Title

Neurotoxicity

ISBN

9780323854344

Authors

Spencer, PS

Lein, PJ

Publication Date

2023

DOI

10.1016/B978-0-12-824315-2.00548-0

Neurotoxicity

PS Spencer[a] **and PJ Lein**[b], [a]Department of Neurology, School of Medicine, and Oregon Institute of Occupational Health Sciences, Oregon Health & Science University, Portland, OR, United States; [b]Department of Molecular Biosciences, School of Veterinary Medicine and the MIND Institute, School of Medicine, University of California, Davis, CA, United States

This is an update of P.S. Spencer, P.J. Lein, Neurotoxicity, Editor(s): Philip Wexler, Encyclopedia of Toxicology (Third Edition), Academic Press, 2014, Pages 489–500, ISBN 9780123864550, https://doi.org/10.1016/B978-0-12-386454-3.00169-X.

Abstract

Neurotoxicity refers to the direct or indirect effect of chemicals that disrupt the nervous system of humans or animals. Numerous chemicals can produce neurotoxic diseases in humans, and many more are used as experimental tools to probe molecular and cellular mechanisms of physiology and pathophysiology in the nervous system of animal tissues in vivo and *in vitro*, and in human neural progenitor cell lines. Some chemicals act directly on neural cells, others interfere with metabolic processes on which the nervous system is especially dependent. Emerging evidence suggests that toxic effects on physiological systems outside the brain, in particular the endocrine system, immune system and gut microbiome, may also adversely impact the nervous system. Some neurotoxic chemicals disrupt neural function, others alter normal developmental trajectories of the brain or cause damage to the adult nervous system. Perturbations may appear and disappear rapidly, evolve slowly over days or weeks and regress over months or years, or cause permanent deficits. Neurotoxicity is usually self-limiting after exposure ceases and rarely progressive in the absence of continued exposure, although there may be a significant delay between exposure and manifestation of neurotoxic effects.

Keywords

Aging; Autism; Bacterium; Blood–brain barrier; Brain; Development; Environment; Fungus; Invertebrate; Nerve; Neurodegeneration; Neuropathy; Occupation; Plant; Vertebrate

Introduction

Neurotoxicity refers to the direct or indirect effect of chemicals that disrupt the nervous system of humans or animals. Numerous chemicals can produce neurotoxic disease in humans, and many more are used as experimental tools to probe physiologic and pathophysiologic processes in the nervous system of animal tissue in vivo and *in vitro* and in human neural progenitor cell lines. Some chemicals act directly on neural cells, others interfere with metabolic processes on which the nervous system is especially dependent. Emerging evidence suggests that toxic effects on physiological systems outside the brain, in particular the endocrine system, immune system and gut microbiome, may also adversely impact the nervous system. Some neurotoxic chemicals disrupt neural function; others alter the developmental trajectory or cause damage to the adult nervous system. Perturbations may appear and disappear rapidly, evolve slowly over days or weeks and regress over months or years, or cause permanent deficits. Neurotoxicity is usually self-limiting after exposure ceases and rarely progressive in the absence of continued exposure, although there may be a significant delay between exposure and manifestation of neurotoxic effects.

Occurrence

Chemicals with the potential to disrupt the mammalian nervous system may occur naturally (neurotoxins) or arise by synthesis (neurotoxicants). While chemicals with neurotoxic potential are conveniently termed 'neurotoxins' or 'neurotoxicants,' this is not an

intrinsic property but rather the description of an effect that may occur if the tissue concentration exceeds a certain threshold. This point is well illustrated by the brain's physiological use of certain chemicals with neurotoxic potential but in concentrations that are required for normal function and which exert no known neurotoxic effect: these include the gaseous neurotransmitters carbon monoxide, nitric oxide and hydrogen sulfide.

Biological chemicals with neurotoxic properties often have high target specificity and toxic potency, discrete biological actions, and are among the best understood mechanistically. Examples of biological chemicals with direct or indirect neurotoxic potential are found in bacteria, algae, fungi, plants, coelenterates, insects, arachnids, molluscs, amphibians, reptiles, fish, and certain mammals (Table 1).

Other less potent naturally occurring substances exhibit neurotoxic effects when encountered in significant concentration for sufficient periods of time. Examples include metals (*arsenic, lead, mercury*) and certain compounds containing these elements (*methylmercury*) (Table 2). Some elements (*manganese, selenium*) and compounds (*vitamin B6*) in this group, while neurotoxic in

Table 1 Naturally occurring substances with mammalian neurotoxic potential.

Life form	*Substance with neurotoxic potential*
Bacterium	Diphtheria toxin
Alga	Anatoxin-a
Fungus	3-Nitropropionic acid
Plant	L-BOAA
Coelenterate	Palytoxin
Insect	Apamin
Arachnid	Scorpion toxins
Mollucs	Conotoxins
Fish	Ciguatoxin
Amphibia	Batrachotoxin
Reptile	Dendrotoxin
Bird	Batrachotoxin
Mammal	Vitamin A

Table 2 Heavy metals and synthetic substances with neurotoxic potential.

Substance	*Primary neurotoxic effects*
Acrylamide	Peripheral neuropathy (axonal degeneration), cerebellar ataxia
Arsenic	Acute encephalopathy (brain swelling and hemorrhage), peripheral neuropathy (axonal degeneration)
Barbiturates	Acute encephalopathy (sedation and coma), chronic encephalopathy, developmental neurotoxicity, facilitated γ-aminobutyric acid neurotransmission
Carbamate pesticides	Acute encephalopathy (cholinergic syndrome), neuromuscular transmission dysfunction, acetylcholinesterase inhibition
Carbon disulfide	Acute psychosis, chronic peripheral neuropathy (axonal degeneration), parkinsonism
Carbon monoxide	Encephalopathy/delayed parkinsonism, neuronal and tissue necrosis secondary to hypoxia
Carbon tetrachloride	Acute encephalopathy, visual dysfunction
Doxorubicin	Progressive ataxia (rodents), sensory neuronal degeneration
Ethanol	Fetal alcohol syndrome, acute encephalopathy (agitation, sedation, ataxia, coma), chronic encephalopathy (cognitive impairment, dementia), myopathy, peripheral neuropathy (vitamin B1 deficiency?)
n-Hexane	Peripheral neuropathy (axonal degeneration)
Lead, inorganic	Peripheral neuropathy (axonal loss and demyelination), acute encephalopathy (seizures), cognitive dysfunction
Manganese, inorganic	Emotional disturbance, psychoses, parkinsonism/dystonia, neuronal degeneration in striatum and globus pallidus
Mercury, inorganic	Cerebellar syndrome (tremor, ataxia), psychobiological reaction (anxiety, personality changes, memory loss)
Methanol	Optic neuropathy (axonal degeneration, primary demyelination), extrapyramidal syndrome (necrosis of putamen), retinopathy (edema)
Methylmercury	Developmental toxicity and teratogenesis, visual dysfunction (tunnel vision), cerebellar syndrome (ataxia), peripheral neuropathy, chronic encephalopathy (cognitive dysfunction)
Organophosphorus compounds (pesticides and warfare agents)	Cholinergic syndrome (certain compounds), peripheral neuropathy (certain compounds only), acetylcholinesterase inhibition
Phenytoin	Fetal phenytoin syndrome, cerebellar syndrome (ataxia, nystagmus), chronic encephalopathy (cognitive dysfunction), extrapyramidal syndrome (chorea, dyskinesia), peripheral neuropathy
Toluene	Acute encephalopathy (sedation, coma), chronic encephalopathy (cognitive dysfunction)
Tricyclic antidepressants	Seizure disorder (myoclonus), psychobiological reaction (serotonin syndrome, anticholinergic syndrome), tremor, extrapyramidal syndrome (dyskinesia)
Trimethyltin	Acute encephalopathy (neuronal degeneration of limbic system) – rodents, chronic encephalopathy (cognitive dysfunction, neuronal loss in hippocampus)

sustained heavy doses, are required in smaller amounts to support normal physiological function, including that of the nervous system. Natural substances (*thiaminase*) that interfere with required chemicals (thiamine) are also associated with neurological disease in animals and humans.

Synthetic chemicals with neurotoxic potential (Table 2) are most commonly encountered in the form of prescription (*dapsone, ethambutol, isoniazid,*) and over-the-counter pharmaceutical agents (*bismuth preparations*), especially anti-neoplastic drugs (*cisplatin, suramin, taxanes, thalidomide, vinca alkaloids*), domestic products used in antidandruff shampoos (*pyridinethione*), fragrance raw materials (*2,6-dinitro-3-methoxy-4-tert-butyltoluene*), pyrolysis products in broiled, baked, or fried food (*acrylamide*), beverages (*ethanol*), workplace chemicals (*n-hexane, trichloroethylene*), pest-control agents (*aldrin, chlorpyrifos*), environmental pollutants (*mercury, persistent organic pollutants*), and substances (*methamphetamine*) used to induce euphoria. Others are associated with special applications, such as chemical warfare (*sarin*) and in military and civilian aerospace (*hydrazine*).

Direct-acting substances with neurotoxic potential are supplemented by other agents that initiate neurological change as a consequence of effects on another organ system on which the brain depends for normal function. Substances that target the liver, kidneys, or lungs fall into this category, as do agents that interfere with the continuous supply of oxygen (*cyanide, azide*) and glucose (*6-chloro-6-deoxyglucose*) required by the nervous system for normal function. Chronic liver failure and manganese intoxication are both associated with high signal abnormalities in the basal ganglia on T1-weighted magnetic resonance images, suggesting that the metal accumulates because it cannot be cleared normally by the liver. Additionally, chemicals that exert toxic effects on physiological systems that modulate neurodevelopment or neural function can also adversely impact the nervous system. Examples include chemicals that target the endocrine system, particularly the thyroid hormone system (*polychlorinated biphenyls (PCBs), perchlorate*), the immune system (*bisphenols, phthalates, tetrachlorodibenzo-p-dioxin, toluene*), and the gut microbiome (*air pollutants, brominated flame retardants, metals, pesticides*).

Neurotoxic effects

The nervous system has a vast repertoire of functional reactions to chemical perturbation, and these responses give rise in the aggregate to a plethora of neurological and psychiatric phenomena, many of which recapitulate the clinical manifestations of disease of nontoxic origins. Large single doses of certain substances, such as organic solvents (*ethanol, toluene*) that intoxicate, induce functional changes in the organism that appear and disappear rapidly. Other agents, such as the *anticholinesterase nerve agents*, induce a subset of functional changes (e.g., parasympathomimetic effects) that reverse when the inhibited target protein (acetylcholinesterase) is reactivated or replaced. Sometimes, as in the case of *methanol*, the latent period (hours) between exposure and neurotoxic effect (retinopathy) is associated with the production and action of toxic metabolite (*formic acid*). Single exposures to large amounts of other substances (*arsenic, mercury, thallium*) may be followed by a latent period of days or weeks before structural and functional changes become evident. While certain substances (*acrylamide*) can induce neurological damage after single large exposures, smaller doses over a long period of time are also effective, but the pattern of neurological deficit may be distinct in the two dosing scenarios. Neurotoxic disorders typically progress during the period of exposure and immediately following exposure, when the pathological events already in progress may take time to unfold before stabilization or recovery can begin. Prospects for functional recovery depend on the presence or absence of tissue damage, the extent of damage, and whether neuropathological changes have occurred in the central nervous system (CNS) (poor prognosis).

An unanswered question is whether, in some instances, disease may progress or recur after chemical exposure has ceased. Certainly, catastrophic and fatal neurodegeneration after a delay period may follow *acute carbon monoxide poisonin*g, but this is an isolated example resulting from a period of acute brain hypoxia. Similarly, in the event of acute intoxication with anticholinesterase nerve agents that result in *status epilepticus*, chronic neurologic consequences, including spontaneous recurrent seizures and cognitive dysfunction, continue to progress for months to years after the neurotoxic chemical has been cleared from the organism. Relapses occur in *ciguatoxicity* presumably because the offending agent is released from fat stores under certain physiological conditions. Progressive visual defects may occur from release of *chloroquine*, which has been sequestered in the choroid layer of the eye. There is also concern that certain substances (*carbon disulfide, organochlorine pesticides, trichloroethylene*) might predispose or accelerate the onset of age-related diseases of the nervous system, such as Parkinson's disease. Finally, research is underway to determine whether certain DNA-damaging agents (*methylazoxymethanol* (MAM)) may predispose neurons to tardive degeneration because of their low capacity for DNA repair. Studies suggest that genotoxins such as MAM have biphasic effects depending on the ability of the targeted cells to divide: cycling cells undergo proliferation as a result of mutation arising from DNA damage (O^6-methylguanine (O^6-mG)), while noncycling neurons respond through related signal transduction pathways with degeneration. MAM is strongly implicated in a progressive neurodegenerative disease (amyotrophic lateral sclerosis and parkinsonism-dementia) that appears clinically years or decades after exposure.

Principles of nervous system vulnerability

In addition to dose and duration of exposure, there are many factors that determine the response of the nervous system to chemicals with neurotoxic potential. Species, sex, genotype, age, nutritional status, body mass and temperature, and activity are

key determinants, as are chemical access, structure, and biotransformation. The time of day has been found to affect the degree of ataxia induced in mice treated with *sodium nitroprusside*.

Metabolic activity of the brain may serve to activate a neurotoxic substance, as in the conversion of *methylphenyltetrahydropyridine* (MPTP) to the mitochondrial toxin *N-methyl-4-phenylpyridinium ion* (MPP^+), which targets substantia nigra neurons and precipitates parkinsonism in humans and other mammals. *β-N-Methylamino-L-alanine* (L-BMAA) is metabolized to formaldehyde in rodent brain tissue, thereby transforming an excitant amino acid to a potential genotoxin. Hepatic biotransformation is broadly concerned with the conversion of lipophilic chemicals to less toxic hydrophilic metabolites (Phase I) and their conjugation (Phase II) prior to excretion. Occasionally, Phase I metabolism may increase the neurotoxic potential of an exogenous substance, as in the case of the stepwise conversion of *n-hexane* to *2,5-hexanedione*, a solvent that targets neuroproteins and causes nerve fiber degeneration in the CNS and peripheral nervous system (PNS). Co-exposure to substances such as *methyl ethyl ketone* or *toluene* may impact Phase I enzyme function and thereby markedly modify (increase or decrease, respectively) the quantitative neurotoxic response to *n-hexane*.

Most mammalian species reproduce the qualitative response of the human nervous system to chemical perturbation, but the sensitivity may differ markedly among species. For example, relative to humans, primates, and cats, rodents are relatively refractory to o*rganophosphate*-induced neuropathy. Sex is a factor in risk for tardive dyskinesia (stereotypic behaviors, dystonia, myoclonus, and tics) associated with the dopamine receptor-blocking properties of *neuroleptic drugs*. Sex as a determinant of biotransformation may be less important in humans than in rats, where marked differences in the metabolism and consequent functional responses to individual chemicals such as *parathion* may be noted. *3,4-Methylenedioxymethamphetamine* (*MDMA*) may depress serotonin more in female than male rats. Differences in genotype may determine the presence of neurotoxic responses, as in the rare mitochondrial polymorphism that results in hypersensitivity to *aminoglycoside-induced deafness*. Aging in humans is generally associated with increased susceptibility to many chemical substances largely because of reductions in biotransformation, renal clearance, and biliary excretion, as well as factors such as reduced body weight, inanition, and polypharmacy that promotes drug and chemical interactions. In addition, neurons display age-related increases of DNA damage, regional nerve cell loss (e.g., substantia nigra), and axonal pathology (e.g., spinal roots). Nutritional status may predispose to human neurotoxicity as seen in minimally nourished Africans who develop spastic paraparesis from a combination of sulfur amino acid deficiency and exposure to *cyanogenic substances*, both of which arise from dietary dependence on the root crop cassava (*Manihot esculenta*). Substances that interfere with vitamin production or utilization, including thiamine (*pyrithiamine*), riboflavin (*quinacrine*), niacin (*3-acetylpyridine*, *6-aminonicotinamide*), or cyanocobalamin (*nitrous oxide*), can produce various types of severe neurological deficit, as does excessive exposure to *vitamin A*. Other types of neurodegeneration are seen with substances (*pyridinethione*, *8-hydroxyquinolines*, *ethambutol*) that chelate physiologically important metals ions, such as zinc. Excessive dietary intake of *sulfur* and *selenium* produces neuronal lesions in ruminants and pigs, respectively. Body temperature is an important determinant of the neurotoxic response of rodents, primates, and possibly humans to the psychostimulant *methamphetamine*: hyperthermia exacerbates and hypothermia attenuates methamphetamine-induced dopamine neurotoxicity. Finally, excessive physical activity has been associated with the acute onset of central motor weakness in cassavism and lathyrism, the latter associated with dietary dependency on grasspea (*Lathyrus sativus*), a legume that contains the excitant amino acid *β-N-oxalylamino-L-alanine* (L-BOAA).

The structure of chemicals and their differential access to the nervous system are of cardinal importance in determining the presence and nature of the neurotoxic response. While access to nervous tissue dictates the possibility of a direct neurotoxic effect, neurotoxicity ultimately depends on the ability of the substance to bind to neural tissue targets and interfere with functional or structural integrity. Structure–activity relationships are therefore of critical importance. For example, *1,2-diacetylbenzene* but not 1,3-diacetylbenzene induces leg weakness in rodents because only the former binds to and cross-links neuroproteins, interferes with anterograde transport of neurofilaments, and causes giant proximal axonal swellings that disrupt nerve conduction. *Triethyltin* targets the myelin sheath and *trimethyltin* damages neurons, but tributyltin lacks neurotoxic properties – another illustration of the critical importance of chemical structure in determining the presence and nature of the neurotoxic response. Even stereological properties of molecules can dictate the presence or absence of neurotoxic properties: L-BOAA (but not D-BOAA) is a potent agonist of neuronal DL-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. Only *levo-naloxone* displays opioid receptor antagonistic activity, and neurotoxicity of chiral PCBs is enantioselective.

The large majority of the nervous system is protected from direct exposure to chemicals in the bloodstream and the cerebrospinal fluid (CSF) by blood–brain/nerve/CSF regulatory interfaces. Structural specialization of blood vessels separates the microenvironment of the brain parenchyma from changes in circulating ion and metabolite concentrations. Regulation of blood–brain/nerve interchange is a key function of capillaries coursing through nervous tissue. Structural specializations of capillary walls in the form of tight junctions between adjacent endothelial cells constitute a diffusion barrier. This allows the endothelium to regulate the selective transport and metabolism of substances from blood to brain and vice versa. While gases (*oxygen*, *carbon monoxide*, *nitrous oxide*) cross capillary walls with ease, many substances are excluded or their access to nervous tissue impeded by the presence of the capillary barrier. Lipophilicity and size are key elements in regulating the passage of macromolecules across the blood–brain barrier. Key nutrients and macromolecules required by the brain cross via facilitated diffusion or specific carriers. Many blood-borne substances attempting passage across brain endothelial cells are stopped by a variety of metabolizing enzymes or expelled back into the capillary lumen by embedded efflux transporters, such as permeability glycoprotein. The epithelial junctions that constitute the blood–CSF barrier at the choroid plexus are somewhat more permeable and allow greater passage of drugs and toxicants into the interstitial fluid that bathes brain tissue.

Many metals are required for normal CNS function and are thus transported across the blood–brain and blood–CSF barriers. However, excess metal may accumulate in endothelial cells and give rise to toxic damage of the cellular barrier. Several metals are known to accumulate in both barriers, including substances with neurotoxic potential such as *lead*, *mercury*, *arsenic*, and *manganese*.

Lead accumulates in the choroid plexus and alters key functions, including transthyretin, the binding protein for thyroid hormone. High concentrations of *lead* damage the blood–brain barrier, cause vascular leakage, and may result in brain swelling accompanied by herniation, ventricular compression, and petechial hemorrhages.

Some regions of the brain (e.g., hypothalamus) and PNS (spinal and autonomic ganglia) lack capillary barriers and are thus directly exposed to chemicals circulating in the bloodstream. Damage to the hypothalamus can have far reaching effects on somatic metabolism, reproductive function, and growth. The adult obesity of rats treated postnatally with *monosodium glutamate* (MSG) exemplifies the effect and raises important questions for human health in regard to past exposure to glutamate-rich foods during postnatal development. In the PNS, the selective loss of sensory neurons in rats treated with *doxorubicin* arises because spinal ganglia lack a protective capillary-nerve barrier.

Certain other substances (*tetanus toxin*) reach nerve cell bodies directly via distal axonal entry and retrograde axonal transport. Tetanus toxin is transported to the spinal anterior horn cell, subsequently translocates and binds to inhibitory presynaptic inhibitory (glycinergic) nerve terminals impinging on the motor nerve cell, and thereby suppresses the inhibition of motor neuron activity leading to hyperexcitation. Violent and sustained muscle contraction (tetany) in response to external stimulation results therefrom. Another example of peripheral entry to the CNS is the transport and delivery of metals (*manganese*, *aluminum*) from the nose along olfactory neurons to the brain of laboratory animals.

The mammalian nervous system has functional design features that predispose it to chemical perturbation. Consequently, neurological dysfunction is among the most common of the toxic responses of humans to chemical substances. Some neurotoxic agents perturb energy generation by interfering with glycolysis (*arsenic*), while others (*3-nitroprionic acid*, *cyanide*, *rotenone*) disrupt sites in the electron transport chain. Some agents (*metronidazole*, *misonidazole*) damage brain regions such as the brainstem nuclei that seem to have a high requirement for glucose.

Architectural design is another important determinant of vulnerability both at the cellular and organ level. Unlike most organs (liver, kidney, testes), the nervous system is regionally organized; motor function, memory, vision, audition, and olfaction require the function and continuous interaction of different brain regions. Whereas small lesions of the liver and kidney have little functional impact, a chemical that damages the hippocampus (*domoic acid*), visual cortex (*methylmercury*), or the peripheral vestibular and auditory system (*streptomycin*), may have devastating effects on function of the organism. Moreover, whereas tissue repair follows damage to many organs, the CNS recovers from injury poorly. Some recovery may be afforded by neurogenesis and/or reorganization of synaptic connections on surviving nerve cells, but regrowth and reconnection of damaged axons are impeded by postinjury proliferation of astrocytes.

Cellular architecture is yet another important factor in determining vulnerability to chemical attack. Unlike most types of mammalian cells, mature neurons and myelinating cells in the CNS (oligodendrocytes) and PNS (Schwann cells) possess elongated cellular processes that expose vast surface areas of membrane to chemicals present in extracellular fluid (Fig. 1). Additionally,

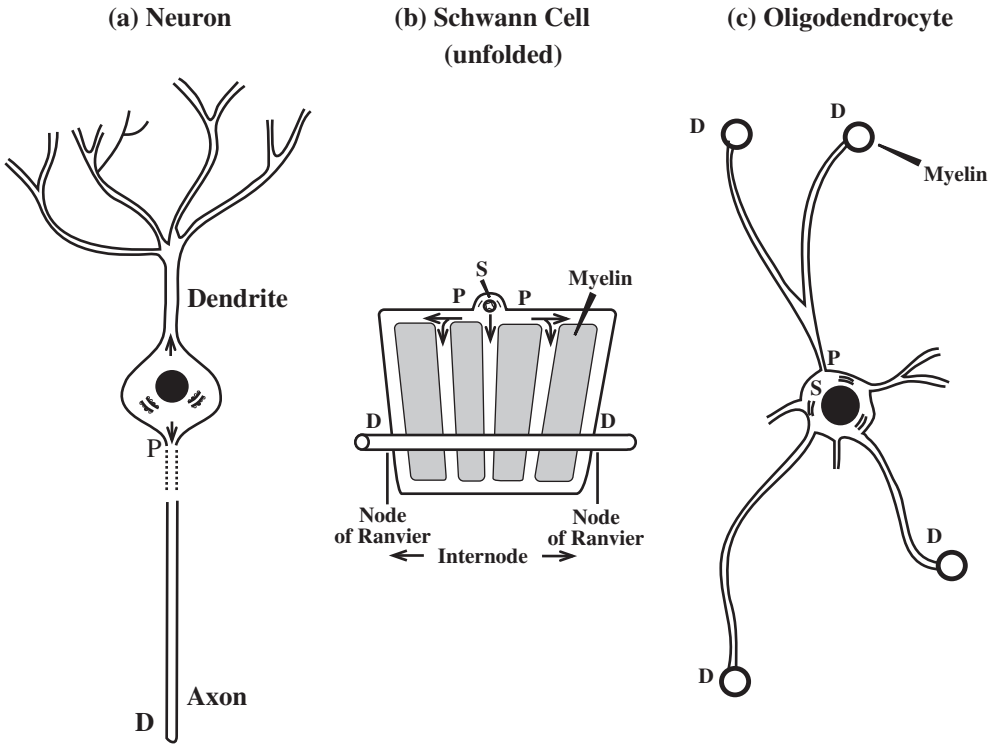

Fig. 1 Diagram of (A) neurons, (B) Schwann cell with myelin sheath 'unrolled,' and (C) oligodendrocyte, to illustrate that each cell has a restricted cell soma (S) and elongated processes that can be divided into proximal (P) and distal (D) portions. The elongated processes of these cells provide a huge area for chemical attack.

each of these cells has a segregated anabolic region (cell body) that is responsible for supplying the metabolic needs of proportionately huge volumes of cytoplasm (axons, dendrites, myelin). The unusual architecture of these cells demands the presence of a distribution system that efficiently transports materials from sites of production to sites of utilization. Within neurons, chemicals that disrupt axonal transport (*taxol*, *vincristine*) can induce axonal degeneration with consequent effects on sensory and motor function. The special vulnerability of the longest and large-diameter axons leads clinically to sensory dysfunction and motor weakness in a stocking-and-glove distribution.

Neural cells are highly interconnected and dependent upon each other's presence and physiological activity for normal function. Thus, chemicals that disrupt Schwann cells (*diphtheria toxin*) or myelin (*hexachlorophene*) secondarily disturb nerve conduction along the axonal processes of neurons with which they are physically associated. Other agents interfere directly with electrical transmission (*pyrethroids*) or the orderly conveyance of signals via synapses (certain *organophosphates*) that connect nerve cells to each other. Similarly, agents (*nitrofurantoin*) that cause peripheral axons to degenerate thereby sever neuronal connections with muscle cells and cause muscle weakness.

Given the nervous system has built-in redundancy, the loss of neurons or axons must exceed a certain threshold for clinical effects to become apparent. That redundancy of nerve cells may be substantially reduced with the advance of age, such that regional loss of CNS striatal dopaminergic neurons is thought to be a factor in dictating susceptibility to agents that can trigger parkinsonism (*carbon disulfide*, *trichloroethylene*) and other neurodegenerative disorders (*manganese)*. Similarly, age-related spinal nerve root demyelination and distal axonal degeneration of long and large-diameter axons may predispose elderly individuals to substances that cause peripheral neuropathy (*vide infra*).

Vulnerability of neurons and their processes

Nerve cells are vulnerable to chemical attack at many loci, including protein synthesis, mitochondrial and nucleic acid function. Somal DNA is predisposed to damage by reactive oxygen species because of the high oxygen consumption and metabolic activity of neurons. The cycad genotoxin MAM, an agent that alkylates DNA with formation of O^6-mG and other adducts, interferes with neuronal development and has been implicated in a long-latency progressive neurodegenerative disease (*vide supra*). Adult neurons have very low levels of the specific DNA repair enzyme, O^6-methylguanine methyltransferase, such that long-term neuronal effects might occur as a consequence of unrepaired DNA damage. Mitochondrial DNA chain growth is blocked by certain anti-HIV drugs (*2′,3′-dideoxyinosine*, *2′,3′-dideoxycytidine*) that cause painful axonal neuropathy. Interference with neuronal protein synthesis occurs with agents (*ricin*) that disrupt polypeptide elongation and trigger axonal degeneration. Chemicals that alter the mitochondrial electron transport chain (*cyanide, 1-methyl-4-phenylpyridinium; 3-nitropoprionic acid; rotenone*) have a propensity to induce basal ganglia damage. Substances (*bromethalin*, *pentachlorophenol*) that uncouple electron transport and oxidative phosphorylation elevate temperature and induce tremor and hyperexcitability. Several neuronal enzymes are key sites of chemical attack, including synaptic acetylcholinesterase (*organophosphates*, *carbamates*) and neuropathy target esterase (*a subset of organophosphates*), with resulting neuroexcitation and axonal degeneration, respectively. *Lead* interferes with oxidative phosphorylation by potently activating protein kinase C. Proteasome inhibitors (*lactacystin, epoxomicin*) induce dose-dependent cell death of dopaminergic neurons in primary ventral mesencephalic culture and loss of nigral neurons in rats.

The excitable membrane of neurons is the target of a rich array of neurotoxins that interfere with ion channels required for the proper functioning of neurons, axons, muscle, and glial cells. These agents are often complex structures generated by invertebrate species and plants presumably for purposes of chemical defense. Voltage-gated sodium ion channels are common targets. Agents that bind to the outer surface of membrane Na^+ channels and prevent ion influx are found in dinoflagellates (*saxitoxin*), vertebrates, including fish, octopus and salamander (*tetrodotoxin*), and cone snails (*geographutoxin*). Lipid-soluble anesthetics (*lidocaine*, *procaine*) bind to a hydrophobic site in the channel and interfere with the gating mechanism. Activation of voltage-gated Na^+ channels by opening or impeding normal closure occurs with exposure to certain plant chemicals (*grayanotoxin*; *pyrethrin*), dinoflagellate chemicals (*ciguatoxin*) stored in fish, amphibian skin toxins (*batrachotoxin*), *scorpion α-toxins*, and synthetic pesticides (*pyrethroids*). Interference with Na^+ channel function is usually heralded by abnormal sensation (paresthesias) in the tongue, around the mouth and in the extremities. While usually transient, *ciguatoxin* is a fat-soluble agent that may cause repeated neurotoxic events after single exposures presumably because of tissue sequestration and periodic release within the affected subject. K^+-channel toxins with selective blocking actions have been identified in the *venom of certain scorpions*, *bees*, and *snakes*. *Thallium* and *bromine* ions are transported by K^+ and Cl^- channels, respectively, with the potential for significant cardiac, neurological and psychiatric consequences for the affected subject. *Calcium channel blockers* are produced by certain plants, insects, spiders, snails, and snakes. *Divalent lead ions* interfere with intracellular processes regulated by Ca^{2+} ions and accumulate in the same intramitochondrial compartment as calcium. Alternative pre-RNA splicing in the gene (*Ca(V)2.2*) that codes for N-type Ca^{2+} channels in nociceptors modulates opioid channel inhibition and spinal analgesia.

Neurotransmitter systems

Nerve cells communicate with each other and with voluntary muscle by chemical neurotransmission. Numerous substances target mechanisms involved in neurotransmitter (NT) synthesis, transport, synaptic release, reuptake, the interaction between NT

and postsynaptic receptor, or the removal of NT from the synaptic gap. Neurotoxicity occurs when the agent reduces or increases NT release, alters NT concentration or resident time, or acts as an agonist or antagonist at a postsynaptic NT receptor.

Acetylcholine synapses at neuromuscular junctions are targets for a number of biologic and synthetic substances (Fig. 2). Neurotransmission is disrupted by agents that interfere with choline transport (*hemicholinium*, *choline*), with acetylcholine synthesis (*triethylcholine*), or synaptic vesicle uptake (*vesamicol*). Other agents (*β-bungarotoxin*, *crotoxin*) target mechanisms involved in presynaptic NT release. Botulism arises from the action of a zinc-endopeptidase (*the light chain of the botulinum toxin dipeptide*) in blocking synaptic transmission by cleaving synaptic-vesicle fusion proteins required for NT exocytosis. Botulinum-induced blockade of normal depolarization-induced NT release at the neuromuscular junction leads to flaccid muscle paralysis. Conversely, *α-latrotoxin* in venom of the black widow spider causes massive NT release at vertebrate neuromuscular junctions resulting in a painful disorder featured by dysarthria, tremor, clonic muscle contraction, and paralysis. Numerous chemicals interfere with acetylcholinesterase, the enzyme that terminates the NT action of acetylcholine. Certain *anticholinesterases* (*edrophonium*) bind directly to the active center of the enzyme and act rather briefly; others (*physostigmine*) carbamylate the enzyme and have longer-lasting actions. The large class of *organophosphates*, which include high-potency agents used in chemical warfare and less hazardous substances employed as agricultural pesticides, interact with the anionic and/or esteratic sites in the active center of acetylcholinesterase to form complexes; the stability of the phosphorylated enzyme is further enhanced by the loss of one of the alkyl groups, a phenomenon known as (chemical) aging. Biological anticholinesterases include the product of a cyanobacterium (*anatoxin-a(s)*) and certain snake toxins (*fasciculins*).

Acetylcholine receptors provide another target for chemicals with neurotoxic potential, most acting as receptor antagonists. *D-Tubocurarine* is the classical nicotinic receptor antagonist, and curare-like substances are found in elapid and hydrophid snakes (*α-neurotoxins*), such as cobra (*α-cobratoxin*) and krait (*α-bungarotoxin*), mollusks (*α-conotoxin*), corals (*lophotoxin*), and certain plants, such as the delphinium (*methyllycaconitine*). *Anatoxin-a* is a potent agonist at neuromuscular, autonomic, and brain nicotinic receptors. Muscarinic receptor antagonists include *atropine*, *scopolamine*, and the synthetic warfare agent *quinuclidinyl benzilate*, which induces dryness of the mouth, blurred vision, confusion, delirium, and coma.

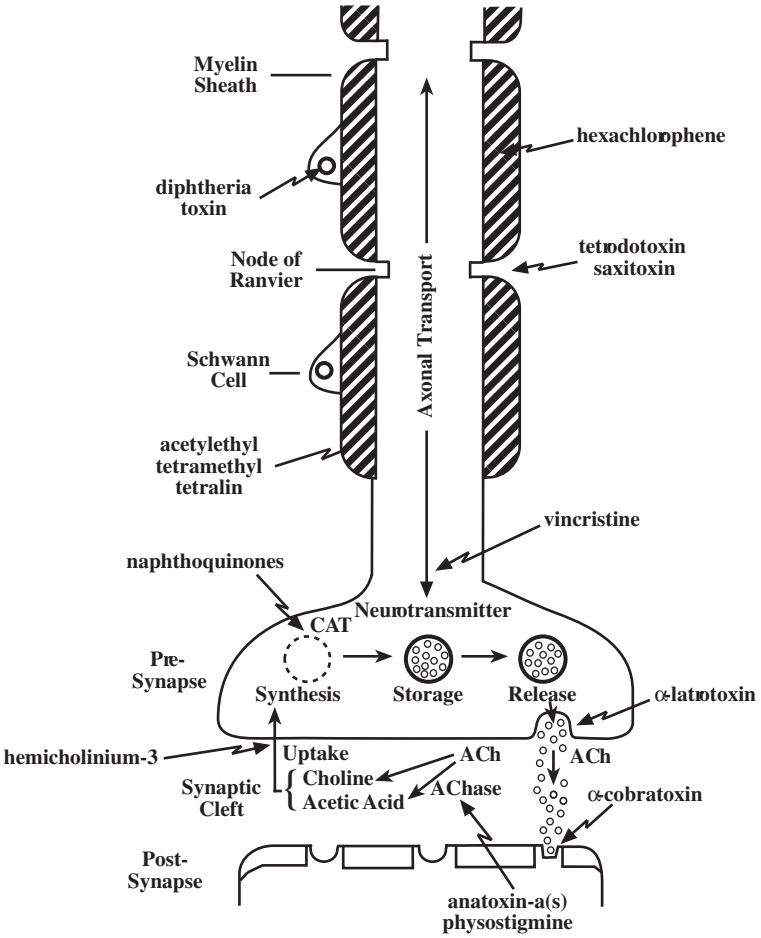

Fig. 2 Targets of neurotoxic agents acting on PNS cholinergic nerve fibers (upper portion), terminals (lower midportion), and neuromuscular synapses (lowest portion). *ACh*, acetylcholine; *AChase*, acetylcholinesterase; and *CAT*, choline acetyltransferase.

Glutamate receptors, which mediate most excitatory neurotransmission in the CNS, are another important target of chemical substances linked to human neurological disease. Fast synaptic transmission is mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors, a target of grasspea L-BOAA that causes pyramidal tract degeneration and spastic paraparesis (lathyrism). Seaweed (*kainic acid*) and dinoflagellate (*domoic acid*) toxins act as agonists at the kainate subclass of glutamate receptors, the latter causing significant CNS neuronal excitotoxicity and CNS degeneration in humans. Glutamate receptors that respond to *N*-methyl-D-aspartate (NMDA) have numerous antagonists, including the anesthetic agent *ketamine* and *phencyclidine* (*angel dust*). Termination of excitatory neurotransmission by removal of synaptic glutamate is perturbed by *dithiocarbamate pesticides*. Ionotropic glutamate receptors also play an important role in mediating neuronal death secondary to energy deficits induced by *hypoxia*.

γ-Aminobutyric acid (GABA), which is synthesized by glutamic acid decarboxylase (GAD), is the major inhibitory NT in the mammalian brain. GAD inhibition (*2-amino-4-pentenoic acid*) induces convulsions. Numerous compounds bind to and act as antagonists (*picrotoxin*, *tetramethylenedisulfotetramine*) or agonists (*muscimol*) at $GABA_A$ receptors, which employ chloride (Cl^-) channels to mediate fast inhibitory postsynaptic potentials. Enhanced GABAergic transmission occurs under the action of *benzodiazepine* and *barbiturate drugs*, while *organochlorine insecticides* (*lindane*, *aldrin*) exert convulsant effects mediated through picrotoxin-binding sites.

Glycine receptors, which mediate inhibitory neurotransmission in the brainstem and spinal cord, have a number of plant-derived antagonists (*strychnine*, *hydrastine*) that elicit hyperreflexia and tetanic muscle contraction among other signs. Tetanus toxin from *Clostridium tetani* triggers generalized muscle rigidity by binding to glycinergic nerve terminals (and inhibiting glycine NT release) after crossing from anterior horn cells that retrogradely transport the agent from a peripheral wound site.

Several other NT systems and pathways are impacted by chemicals that exhibit neurotoxicity. Dopamine mediates communication in a number of important pathways, including the nigrostriatal tract (a key component of basal ganglia mechanisms for control of the quality of motor movement) that degenerates in Parkinson's disease and MPP^+-induced toxicity. Extrapyramidal movement disorders, both acute (dystonia, akathisia, and parkinsonism) and chronic (tardive dyskinesia), can occur as a side effect of a number of therapeutic drugs (antipsychotics) acting on dopaminergic pathways via dopamine-receptor blockade. Chemicals that perturb adrenergic function may interfere with NT synthesis (*α-methyltyrosine*), serve as false NTs (*methyl-dopa*), block vesicular uptake (*reserpine*), inhibit the cleavage enzyme catechol-*O*-methyltransferase (*pyrogallol*), or promote NT release (*amphetamine*). *Substituted amphetamines* (MDMA) damage axons derived from the dorsal raphe serotonergic projection system of rodents and primates. Natural substances in plants interfere with substance P neurotransmission (*capsaicin*), cannabinoid receptors (*cannabis*), purinoceptors, and adenosine receptors (*caffeine*). Caffeine intoxication is characterized by anxiety, sleep disturbance, and mood changes, while caffeine withdrawal in sensitive subjects leads to vascular headache, drowsiness, and fatigue.

Axonal transport

Disruption of the transport of materials along axons is another method by which chemicals can perturb neural function and induce changes, including focal axonal pathology and axonal degeneration (axonopathy). Some agents interfere with the anterograde transport of materials from sites of synthesis (cell body) to sites of utilization (axon and terminal) (*β-β′-iminodipropionitrile*, *1,2-diacetylbenzene*), with retrograde axon transport (*acrylamide*), or with the return of materials from nerve terminals (*zinc pyridinethione*). Certain plant-derived chemicals (*colchicine*, *vinca alkaloids*) bind to tubulin, inhibit microtubule-based function, and thereby block bidirectional axonal transport. *Taxol*, originally isolated from the Pacific yew tree, interferes with axonal transport by promoting microtubule assembly and stabilization. Retrograde axonal transport may also serve to ferry foreign substances (*ricin*, *tetanospasmin*, *certain metals*) to targets in neuronal somata.

Neuroglia and myelin

Neuroglia include (1) ependymal cells lining the ventricles of the brain and the central canal of the spinal cord; (2) cells of the PNS (Schwann cell) and CNS (oligodendrocyte) that wrap around axons to form compacted plasma membranes (myelin) that provide electrical insulation to speed nerve conduction; (3) cells (astrocytes) that interface between nerve cells and capillaries in the CNS, regulate interstitial water content, K^+ concentration, remove and metabolize certain NT molecules, and proliferate following injury; and (4) microglia, which are phagocytic cells that function as endogenous immune cells in the brain.

Ependymal cells are susceptible to agents (*amoscanate*) present in CSF. Astrocyte foot processes investing cerebral capillaries undergo marked swelling in *water* intoxication, *lead* encephalopathy and in *hypercapnia*, and after the experimental administration of *6-aminonicotinamide*, *isoniazid*, *misonidazole*, or *ouabain*. Astrocytes increase their glycogen content in a variety of insults (*methionine sulfoximine*), form intranuclear inclusion bodies in *lead* intoxication, and greatly increase the relative size of the nuclear compartment in hepatic encephalopathy, which is thought to be triggered by *hyperammonemia*. The food-associated mycotoxin *ochratoxin A* inhibits glutamate uptake by astrocytes, which has been proposed to increase extracellular glutamate, excitotoxicity, and neuronal loss. Ochratoxin A may be found worldwide in agricultural products such as cereal grains, dried fruit, wine and coffee.

Myelinating cells are susceptible to substances that disrupt the synthesis of myelin components, the best example of which is *diphtheria toxin*, which has access to peripheral nerves where it inhibits Schwann cell protein synthesis and causes primary demyelination. Oligodendrocyte demyelination can be induced experimentally by diphtheria toxin and by other protein synthesis inhibitors, such as *ethidium bromide* and *actinomycin D*. The latter induces widespread status spongiosus of white matter, with edema fluid in the periaxonal space and between myelin membranes split open at the intraperiod line. *Cuprizone* (*biscyclohexanone oxalylhydrazone*) induces oligodendrocyte degeneration with intramyelinic edema. Several other agents (*cycloleucine*, *hexachlorophene*, *triethyltin*) trigger reversible changes in CNS and/or PNS myelin without apparent damage or loss of the myelin-forming cells.

System vulnerability

Developing nervous system

The dominant host factor influencing response of the nervous system to exogenous chemicals is developmental age. The intrinsic neural factors that determine or influence response to neurotoxic chemicals differ in development, at maturity, and in late life. Thus, physiological differences between developing and adult organisms underlie potentially significant differences in distribution, metabolism, and excretion of neurotoxic agents. Neurotoxic effects observed following developmental exposure can differ both quantitatively and qualitatively from those seen following adult exposure. Quantitatively, the developing nervous system is generally more sensitive to neurotoxic agents. Studies of both humans and rodents indicate that intrauterine exposure to *lead* or *PCBs* is more damaging to the nervous system than exposure later in life. Neurotoxic effects may also differ qualitatively as a function of developmental age. For example, infants of mothers treated with the anticonvulsant drug *sodium valproate* may display congenital malformations, including neural tube defects, whereas neurotoxicity in the adult is manifest as tremor, a confusional state and, in rare cases, parkinsonism.

The influence of developmental age on the response to neurotoxic agents largely reflects the susceptibility of developmental processes occurring in the developing brain. Neurodevelopment is a complex process that is precisely regulated in time and space, with the basic framework of the nervous system laid down in a step-by-step process in which each step is dependent upon the proper completion of the previous one. In both humans and rodents, normal neurodevelopment begins very early in the fetus and is not complete until puberty. Active organogenesis, which takes place during the period from implantation through mid-gestation, requires the concomitant and coordinated ontogeny of cell proliferation, differentiation, and migration. During late gestation and the early neonatal period, the processes of synaptogenesis, apoptosis, and myelination are predominant. These processes continue throughout later stages of neurodevelopment, and together with elimination of extraneous synapses via axon and dendrite retraction, function to refine patterns of neuronal connectivity. Experimental and clinical data suggest that errors in timing, spatial resolution, or magnitude of any of these developmental events can have functional consequences. For example, magnetic resonance imaging studies of patients with schizophrenia (modeled in rats by single treatment with MAM on embryonic day 17) reveal excessive pruning of axons and dendrites in the cerebral cortex during late adolescence, coincident with the onset of symptoms. Substances have been identified that interfere with each of these processes in animal models and, in some instances, humans. The outcome of such interactions ranges from death to gross structural abnormalities to subtle defects in structure or function: early gestational exposure to substances such as *fumonisins* can produce neural tube defects; exposure to ionizing radiation alters brain morphology and induces mental retardation; exposure to high concentrations of *ethanol* causes mental retardation, while moderate levels of alcohol exposure can delay motor development; intrauterine exposure to *cocaine* causes excessive outgrowth of dendrites; and exposure of infants and children to relatively low levels of *lead* is linked to reduced scores on tests of cognitive development and to increased aggressive tendencies.

A critical determinant of the pattern of damage induced by neurotoxic agents is the timing of exposure relative to ongoing ontogenetic events. For example, *vitamin A* and other *retinoic acid derivatives* cause marked and irreversible abnormalities, including anencephaly and spina bifida, when exposure occurs during gestation days 5–10 in rats. In contrast, administration of retinoic acid on gestational day 12 fails to perturb brain development. There are at least four scenarios to explain how timing of exposure influences neurotoxic outcome. First, later stages of neurodevelopment depend on successful completion of early stages; therefore, even relatively minor disturbances early in neurodevelopment may cause significantly more damage than perturbations occurring at later stages. For example, inhibition of cellular proliferation by agents, such as *MAM*, *lead*, *mercury*, or *ethanol*, can subsequently impact migration, differentiation, and ultimately neuronal connectivity. Second, individual neurodevelopmental events may be differentially vulnerable to specific substances. It has been clearly demonstrated that specific brain regions are vulnerable to antimitotic agents during the developmental period when regional cells are actively proliferating. The DNA-damaging agent MAM disrupts regional mouse brain development in an O^6-mG-dependent manner, with little change from normal in MAM-treated animals that overexpress the specific DNA-repair enzyme O^6-mG methyltransferase. Third, since different brain regions develop on different timelines during prenatal and postnatal life, a chemical may produce impairment in different functional domains depending on the time of exposure. Changing the time at which neonatal rats are exposed to *ethanol* can trigger neuronal cell loss via apoptosis in different brain regions, thereby giving rise to different profiles of functional deficits. Fourth, the expression and/or function of target proteins can vary in the developing versus adult brain. The alpha subunit of the glycine receptor exists in several isoforms that are transcriptionally regulated during development. The adult isoforms of the alpha subunit have a higher affinity for strychnine than the neonatal isoforms; thus, the developing nervous system is less vulnerable to *strychnine* than the adult. Another important example includes NTs and enzymes that metabolize NTs, such as acetylcholinesterase. In the adult nervous

system, these proteins function in neurotransmission; however, during development, NTs and acetylcholinesterase act as morphogenic factors that modulate patterns of neuronal connectivity. Therefore, substances that target NT systems (*chlorpyrifos*) may have quite different effects on the developing fetus or child compared to the adult, and this has been demonstrated in animal models.

The potential for chemical exposure to the fetus begins before conception in that prior parental exposure to toxicants can have a major impact on the developing fetus. Parental exposures threaten fetal health by either altering maternal or paternal reproductive organs directly or via release of stored neurotoxic agents from maternal tissues during pregnancy. Yusho disease is a tragic example of preconception exposure to *PCBs* influencing fetal neurodevelopment. Women in the Japanese town of Yusho who consumed cooking oil contaminated with PCBs up to a year prior to conception gave birth to infants exhibiting a constellation of symptoms including dysmorphism, skin lesions, hepatic dysfunction, and cognitive abnormalities. PCBs stored in maternal tissues were mobilized during pregnancy. Similarly, *lead* can be mobilized from storage depots in bone during pregnancy. Once in the maternal blood supply, chemicals may diffuse across the placenta and enter the fetal circulation. Some agents (*organoarsenicals*) accumulate in the placenta, which shields the offspring from exposure. However, the placenta does not block small molecular weight compounds (*carbon monoxide*), lipophilic compounds (*PCBs*, *ethanol*, *methylmercury*), or compounds using active transport mechanisms (*lead*). Once in the fetal circulation, the developing brain is protected by the same brain barrier mechanisms that protect the adult brain. Contrary to a long-standing belief, functional brain barrier mechanisms are in place after neural tube closure. The vulnerability of the developing brain may be enhanced, however, by inward transport mechanisms for nutrients such as amino acids, glucose, and trace metals, which are more active than in the adult brain and thus may facilitate the entry of neurotoxins such as mercury, lead, and manganese. The lower concentration of proteins in fetal and neonatal plasma may allow their binding capacity for drugs and toxicants (heavy metals) to be exceeded more easily than in the adult. In addition, the presence of transport mechanisms that are unique to the developing brain may similarly increase the vulnerability of the developing brain. Prior to keratinization of the human fetal epidermis, beginning at 20 weeks of gestation, exogenous chemicals may also diffuse from the amniotic fluid into the developing fetus. After birth, exposure to potential toxicants may occur via breastfeeding and consumption of other contaminated foodstuffs, oral contact via hand-to-mouth activity, dermal contact, or inhalation. Compared with adults, children in all postnatal developmental stages have higher rates of respiration and energy consumption per kilogram of body weight, which increases their exposure rates. In addition, skin is highly permeable during the newborn period, and several epidemics of developmental neurotoxicity have been described involving percutaneous absorption of chemicals. These include hypothyroidism from iodine in *povidone iodine* (*Betadine*) scrub solutions and myelin disorders consequent to bathing infants in *hexachlorophene*. The expression of Phase I and Phase II metabolic enzymes is also developmentally regulated, resulting in altered abilities of developing organisms to detoxify and excrete chemicals relative to adults. This difference may confer increased resistance when a substance must be metabolized to an active metabolite. But, more frequently, the metabolic differences manifest as a decreased capacity of children to excrete toxins as compared to adults, and thus they are more vulnerable to certain neurotoxic agents. The lack of functional paraoxonase, the enzyme that detoxifies many *organophosphates*, contributes to the increased vulnerability of the developing nervous system to the neurotoxic effects of these agents.

Many chemicals are recognized teratogens in animals; a significantly smaller subset of these is known or suspected to cause toxic effects in the developing human nervous system. Some of the more significant human developmental neurotoxicants include *ethanol*, which causes a constellation of effects ranging from fetal alcohol syndrome to alcohol-related neurodevelopmental disorder; maternal *tobacco* use (fetal tobacco syndrome); *excess vitamins A and D*; metals, particularly *inorganic and organic mercury*, *lead*, *arsenic*, *magnesium*, and *cadmium*; anticonvulsants, such as *phenytoin*, *valproate*, *phenobarbital*, *carbamazepine*, and *primidone*; drugs of abuse, including *cocaine*, *cannabis*, and *mescaline*; persistent *aromatic hydrocarbons*, especially *PCBs and polybrominated diphenyl ethers (PBDEs)*; and both *organochlorine* and *organophosphate pesticides*.

Detecting effects in the human population is difficult because they may be subtle (small shifts in IQ scores, slight changes in behavior) or because neurotoxicity does not manifest until a significant period of time after the developmental exposure. Delayed neurotoxicity may arise via two different mechanisms. One of these involves a developmental insult in which anatomical and/or functional effects may be masked or attenuated initially because of compensatory mechanisms or plasticity. However, these developmental perturbations predispose the individual to neural deficits following subsequent insults, such as chemical exposure, disease, or aging because of decreased reserve capacity. This phenomenon has been demonstrated in animal models for *organochlorine pesticides*, and in both animal models and humans following developmental exposures to *methyl mercury*. The second mechanism involves the occurrence of a toxic insult early in neurodevelopment, but with manifestation of the pathological change much later in neurodevelopment when function of the affected cells is normally activated. An agent that causes this type of delayed neurotoxicity experimentally is the food additive *monosodium glutamate* (MSG). Developmental exposure to MSG causes excessive apoptosis of neurons via activation of NMDA glutamate receptors in the developing hypothalamus. However, the fetal loss of these hypothalamic neurons becomes evident (as hypogonadism and infertility) only in adolescence when the neuroendocrine function of these neurons is normally activated.

The identification of the food additive MSG as a potential developmental neurotoxicant triggered studies of other substances that modulate excitatory and/or inhibitory neurotransmission in the developing brain. The culmination of this work is the recognition that neuroactive compounds that either block NMDA glutamate receptors or promote inhibitory neurotransmission at $GABA_A$ receptors can also trigger neuronal apoptosis in the immature rodent brain. *Ethanol*, which has both NMDA antagonist and GABA mimetic properties, causes enhanced neuronal apoptosis relative to compounds with only one of these activities. The period of peak vulnerability for this toxic effect coincides with the developmental period of rapid synaptogenesis, also known as the brain growth spurt, which occurs in the early postnatal period in mice and rats, and from the third trimester to several years after

birth in humans. These studies have generated significant concern in the clinical setting because a number of anesthetics used in pregnant women and infants function by blocking NMDA receptors (*ketamine*) or promoting GABA neurotransmission (*midazolam*, *diazepam*, *propofol*). These anesthetic drugs have been shown to cause widespread developmental neurodegeneration via apoptosis and persistent cognitive deficits in rodents exposed to clinically relevant levels, and similar effects have been demonstrated for ketamine in nonhuman primates. Epidemiological studies support a link between brief anesthesia exposure during early infancy and poor neurodevelopmental outcome; however, interpretation is confounded by the potential impact of the surgical procedure, concurrent illness, or coexisting pathology. This case study is an illustration of some of the issues associated with confirming developmental neurotoxicity in humans, even for compounds with known mechanisms of action.

Additional emerging issues in developmental neurotoxicology that merit mention include endocrine disruption as a mechanism of developmental neurotoxicity and gene–environment interactions as determinants of risk and/or severity of neurodevelopmental disorders. With respect to the former, sex steroids, thyroid hormones, and neuropeptides are known to modulate key neurodevelopmental processes including neuronal differentiation, migration, survival and apoptosis, neuronal connectivity, and synaptic plasticity. Alteration of hormonal status during brain development can cause functional deficits in adolescence and adulthood, as illustrated by cognitive dysfunction associated with *fetal hypothyroidism*. While a number of chemicals with known endocrine-disrupting activity has been shown to interfere with neurodevelopment (*PCBs*, *bisphenol A*), it remains to be established whether the developmental neurotoxicity of these compounds is mediated by endocrine disruption and whether endocrine disruption alters neurodevelopment in humans.

The second emerging issue in developmental neurotoxicology reflects a growing consensus that environmental factors, potentially including environmental pollutants, drugs, or dietary factors, interact with genes of susceptibility to determine risk and/or variable expression of complex neurodevelopmental disorders, including autism spectrum disorder (ASD), attention-deficit hyperactivity disorder (ADHD), and schizophrenia. This paradigm shift is driven in part by evidence that the prevalence of neurodevelopmental disorders is increasing. For example, decades ago, ASD was considered rare, occurring in approximately 1 in 2500 individuals. The Centers for Disease Control and Prevention estimates the current prevalence of ASD among 8-year old children in the United States to be 1 in 54. While this increased prevalence can be attributed in part to the broadening of diagnostic criteria and greater awareness of ASD in recent years, these factors alone do not fully explain the observed increase in the prevalence of ASD. Additionally, while a strong hereditary component for ASD has been demonstrated, genetic research has also shown that genes linked to ASD rarely segregate in a simple Mendelian fashion and that single genetic anomalies account for a very small proportion of cases. Moreover, there is incomplete concordance of ASD diagnosis in monozygotic twins, and even in genetic syndromes strongly associated with ASD, a significant percentage of carriers do not express autistic phenotypes. These observations are consistent with a model in which environmental factors act as modifiers of ASD risk genes.

Direct 'proof-of-concept' evidence in support of the hypothesis that environmental factors influence risk of neurodevelopmental disorders comes from clinical and epidemiological studies linking increased ASD risk with perinatal exposure to viruses such as rubella and cytomegalovirus, chronic inflammation, and gestational exposure to potent teratogens including *thalidomide*, *valproic acid*, and *hydantoin*, Additional environmental chemicals postulated to confer risk for neurodevelopmental disorders include legacy chemicals known to be toxic to the developing human nervous system (*lead*, *mercury*, *PCBs*), as well as more contemporary contaminants, including pesticides (*organophosphorus cholinesterase inhibitors*, *organochlorine insecticides*, *neonicotinoids* and *pyrethroids*), flame retardants (*PBDEs*), plasticizers (*phthalates*. *bisphenol A*), and complex environmental mixtures such as air pollution.

Diverse pathophysiologic mechanisms have been hypothesized to mediate interactions between environmental chemicals and genetic risk factors that confer risk for ASD and other neurodevelopmental disorders. These include: a) mutations in genes that encode proteins involved in detoxification of endogenous toxins and xenobiotics, such as the enzyme paraoxonase 1 (PON1), which hydrolyzes OP pesticides and has been associated with increased risk of autism; b) endocrine disruption, based on the rationale that ASD and ADHD disproportionately affect males, and the requirement for thyroid hormone for normal neurodevelopment; c) chemical perturbation of the gut microbiome, derived from evidence suggesting that gut microbiota may regulate responses to xenobiotics and influence brain function, and that the microbiota may differ in children with neurodevelopmental disorders versus typically developing children; d) mitochondrial dysfunction and oxidative stress; and e) immune dysregulation. Experimental evidence has demonstrated that environmental chemicals can disrupt endocrine signaling (*chlorpyrifos, PCBs*), alter the gut microbiome (*air pollutants, metals, neonicotinoid pesticides, PBDEs*) and cause oxidative stress (*methylmercury*) and immune dysregulation (*bisphenol A, phthalates*). Clinical evidence indicates that neurodevelopmental disorders are associated with endocrine disruption, altered gut microbiota, increased expression of biomarkers of oxidative stress in the central nervous system, and a dysregulated immune system. However, causal relationships between the chemical effect on these endpoints and increased risk of neurodevelopmental disorders are largely lacking.

Perhaps the most theoretically straightforward mechanisms for explaining how environmental chemicals interact with genetic factors to increase risk of neurodevelopmental disorders involve physical interactions between chemicals and DNA (e.g., chemically-induced de novo mutations) or chromatin (e.g., epigenetic changes in miRNA expression, DNA methylation patterns and/or histone acetylation). While experimental and clinical studies clearly demonstrate that epigenetic mechanisms are critically involved in regulating normal neurodevelopment, synaptic plasticity and cognitive function, and that epigenetic changes in the brain are associated with neurodevelopmental disorders, there are as yet few data causally linking environmental chemicals to neurodevelopmental disorders via epigenetic mechanisms. One of the few potentially relevant examples is the recent demonstration that the non-dioxin-like (NDL) PCB congener, *PCB 95*, increased synaptogenesis via upregulated expression of miR132. miR132 represses expression of the transcriptional repressor, methyl-CpG-binding protein-2 (MeCP2), dysfunction of which is linked to

dendritic and synaptic dysregulation. Mutations in MeCP2 are the genetic cause of Rett syndrome and are associated with increased ASD risk. miR132 also interacts with fragile X mental retardation protein (FMRP) to regulate synapse formation, and expression of miR132 is dysregulated in schizophrenia. Intriguingly, an analysis of persistent organic pollutants in children's brains found significantly higher levels of *PCB 95* in postmortem brains of children with a syndromic form of autism, as compared to neurotypical controls. In contrast, levels of other PCBs and PBDEs were comparable across groups. The samples with detectable PCB 95 levels were almost exclusively those with maternal 15q11-q13 duplication (Dup15q), which is a known genetic cause of ASD. Dup15q was the strongest predictor of PCB 95 exposure across age, gender, or year of birth. Dup15q brain samples had lower levels of repetitive DNA methylation as measured by LINE-1 pyrosequencing, suggesting the possibility that NDL PCBs contribute to an increased risk of neurodevelopmental disorders via epigenetic mechanisms.

Adult nervous system

Chemicals generally perturb neurological function of the adult by interfering differentially with the structure and function of specific neural pathways, circuits, and systems. Vulnerable circuits within the brain include those that modulate and effect efferent output. Most commonly affected, however, are the peripheral neurons in pathways that relay information to and from the brain.

The special senses of vision, audition, balance, gustation, and olfaction depend on neural pathways that originate in peripheral receptors and terminate in the brainstem or cerebral cortex. Afferent pathways for taste, smell, hearing, and balance employ sensory neurons in ganglia that lack a blood–nerve barrier. However, chemicals that perturb the special senses seem most commonly to interfere with the structure or function of the peripheral sensory receptors. Olfaction and gustation are subserved by cilia-bearing sensory neurons that are continuously generated from stem cells, a process of cellular replacement that can be disrupted by antiproliferative drugs such as *vincristine* and *doxorubicin*. For vision, the function of retinal cells is perturbed by a large number of substances, some of which produce reversible change (*cardiac glycosides* and *trimethadione*), while others (*aminophenoxyalkanes*) elicit morphological damage or developmental changes that persist throughout life (*cycasin, MAM*). For retinal ganglion cells, the nerve fibers that form the optic pathway are sites of vulnerability to toxic attack. Substances that impair energy metabolism (*thallium, cyanide*) tend to damage proximal regions of axons projecting into the optic nerve from the papillomacular bundle, while distal axonal degeneration, with damage to the optic tracts, occurs with drugs such as *clioquinol* and *ethambutol*. Vestibular and auditory function may be affected concurrently by agents that target receptor cells in the inner ear of rodents (*2-butenenitrile*, *2-pentenenitrile*) and humans (*streptomycin*). Other potentially ototoxic substances include *cisplatin*, *furosemide*, and *imipramine*. *Noise* may exacerbate the neurotoxic effects of some ototoxic agents. Disturbance of human oculomotor function may take the form of nystagmus (*carbon monoxide*) or opsoclonus (*chlordecone*), two types of abnormal eye movement. Certain neurotoxic substances produce lesions of vestibular and cochlear nuclei in rodents (*6-chloro-6-deoxyglucose*) and primates (*1-amino-6-chloropropane*).

Sensorimotor function is altered by a number of chemicals that act at different sites in the neuraxis. Most affect the axons or somata of lower motor neurons in the anterior horn of the spinal cord or primary sensory neurons in dorsal root ganglia. Some substances target the nerve cell body of sensory neurons that detect touch and vibration (*methylmercury*), position sense (*pyridoxine*), or pain (*capsaicin*), or of neurons that regulate cardiac muscle (*doxorubicin*) or voluntary muscle function (*domoic acid, β-N-methylamino-L-alanine*). Others target motor nerve terminals (*botulinum toxin*, *α-latrotoxin*) or the enzyme that breaks down acetylcholine NT (*anticholinesterases*), both of which produced acute alterations associated with reduced or enhanced synaptic transmission. Temporary disruption of electrical impulse conduction is another neurotoxic effect. Agents (*pyrethroids*, *ciguatoxin*, *tetrodotoxin*) that perturb electrical activity in the excitable membrane (axolemma) of the nerve cell produce rapidly reversible sensory abnormalities (paresthesias) in the distribution of trigeminal and elongate peripheral nerves. Those substances (*hexachlorophene*, *ethidium bromide*) that attack the myelin sheath or myelinating cell precipitate focal demyelination and remyelination, with consequent disruption and subsequent restoration of nerve conduction and associated sensory motor phenomena over a period of several weeks. Focal demyelination and remyelination may also result from exposure to chemicals that block neurofilament transport and cause focal axonal swellings to develop proximally (*1,2-diacetylbenzene, 3,4-dimethyl-2,5-hexanedione*) or distally (*2,5-hexanedione*, *carbon disulfide*, *acrylamide*). Chemicals that produce distal (*acrylamide*), but not proximal (*β-β′-iminodipropionitrile*) giant axonal neurofilaments swellings trigger retrograde distal axonal degeneration (distal axonopathy). Long and large-diameter myelinated axons in the PNS and CNS are vulnerable to distal axonopathy caused by a number of compounds (*organophosphates*, *isoniazid*, *thalidomide*), the resulting clinical picture being one of symmetrical sensory loss and muscle weakness in the distal extremities (stocking-and-glove polyneuropathy). Shorter and thinner nerve fibers, including postganglionic nerves of the autonomic nervous system, may become involved in distal axonopathies. This common type of neurodegeneration usually occurs after repeated exposure, evolves during and shortly after the period of intoxication, and then reverses as regenerating axons reestablish functional contact with denervated sensory terminals and muscle. Significant atrophy may result from prolonged skeletal muscle denervation, and recovery of sensory-motor function may be slow, progressive, and incomplete. When central motor pathways are heavily impacted by distal axonopathy (*leptophos*, *clioquinol*), those affected may display permanent residual spasticity.

The motor pathway from brain to muscle is modulated by two other CNS structures that are vulnerable to substances with neurotoxic potential, namely the basal ganglia and cerebellum. Damage to the cerebellum may result in loss of coordination of limb and eye movement and in an ataxic gait (*methylmercury*, *3-acetylpyridine*). *Sida carpinifolia* and *Ipomea carnea* are rich in *swainsonine*, a toxin that inhibits the enzyme alpha mannosidase and induces a cerebellar syndrome. The cerebellum and basal ganglia are both sensitive to *hypoxia* and related states induced by agents that impair energy metabolism (*cyanide*) and promote glutamate-mediated excitotoxicity. Other energy-disrupting agents elicit neuronal damage in the putamen (*3-nitropropionic acid*,

methanol), pallidum (*carbon monoxide*), substantia nigra (*MPTP*, *paraquat*), or other parts of the basal ganglia (*manganese*). These types of neurotoxicity may find clinical expression as parkinsonism, tremor, dystonia, and other clinical signs of extrapyramidal dysfunction. Movement disorders of various types may result from the side effects of several therapeutic agents (*amphetamines, anticonvulsants, anticholinergics, neuroleptics, dopamine agonists, lithium, tricyclic antidepressants*). Atypical parkinsonism has been linked to ingestion of fruit of the Annonaceae family (corossol, soursop), which contain *isoquinolinic alkaloids* and *acetogenins*. A large component of aspartate, together with glutamate, is found in water-soluble component of the Yellow Star thistle (*Centaurea solstitialis*), which together with Russian knapweed (*Acroptilon repens*), induces a degenerative extrapyramidal disorder in horses known as equine nigropallidal encephalomalacia.

Autonomic regulation of the pupil, lacrimal and salivary glands, airway, heart, gut, bladder, genitalia, and blood vessels involves sympathetic, parasympathetic, and enteric neurons. Unlike their somatic counterparts in the spinal cord, efferent neurons of the autonomic nervous system are located in peripheral ganglia with permeant blood vessels. Autonomic function is disrupted by agents that target synapses that utilize acetylcholine since this is the principal NT for all preganglionic autonomic fibers, all postganglionic parasympathetic fibers, and some postganglionic sympathetic fibers. Drugs that selectively block nicotinic receptors (*curare*) curtail ganglionic output, while muscarinic agents (*atropine*) block transmission to effector cells. Anticholinesterase agents (*fasciculins, organophosphates*) stimulate sympathetic and parasympathetic activity, sometimes with the dramatic clinical consequences of a cholinergic crisis (*nerve agents*). Direct contact of anticholinesterase nerve agents (*sarin, O-ethyl S-2-diisopropylaminoethyl methylphosphonothioate*, or *VX*) with the eye, nasal passages, and bronchi leads to pupillary constriction (miosis), blurred vision, rhinorrhea, bronchoconstriction, and increased secretions. Systemic exposure results in increased salivation, bradycardia, enhanced lacrimation, urination, and defecation. Attendant muscle fasciculation, weakness, and seizures arise from peripheral somatic and CNS actions of anticholinesterase nerve agents.

The autonomic nervous system and endocrine function are regulated by the hypothalamus and associated limbic structures of the brain. Hypothalamic functions, including the regulation of temperature, heart rate, blood pressure, blood osmolarity, circadian control, and water and food intake, may be impacted by a range of chemicals. Parts of the hypothalamus lack a blood–brain barrier: Infant mice treated with excitotoxic agents (*glutamate*, *aspartate*, *cysteic acid*) display extensive damage of the arcuate nucleus and develop a syndrome of obesity, skeletal stunting, reproductive failure, and gonadal hypoplasia. The hypothalamus receives major input from the hippocampus, which functions in the storage of declarative memory, uses cellular circuitry that involves glutamatergic synapses vulnerable to excitotoxins (*domoic acid*) and certain other agents (*trimethyltin*, *trimethyl lead*, *soman*), the latter possibly as a result of seizure-associated *hypoxia*.

See also: Acrylamide; Aluminum; Amiodarone; Amphetamines; Anesthetics; Animals, poisonous and venomous; Anticancer therapeutic agents; Anticholinergics; Antimony; Antimony trioxide; Arsenic; Arsine; Aspartame; Atropine; Avermectin; Barbiturates; Barium; Batrachotoxin; Behavioral toxicology; Bismuth; Bromine; Caffeine; Carbamate pesticides; Carbon disulfide; Carbon monoxide; Chlorpyrifos; Cholinesterase inhibition; Cisplatin; CN gas; Cocaine; Colchicine; Common mechanisms of toxicity in pesticides; Curare (d-tubocurarine); Cyclosarin (GF); Dichlorvos; Dieldrin; Drugs of abuse; Endosulfan; Endrin; Ethanol; Ethylene oxide (EO); Fetal alcohol spectrum disorders; Food additives; Food safety and toxicology; Ginger jake; G-series nerve agents; Hexachlorophene; Hexane; Hydrogen sulfide; Isoniazid; Jet fuels; Lead; Lithium; LSD (lysergic acid diethylamide); Maneb; Manganese; Marijuana; Marine venoms and toxins; Mercury; Mescaline; Metals; Methanol; Methyl ethyl ketone; Methylenedioxymethamphetamine; Methylmercury; Metronidazole; Monosodium glutamate (MSG); Mushrooms, ibotenic acid; Mycotoxins; Nerve agents; Nicotine; Nitrous oxide; Occupational toxicology; Okadaic acid; Opium and the constituent opiates; Pesticides and its toxicity; Petroleum hydrocarbons; Plants, poisonous (humans); Pyrethrins/pyrethroids; Pyridoxine; Red tide; Ricin and other toxalbumins; Rotenone; Sarin; Saxitoxin; Sedatives; Solvents; Soman; Strychnine; Tabun; Tellurium; Tetrachloroethylene; Tetrodotoxin; Thalidomide; Thallium; Tin; Toluene diisocyanate; Trichloroethane; Tricyclic antidepressants; Trinitrotoluene; V-series nerve agents other than VX; VX; Xylene

Further reading

Aschner M and Costa LG (2015) *Environmental Factors in Neurodevelopmental and Neurodegenerative Disorders*. Oxford, UK: Elsevier.

Aschner M and Costa LG (2021) *Neurotoxicity of Metals: Old Issues and New Developments*. New York: Elsevier.

Banach M, Juranek JK, and Zygulska AL (2016) Chemotherapy-induced neuropathies-a growing problem for patients and health care providers. *Brain and Behavior: A Cognitive Neuroscience Perspective* 7(1): e00558. https://doi.org/10.1002/brb3.558.

Bentea E, Verbruggen L, and Massie A (2017) The proteasome inhibition model of Parkinson's Disease. *Journal of Parkinson's Disease* 7: 31–63. https://doi.org/10.3233/JPD-160921.

Brust JCM (2004) *Neurological Aspects of Substance Abuse*, 2nd edn. Butterworth-Heinemann: Boston, MA.

De Miranda BR, Castro SL, Rocha EM, Bodle CR, Johnson KE, and Greenamyre JT (2021) The industrial solvent trichloroethylene induces LRRK2 kinase activity and dopaminergic neurodegeneration in a rat model of Parkinson's disease. *Neurobiology of Disease* 153: 105312. https://doi.org/10.1016/j.nbd.2021.105312.

Delp J, Cediel-Ulloa A, Suciu I, Kranaster P, van Vugt-Lussenburg BM, Munic Kos V, van der Stel W, Carta G, Bennekou SH, Jennings P, van de Water B, Forsby A, and Leist M (2021) Neurotoxicity and underlying cellular changes of 21 mitochondrial respiratory chain inhibitors. *Archives of Toxicology* 95: 591–615. https://doi.org/10.1007/s00204-020-02970-5.

Dempsey JL, Little M, and Cui JY (2019) Gut microbiome: An intermediary to neurotoxicity. *NeuroToxicology* 75: 41–69. https://doi.org/10.1016/j.neuro.2019.08.005.

D'Mello JPF (2019) *A Handbook of Environmental Toxicology: Human Disorders and Ecotoxicology*. Wallingford, Oxfordshire, United Kingdom: CABI.

Feldman RG (1999) *Occupational and Environmental Neurotoxicology*. Philadelphia, PA: Lippincott-Raven.

Fox DA, Grandjean P, de Groot D, and Paule MG (2012) Developmental origins of adult diseases and neurotoxicity: Epidemiological and experimental studies. *Neurotoxicology* 33: 810–816. https://doi.org/10.1016/j.neuro.2011.12.016.

Harischandra DS, Ghaisas S, Zenitsky G, Jin H, Kanthasamy A, Anantharam V, and Kanthasamy AG (2019) Manganese-induced neurotoxicity: New insights into the triad of protein misfolding, mitochondrial impairment, and neuroinflammation. *Frontiers in Neuroscience* 26(13): 654. https://doi.org/10.3389/fnins.2019.00654.

Ijomone OM, Ijomone OK, Iroegbu JD, Ifenatuoha CW, Olung NF, and Aschner M (2020) Epigenetic influence of environmentally neurotoxic metals. *NeuroToxicology* 81: 51–65. https://doi.org/10.1016/j.neuro.2020.08.005.

Keil KP and Lein PJ (2016) DNA methylation: a mechanism linking environmental chemical exposures to risk of autism spectrum disorders? *Environmental Epigenetics* 2(1): dvv012. https://doi.org/10.1093/eep/dvv012.

Kostrzewa RM (2021) *Handbook of Neurotoxicity*. Berlin, Germany: Springer.

Mantovani A and Fuci A (2020) *Challenges in Endocrine Disruptor Toxicology and Risk Assessment*. U.K.: Royal Society of Chemistry.

Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, Park BY, Snyder NW, Schendel D, Volk H, Windham GC, and Newschaffer C (2017) The changing epidemiology of Autism Spectrum Disorders. *Annual Review of Public Health* 38: 81–102. https://doi.org/10.1146/annurev-publhealth-031816-044318.

Pałasz A, Menezes IC, and Worthington JJ (2021) The role of brain gaseous neurotransmitters in anxiety. *Pharmacological Reports* 73: 357–371. https://doi.org/10.1007/s43440-021-00242-2.

Panesar HK, Kennedy CL, Keil Stietz KP, and Lein PJ (2020) Polychlorinated biphenyls (PCBs): Risk factors for Austism Spectrum Disorder? *Toxics* 8: E70. https://doi.org/10.3390/toxics8030070.

Panzica GC, Bo E, Martini MA, Miceli D, Mura E, Viglietti-Panzica C, and Gotti S (2011) Neuropeptides and enzymes are targets for the action of endocrine disrupting chemicals in the vertebrate brain. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews* 14: 449–472. https://doi.org/10.1080/10937404.2011.578562.

Pugsley K, Scherer SW, Bellgrove MA, and Hawi Z (2021) Environmental exposures associated with elevated risk for autism spectrum disorder may augment the burden of deleterious de novo mutations among probands. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-021-01142-w.

Rice D and Barone S (2000) Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental Health Perspectives* 108: 511–533.

Slikker W Jr, Paule MG, and Wang C (2018) *Handbook of Developmental Neurotoxicology*, 2nd edn. Amsterdam: Elsevier, Inc.

Spencer PS and Palmer VS (2021) Direct and indirect neurotoxic potential of metal/metalloids in plants and fungi used for food, dietary supplements, and herbal medicine. *Toxics* 9: 57. https://doi.org/10.3390/toxicvs9030057.

Spencer PS, Palmer VS, and Kisby GE (2020) Western Pacific ALS-PDC: Evidence implicating cycad genotoxins. *Journal of the Neurological Sciences* 419: 117185. https://doi.org/10.1016/j.jns.2020.117185.

Spencer PS and Schaumburg HH (2000) *Experimental and Clinical Neurotoxicology*. New York: Oxford.

Tsai YH and Lein PJ (2021) Mechanisms of organophosphate neurotoxicity. *Current Opinion in Toxicology* 26: 49–60. https://doi.org/10.1016/j.cotox.2021.04.002.

Tu AT (1984–1992) *Handbook of Natural Toxins*, vols. 2–7. New York: Marcel Dekker.

Zawia NH (2004) *Molecular neurotoxicology: Environmental agents and transcription-transduction coupling*. Boca Raton, FL: CRC Press.

Zoeller RT (2007) Environmental chemicals impacting the thyroid; targets and consequences. *Thyroid* 17(9): 811–817. https://doi.org/10.1089/thy.2007.0107.