittmatter SM, Snyder SH. 1985. Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc. Natl. Acad. Sci. USA* 82:2173–77
102. Uhl GR. 1998. Hypothesis: the role of dopaminergic transporters in selective vulnerability of cells in Parkinson's disease. *Annu. Neurol.* 43(5):555–60
103. El Mestikawy S, Wehrlé R, Masson J, Lombard MC, Hamon M, Sotelo C. 1997. Distribution pattern and ultrastructural localization of Rxt1, and orphan Na^+/Cl^--dependent transporter, in the central nervous system of rats and mice. *Neuroscience* 77:319–33
104. Renick SE, Kleven DT, Chan J, Stenius K, Milner TA, et al. 1999. The mammalian brain high-affinity L-proline transporter is enriched preferentially in synaptic vesicles in a subpopulation of excitatory nerve terminals in rat forebrain. *J. Neurosci.* 19:21–33
105. Hall DH, Hedgecock EM. 1991. Kinesin-related gene *unc-104* is required for axonal transport of synaptic vesicles in *C. elegans*. *Cell* 65:837–47
106. Patel N, Thierry-Mieg D, Mancillas JR. 1993. Cloning by insertional mutagenesis of a cDNA encoding *Caenorhabditis elegans* kinesin heavy chain. *Proc. Natl. Acad. Sci. USA* 90:9181–85
107. Byrd DT, Kawasaki M, Walcoff M, Hisamoto N, Matsumoto K, Jin Y. 2001. UNC-16, a JNK-signaling scaffold protein, regulates vesicle transport in *C. elegans*. *Neuron* 32:787–800
108. Koushika SP, Nonet ML. 2000. Sorting and transport in *C. elegans*: a model system with a sequenced genome. *Curr. Opin. Cell Biol.* 12:517–23
109. Nonet ML. 1999. Visualization of synaptic specializations in live *C. elegans* with synaptic vesicle protein-GFP fusions. *J. Neurosci. Methods* 89:33–40
110. Yonekawa Y, Harada A, Okada Y, Funakoshi T, Kanai Y, et al. 1998. Defect in synaptic vesicle precursor transport and neuronal cell death in KIF1A motor protein-deficient mice. *J. Cell Biol.* 141:431–41
111. Tanaka Y, Kanai Y, Okada Y, Nonaka S, Takeda S, et al. 1998. Targeted disruption

of mouse conventional kinesin heavy chain, kif5B, results in abnormal perinuclear clustering of mitochondria. *Cell* 93:1147–58

112. Saifee O, Wei L, Nonet ML. 1998. The *Caenorhabditis elegans* unc-64 locus encodes a syntaxin that interacts genetically with synaptobrevin. *Mol. Biol. Cell* 9:1235–52
113. Nonet ML, Saifee O, Zhao H, Rand JB, Wei L. 1998. Synaptic transmission deficits in *Caenorhabditis elegans* synaptobrevin mutants. *J. Neurosci.* 18:70–80
114. Deken SL, Beckman ML, Boos L, Quick MW. 2000. Transport rates of GABA transporters: regulation by the N-terminal domain and syntaxin 1A. *Nat. Neurosci.* 3:998–1003
115. Saunders C, Ferrer JV, Shi L, Chen J, Merrill G, et al. 2000. Amphetamine-induced loss of human dopamine transporter activity: an internalization-dependent and cocaine-sensitive mechanism. *Proc. Natl. Acad. Sci. USA* 97:6850–55
116. Daws LC, Callaghan PD, Moron JA, Kahlig KM, Shippenberg TS, et al. 2002. Cocaine increases dopamine uptake and cell surface expression of dopamine transporters. *Biochem. Biophys. Res. Commun.* 290:1545–50
117. Johnsen R, Baillie DL. 1997. Mutation. See Ref. 157, pp. 79–95
118. Anderson P. 1995. Mutagenesis. See Ref. 158, pp. 31–58
119. Vasudevan G, Ullman B, Landfear SM. 2001. Point mutations in a nucleoside transporter gene from Leishmania donovani confer drug resistance and alter substrate selectivity. *Proc. Natl. Acad. Sci. USA* 98:6092–97
120. Edgley M, Baillie DL, Riddle DL, Rose AM. 1995. Genetic balancers. See Ref. 158, pp. 147–84
121. Wyllie AH, Kerr JF, Currie AR. 1980. Cell death: the significance of apoptosis. *Int. Rev. Cytol.* 68:251–306
122. Pettmann B, Henderson CE. 1998. Neuronal cell death. *Neuron* 20:633–47
123. Ellis HM, Horvitz HR. 1986. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* 44:817–29
124. Jacobson MD, Weil M, Raff MC. 1997. Programmed cell death in animal development. *Cell* 88:347–54
125. Chen F, Hersh BM, Conradt B, Zhou Z, Riemer D, et al. 2000. Translocation of *C. elegans* CED-4 to nuclear membranes during programmed cell death. *Science* 287:1485–89
126. Yang X, Chang HY, Baltimore D. 1998. Essential role of CED-4 oligomerization in CED-3 activation and apoptosis. *Science* 281:1355–57
127. Jellinger KA. 2000. Cell death mechanisms in Parkinson's disease. *J. Neural Transm.* 107:1–29
128. Banati R, Blunt S, Graeber MB. 1999. What does apoptosis have to do with Parkinson's disease. *Mov. Disord.* 14:384–85
129. Hengartner MO. 2001. Apoptosis: corralling the corpses. *Cell* 104:325–28
130. Lotharius J, Dugan LL, O'Malley KL. 1999. Distinct mechanisms underlie neurotoxin-mediated cell death in cultured dopaminergic neurons. *J. Neurosci.* 19:1284–93
131. Choi WS, Yoon SY, Oh TH, Choi EJ, O'Malley KL, Oh YJ. 1999. Two distinct mechanisms are involved in 6-hydroxydopamine- and MPP^+-induced dopaminergic neuronal cell death: role of caspases, ROS, and JNK. *J. Neurosci. Res.* 57:86–94
132. Armstrong JS, Hornung B, Lecane P, Jones DP, Knox SJ. 2001. Rotenone-induced G2/M cell cycle arrest and apoptosis in a human B lymphoma cell line PW. *Biochem. Biophys. Res. Commun.* 289:973–78
133. Shimizu S, Eguchi Y, Kamiike W, Waguri S, Uchiyama Y, et al. 1996. Retardation of chemical hypoxia-induced necrotic cell death by Bcl-2 and ICE inhibitors: possible involvement of common mediators in apoptotic and necrotic signal transductions. *Oncogene* 12:2045–50

134. Yuki K, Miyauchi T, Kakinuma Y, Murakoshi N, Suzuki T, et al. 2000. Mitochondrial dysfunction increases expression of endothelin-1 and induces apoptosis through caspase-3 activation in rat cardiomyocytes in vitro. *J. Cardiovasc. Pharmacol.* 36:S205–8
135. Hall DH, Gu G, Garcia-Anoveros J, Gong L, Chalfie M, Driscoll M. 1997. Neuropathology of degenerative cell death in *Caenorhabditis elegans*. *J. Neurosci.* 17:1033–45
136. Shaham S. 1998. Identification of multiple *Caenorhabditis elegans* caspases and their potential roles in proteolytic cascades. *J. Biol. Chem.* 273:35109–17
137. Lorenzo HK, Susin SA, Penninger J, Kroemer G. 1999. Apoptosis inducing factor (AIF): a phylogenetically old, caspase-independent effector of cell death. *Cell Death Differ.* 6:516–24
138. Joza N, Susin SA, Daugas E, Stanford WL, Cho SK, et al. 2001. Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature* 410:549–54
139. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, et al. 1999. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397: 441–46
140. Nass R, Hall DH, Miller DM, Blakely RD. 2001. *Modeling Parkinson's disease in C. elegans: studies of neurotoxin induced DA neuron degeneration*. Presented at Soc. Neurosci., Los Angeles, CA
141. Culetto E, Sattelle DB. 2000. A role for *Caenorhabditis elegans* in understanding the function and interactions of human disease genes. *Hum. Mol. Genet.* 9:869–77
142. Feany MB, Bender WW. 2000. A *Drosophila* model of Parkinson's disease. *Nature* 404:394–98
143. Tabrizi SJ, Orth M, Wilkinson JM, Taanman JW, Warner TT, et al. 2000. Expression of mutant alpha-synuclein causes increased susceptibility to dopamine toxicity. *Hum. Mol. Genet.* 9:2683–89
144. Mizuno Y, Hattori N, Mori H. 1999. Genetics of Parkinson's disease. *Biomed. Pharmacother.* 53:109–16
145. Dawson TM. 2000. New animal models for Parkinson's disease. *Cell* 101:115–18
146. Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK, Fischman AJ. 1999. Dopamine transporter density in patients with attention deficit hyperactivity disorder. *Lancet* 354:2132–33
147. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* 3:1301–6
148. Link EM, Hardiman G, Sluder AE, Johnson CD, Liu LX. 2000. Therapeutic target discovery using *Caenorhabditis elegans*. *Pharmacogenomics* 1:203–17
149. Rand JB, Johnson CD. 1995. Genetic pharmacology: interactions between drugs and gene products in *Caenorhabditis elegans*. *Methods Cell Biol.* 187–204
150. Lewis JA, Wu CH, Berg H, Levine JH. 1980. The genetics of levamisole resistance in the nematode *Caenorhabditis elegans*. *Genetics* 95:905–28
151. Lewis JA, Elmer JS, Skimming J, McLafferty S, Fleming J, McGee T. 1987. Cholinergic receptor mutants of the nematode *Caenorhabditis elegans*. *J. Neurosci.* 7:3059–71
152. Morgan PG, Sedensky MM, Meneely PM. 1991. The genetics of response to volatile anesthetics in *Caenorhabditis elegans*. *Ann. NY Acad. Sci.* 625:524–31
153. Alfonso A, Grundahl K, McManus JR, Rand JB. 1994. Cloning and characterization of the choline acetyltransferase structural gene (cha-1) from *C. elegans*. *J. Neurosci.* 14:2290–300
154. Hartman PS. 1987. Caffeine-resistant mutants of *Caenorhabditis elegans*. *Genet. Res.* 49:105–10
155. Avery L, Horvitz HR. 1990. Effects of

starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *J. Exp. Zool.* 253:263–70

156. McIntire SL, Jorgensen E, Kaplan J, Horvitz HR. 1993. The GABAergic nervous system of *Caenorhabditis elegans*. *Nature* 364:337–41
157. Riddle DL, Blumenthal T, Meyer BJ, Priess JR, eds. 1997. *C. elegans II.* New York: Cold Spring Harbor Press
158. Epstein H, Shakes DC, eds. 1995. *Caenorhabditis elegans: Modern Biological Analysis of an Organism.* San Diego, CA: Academic

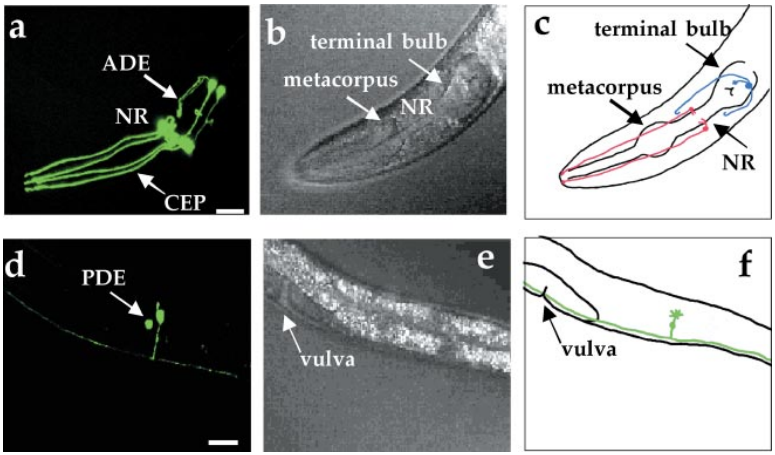

Figure 1 Visualization of all eight DA neurons in living, adult *C. elegans* hermaphrodites using DAT-1::GFP transcriptional fusions. (*a*) 3-D reconstruction of confocal epifluorescence from head DA neurons in a $P_{dat\text{-}1}$::GFP transgenic line. Arrows identify CEP and ADE processes. NR refers to the nerve ring. (*b*) DIC image of animal in panel (*a*). (*c*) Schematic drawing showing location of DA neurons in the head relative to the pharynx. In this top view, two pairs of CEP neurons (red) project dendritic endings to the tip of the nose and one pair of ADE neurons (blue) extend ciliated processes to amphids adjacent to the terminal bulb of the pharynx. (*d*) 3-D reconstructions of confocal epifluorescence of the PDE neurons. Both PDE cell bodies are apparent. (*e*) DIC image of animal in panel (*d*). (*f*) Schematic drawing showing left hand member of pair of PDE neurons (green) in lateral location posterior to vulva. All scale bars = 25 μm. Anterior is to the left. See Reference (3) for details. Reproduced with permission from PNAS (3).

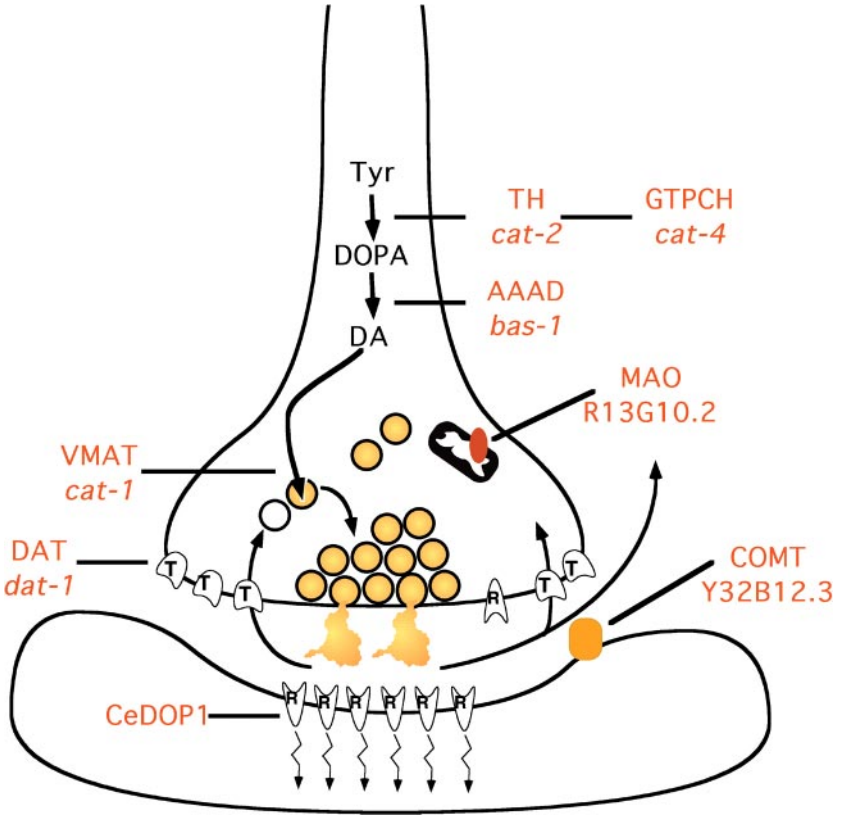

Figure 2 Schematic model of a *C. elegans* DA neuron containing known and predicted genes involved in DA biosynthesis and metabolism. Genetic mutants are indicated in italics. All protein locations are putative. TH, tyrosine hydroxylase; GTPCH, GTP cyclohydrolase; AAAD, aromatic L-amino acid decarboxylase; MAO, monoamine oxidase; VMAT, vesicular monoamine transporter; DAT-1, dopamine transporter; CeDOP1, D1-like DA receptor; COMT, cachol-O-methyltransferase.

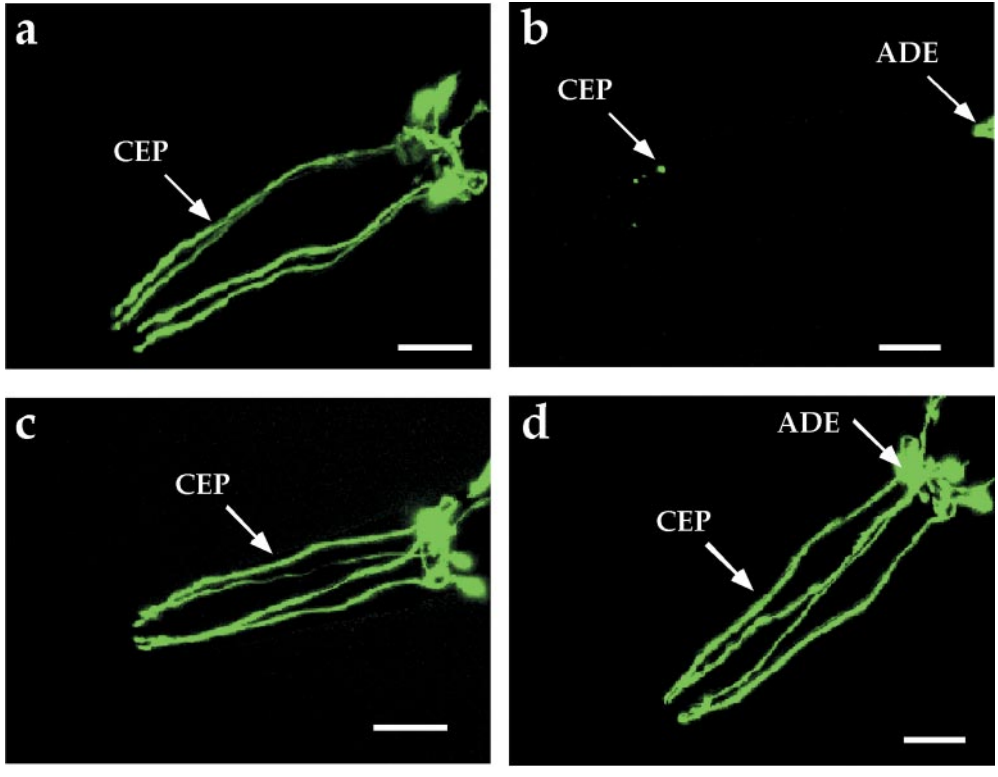

Figure 3 Suppression of 6-OHDA sensitivity of DA neurons. (*a*) $P_{dat\text{-}1}$::GFP animals exposed to vehicle; (*b*) $P_{dat\text{-}1}$::GFP worms exposed to 6-OHDA; (*c*) $P_{dat\text{-}1}$::GFP, *dat-1*(ΔDAT-1 worms) exposed to vehicle; (*d*) $P_{dat\text{-}1}$::GFP, *dat-1* worms exposed to 6-OHDA. Scale bars = 25 μm. See Reference (3) for details. Reproduced with permission from PNAS (3).

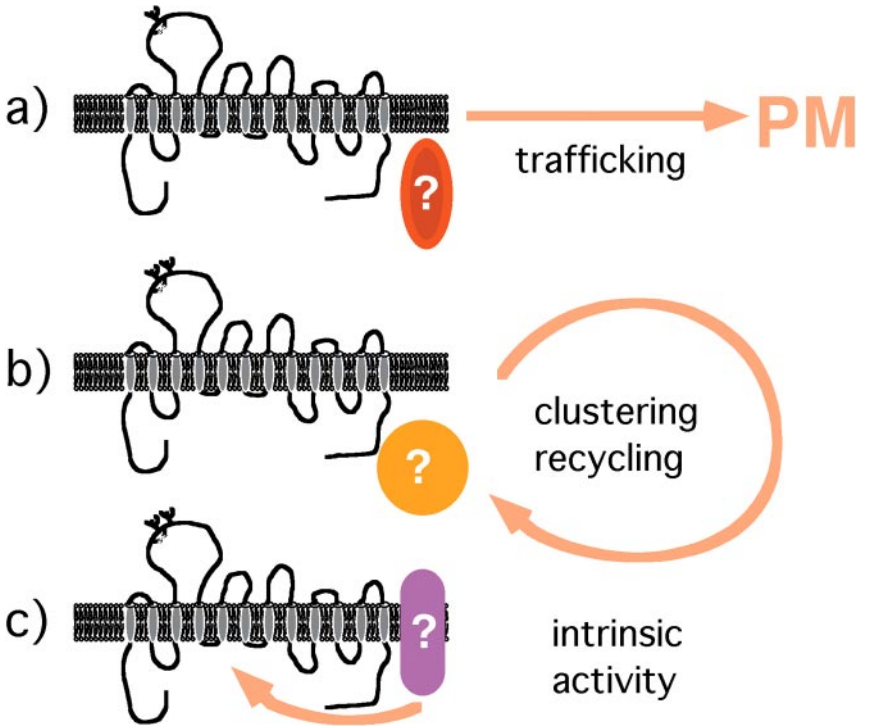

Figure 4 Predicted DAT-1 affected mutants generated from our 6-OHDA dependent DA neurodegeneration screen. The screen could yield mutants affecting DAT-1 trafficking (*a*), recycling from synaptic vesicles to the plasma membrane (*b*), or activity (*c*).