Toxic Responses of the Fish Nervous System

Steven P. Bradbury
Richard W. Carlson
Tala R. Henry
Stephanie Padilla
John Cowden
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Introduction

Few of the approximately 70,000 chemicals on the Toxic Substances Control Act inventory or the 1000 to 1600 new chemicals introduced each year in the United States have been tested for neurotoxicity to support risk assessments (NRC, 1992), even though it is estimated that 5 to 10% of them are likely to be neurotoxic. Neurotoxicity has been defined as adverse effects of physical, biological, or chemical agents on the structure or function of the nervous system in developing or adult organisms (Philbert et al., 2000). From a human health risk assessment perspective, the potential for neurotoxic effects associated with synthetic chemicals has led to the development of valid, sensitive, and reproducible methods to identify neurotoxic chemicals, to characterize neurological effects, and to determine the mechanisms by which chemicals produce neurotoxicity. Similar efforts to develop methods for assessing neurotoxicity in fish may yield further insights into neurotoxic mechanisms (Carlson et al., 1998; Drummond and Russom, 1990; Featherstone et al., 1991, 1993; Rice et al., 1997; Timme-Laragy et al., 2006; Weber et al., 1997) in addition to addressing ecological risk concerns.

This chapter aims to provide a framework from which to approach questions concerning the neurotoxic effects of chemicals in fish. First, a brief summary of structural and functional attributes of the nervous system is provided, followed by an overview of neurotoxic mechanisms of action. The final section of the chapter summarizes mechanisms of action and manifestations of neurotoxic effects for several classes of compounds. In this latter section, an attempt is made to highlight examples where structural and functional alterations to the nervous system at the subcellular to cellular level can be linked to physiological and behavioral effects. The integration of effects across levels of biological organization is essential for establishing the mechanistic basis underlying neurotoxicity, as well as for identifying and quantifying ecologically relevant neurotoxic effects in fish.

Overview of Fish Nervous System Development, Structure, and Function

Development of the Fish Nervous System

The development of the fish nervous system follows the same general design as the development of all other vertebrate nervous systems (Figure 9.1). Beginning with gastrulation, in a process known as neural induction, ectoderm is specified into either surface epidermis or neuroectoderm. Surface epidermis ultimately forms skin, while the neuroectoderm becomes neural tissue. Evidence from several model organisms, including the widely used developmental model, zebrafish (Danio rerio), indicates that neural induction is mediated through the bone morphogenetic protein (BMP) signaling pathway. Surface epidermis is induced when ectoderm is exposed to BMP signaling. Extracellular antagonism of BMP ligands prevents BMP signaling, allowing the ectoderm to adopt a neuroectodermal fate (Blader and Strähle, 2000; Lewis and Eisen, 2003).

Following induction, the neuroectoderm undergoes several morphogenetic movements to form the rudimentary nervous system (Lowery and Sive, 2004). Initially, the neuroectoderm forms a flat epithelial sheet called the neural plate (Figure 9.1A). As morphogenesis begins, the lateral edges of the neural plate rise to form the neural folds (Figure 9.1B). Specialized neural crest cells are induced at the apex of each neural fold. These neural crest cells migrate from the neural folds and ultimately give rise to several structures, including neurons and glia of the peripheral nervous system (PNS). The neural folds are brought into apposition and ultimately join at the midline. Unlike other vertebrates, fish neural fold fusion produces a solid rod of cells called the neural keel (Figure 9.1C). Although the midline is distinct during neural keel formation, cells within the neural keel are capable of crossing the midline. Once covered by surface epidermis, the neural keel subsequently undergoes programmed cell death to become
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A hollow neural tube (Figure 9.1D). At the anterior end of the neural tube, bulging begins the subdivision of the brain into the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain) (Figure 9.2). The remainder of the neural tube becomes the spinal cord. Within the spinal cord, progenitor cells are patterned by secreted signaling molecules along the anterior–posterior and dorsal–ventral axes. Anterior–posterior patterning of the neuroectoderm begins during gastrulation in response to signals derived from the organizer (Blader and Strähle, 2000). Forebrain is thought to be the default state of neural tissue, with subsequent posteriorization involving retinoic acid, fibroblast growth factor, and Wnt signaling (Lewis and Eisen, 2003). Along the dorsal–ventral axis, distinct neuronal cell types are located at stereotypic positions in the spinal cord, with sensory neurons specified dorsally and motorneurons specified ventrally (Blader and Strähle, 2000; Lewis and Eisen, 2003). As with other vertebrates, sonic hedgehog protein secreted from the floor plate and notochord induces ventral motor-neuron differentiation in a concentration-dependent manner (Blader and Strähle, 2000; Lewis and Eisen, 2003). In the dorsal neural tube, BMP signaling from the roof plate and adjacent non-neural ectoderm are hypothesized to induce sensory neuron fates in a concentration-dependent manner (Blader and Strähle, 2000; Lewis and Eisen, 2003).

Once neuronal fates have been specified in appropriate anterior–posterior and dorsal–ventral locations, the next step in nervous system development is axonogenesis, when neurons extend axons to their innervation targets. Embryonic nervous system development has been particularly well characterized in zebrafish (Danio rerio) (Bernhardt, 1999); therefore, the summary of axonogenesis given here is necessarily restricted to zebrafish, although many facets of zebrafish axonogenesis are likely to be applicable.
to other teleosts. A tremendous amount of descriptive information is known about zebrafish axonogenesis, with axonal pathways and targets characterized for several neuron classes (Bernhardt, 1999; Hutson and Chien, 2002; Lewis and Eisen, 2003). Unfortunately, the cellular and molecular basis for axon guidance is not as well understood. Recent genetic screens in zebrafish have identified mutants with defective axonogenesis (Hutson and Chien, 2002) and several axonal guidance gene homologs have been identified in the zebrafish genome (Bernhardt, 1999; Chilton, 2006). Although the presence of these homologs suggest that fish axon migration is regulated by integrating signals in the growth cone, the in vivo significance of these homologs remains to be determined.

The final step in embryonic nervous system development occurs when an axon reaches its innervation target. Active synapses are formed between neurons and their targets in a process known as synaptogenesis. Synaptogenesis has been heavily studied in higher vertebrates, with a generally accepted model of presynaptic neurons responding to both diffusible factors and distinct cell adhesion molecules in the target cell (Waites et al., 2005). Genes that play critical roles in vertebrate synaptogenesis are conserved in zebrafish (*Danio rerio*), although little functional assessment has been made (Hutson and Chien, 2002). Further insights into teleost synaptogenesis will likely come from real-time in vivo imaging of synaptogenesis in zebrafish (Hutson and Chien, 2002).

In the adult fish, the central nervous system (CNS) includes the neuronal structures encased within the skull and the spinal column. The PNS is comprised of nerve ganglia lying outside the spinal column as well as nerve processes found elsewhere throughout the organism. As noted above, this subdivision of the nervous system occurs during embryogenesis through ectodermal morphogenesis and patterning. The coordination and control of physiological and behavioral processes reflects the integration of structural and functional attributes of the entire nervous system.

**Central Nervous System Anatomy**

Although significant differences exist in anatomical features across fish species, the basic structural components of the developing CNS include the prosencephalon (forebrain), mesencephalon (midbrain), rhombencephalon (hindbrain), and the spinal cord (Figure 9.2).
Prosencephalon

The prosencephalon is comprised of the telencephalon and the diencephalon (Bond, 1996; Wullimann, 1998). The telencephalon possesses rostrally located olfactory bulbs, olfactory lobes, and, more caudally, cerebral hemispheres. The telencephalon is the site where the olfactory nerve (cranial nerve I) enters the olfactory bulbs. In actinopterygian fish, the cerebral hemispheres can be divided into dorsal and ventral components, which are homologous to the pallium and subpallium, respectively, of other vertebrates. The majority of afferent neurons entering the telencephalon are olfactory; however, there is some indication that non-olfactory ascending inputs exist, although their sites of integration have not been identified. It is now generally accepted that the telencephalon contributes to the processing of visual inputs, in addition to olfactory information, and plays a role in feeding, defense, schooling, aggressive, and reproductive behaviors (Bernstein, 1970; Bond, 1996; Davis and Kassel, 1983; Northcutt and Davis, 1983; Wullimann, 1998). Numerous studies have documented the presence of a variety of neurotransmitters in the telencephalon, as well as acetylcholine, gamma-aminobutyrate (GABA), catecholamines, excitatory amino acids (e.g., glutamate), and nitric oxide (Bissoli et al., 1989; Brantley and Bass, 1988; Byrd and Brunjes, 1995; Khan et al., 1996; Parent, 1983; Reiner and Northcutt, 1992; Sas et al., 1990; Schober et al., 1994; Sloley and Rehnberg, 1988; Sloley et al., 1992; Smith 1984). Catecholamines that have been identified in fish include noradrenaline, dopamine, and 5-hydroxytryptamine. Neuropeptides, including substance P, neuropeptide Y, and leucine-enkephalin (Byrd and Brunjes, 1995; Reiner and Northcutt, 1992), have also been found in fish nervous tissue.

The diencephalon in actinopterygian fish has been subdivided into the preoptic area, hypothalamus, thalamus, epithalamus, and synencephalon (Bradford and Northcutt, 1983). Wullimann (1998), however, considers the preoptic area an intermediate zone between the telencephalon and the diencephalon in the teleost brain. Wullimann (1998) also considers the synencephalon as part of the pretectum (see below), which is intermingled with diencephalic cell groups. Below the hypothalamus lies the pituitary gland, and the pineal organ arises from the epithalamus. The primary afferent neurons to the thalamus are the optic nerves (cranial nerve II), which enter the brain and cross anterior to the diencephalon. The hypothalamus receives a number of afferents from the telencephalon; the thalamus receives afferents from the cerebellum and serves as a relay center for the transfer of olfactory and striate-body impulses to other parts of the diencephalon and lower brain centers. In general, the diencephalon appears to serve as a coordination center for incoming and outgoing signals associated with internal homeostasis and for affecting the endocrine system through the pituitary gland (Bernstein, 1970; Bond, 1996; Bradford and Northcutt, 1983; Wullimann, 1998). Acetylcholine (Brantley and Bass, 1988), GABA, glutamate (Sloley et al., 1992), nitric oxide (Holmqvist and Ekstrom, 1997), noradrenaline, dopamine, and 5-hydroxytryptamine (Khan et al., 1996; Parent, 1983; Sas et al., 1990) have been identified as neurotransmitters in the diencephalon, primarily in the hypothalamus.

Mesencephalon

The mesencephalon, comprised of the optic tectum and the ventral tegmentum, is the primary site for processing impulses received from the optic nerve. Extensive electrophysiological and evoked response studies have been undertaken to create maps of visual fields on the optic tectum in cyclostomes, plagiostomes, and actinopterygians. The optic nerve is decussated, with projections from the right eye projecting to the left tectal hemisphere and from the left eye projecting to the right tectal hemisphere. Ascending efferent fibers from the optic tectum have been traced to the thalamus and pretectum, while descending efferent fibers proceed to the dorsolateral region of the ventral tegmentum. Some tectal outputs provide terminals to the reticular formation and cell groups in the medulla. Electrical stimulation and lesion studies support the conclusion that the optic tectum contains neuronal units that coordinate visual inputs with locomotor activity (Bernstein, 1970; Bond, 1996; Vanegas, 1983; Wullimann, 1998). Reports suggest that acetylcholine (Brantley and Bass, 1988), GABA (Rio et al., 1996), glutamate (van Deusen and Meyer, 1990), nitric oxide (Holmqvist and Ekstrom, 1997), and glycine (Becker et al., 1991) are predominant neurotransmitters within the optic tectum. Moderate catecholaminergic innervation has also been documented (Sas et al., 1990).
Rhombencephalon

The rhombencephalon is comprised of the metencephalon and the myelencephalon. The metencephalon includes the cerebellum and the pons, with the cerebellum being the major component. The structure of the cerebellum is highly variable in fish. In teleosts, it can be divided into three major sections that are arranged similarly to that of the cerebellar cortex in other vertebrates. These areas are termed the valvula cerebelli, corpus cerebelli, and vestibulolateral lobe. Each area contains a molecular layer, a Purkinje (ganglion) cell layer, and a granule cell layer. Primary sensory fibers in hair cell sensory systems (see sensory organ systems discussion below) and neurons from the spinal cord are important ascending inputs to the cerebellum. Efferents from the cerebellum project to the ventral tegmentum, the thalamus, and the reticular formation. The cerebellum is associated with muscle tone, motor control, and lateral line sensory input (Bernstein, 1970; Bond, 1996; Finger, 1983; Wullimann, 1998). The cerebellum has been reported to contain glutamate, aspartate, GABA, and glycine, as well as catecholaminergic neurotransmitters (Ma, 1994; Sas et al., 1990). The myelencephalon, although containing portions of the cerebellum, is predominately comprised of the medulla oblongata (Wullimann, 1998). The boundary between the medulla oblongata and spinal cord in fish is generally indistinct and can be most readily defined by the types of information transmitted with associated nerve columns. Information derived from general sensory, cutaneous, vestibular, lateral line, and trigeminal (cranial nerve V) nerve fibers is carried in the somatic sensory column. Nerve fibers derived from chemoreceptors and nerves arising in the viscera are associated with the visceral sensory column. Sensory inputs associated with sight and olfaction do not input directly to the medulla. A visceral motor column in the medulla carries efferent fibers derived from the facial (cranial nerve VII), glossopharyngeal (cranial nerve IX), and vagus (cranial nerve X) nerves to the glands and musculature of the viscera. A somatic nerve column carries efferent nerve fibers to the ocular muscles and muscles of the pharyngeal complex. The medulla of the actinopterygians contains a pair of Mauthner cells, which are giant interneurons at the level of the vestibulocochlear nerve (cranial nerve VIII) that coordinate the startle response associated with sensory inputs. The dendrites of the Mauthner cells connect with fibers of the trigeminal nerve, facial nerve, glossopharyngeal nerve, and vagus nerve to the cerebellum and the optic tectum. Their axons pass the length of the spinal cord and coordinate musculature in the tail and associated rapid swimming movements (Bernstein, 1970; Bond, 1996). Serotonin and catecholamines have been reported to be neurotransmitters in the medulla (Parent, 1983; Sas et al., 1990), in addition to glycine, GABA (Becker et al., 1991; Faber and Korn, 1988; Legendre and Korn, 1994, Triller et al., 1993), and nitric oxide (Schober et al., 1994).

Spinal Cord

In fish, the spinal cord occupies the entire vertebral canal. The cord consists of a series of segments that form dorsal and ventral roots. The dorsal and ventral horns correspond to regions of sensory input (dorsal root) and motor output (ventral root). As is generally noted within vertebrates, these roots join to form the spinal nerves. Differentiation of the spinal cord increases with evolutionary progression from Cyclostomata (lampreys and hagfish) to Chondrichthyes (cartilaginous fish) to Osteichthyes (bony fish). In the latter fishes, the gray matter is clearly divided into dorsal and ventral horns. The dorso medial gray matter innervates the trunk musculature and specialized areas, such as the pectoral fin. Many fibers ascend to the medulla oblongata and cerebellum. The descending fibers consist of many vestibulospinal and reticulospinal fibers and the giant Mauthner cells, which contain large-diameter, well-myelinated axons. The dendrites and cell bodies of Mauthner cells are found in the medulla oblongata. These cells have many collaterals that have extensive connections with interneurons and motorneurons in the spinal cord. The role of the Mauthner cells is to mediate sensory inputs through the startle response, as mentioned previously. As a final note, spinal cords of adult and larval fish are unique from mammals in their capacity for anatomical and physiological regeneration (Bernstein, 1970; Bond, 1996).

Peripheral Nervous System Anatomy and Function: The Autonomic Nervous System

The discussion of the central nervous system and associated components in the peripheral nervous system has made reference to aspects of both the somatic and autonomic nervous system, which are responsible for “voluntary” and “involuntary” actions. In mammals, the autonomic system contributes to the regulation
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of organs and tissues such as the gut, heart, and blood vessels. In fish, the autonomic nervous system is similarly involved in the control of the gut and circulatory systems, as well as control of the swim bladder and melanophores. Studies in teleosts suggest that functional attributes of the autonomic nervous system are similar to that noted in mammals; however, the presence of gills and a swim bladder in fish and a smaller number of nerves and neurotransmitters highlight some important differences (Donald, 1998; Nilsson and Holmgren, 1998).

An anatomical differentiation of the fish autonomic nervous system into sympathetic and parasympathetic subsystems is difficult (reviewed in Donald, 1998; Nilsson and Holmgren, 1998). Instead, a terminology that differentiates cranial nervous pathways from spinal nervous pathways has been proposed. In both the cranial and spinal pathways, a preganglionic neuron runs from the central nervous system to an autonomic ganglion. A postganglionic neuron then serves as the afferent to the appropriate tissue or organ. The spinal autonomic ganglia are typically located close to the spinal column in higher vertebrates and teleosts, while autonomic ganglia associated with cranial pathways are usually located near or within the effector organ. Within the fishes, there is great variability in the anatomy of the autonomic nervous system. In the cyclostomes, the autonomic nervous system is poorly developed, with no spinal ganglia readily apparent, and it is difficult to differentiate sensory and autonomic nerve fibers. In the elasmobranchs and teleosts, autonomic ganglia are observed along the spinal column. Cranial autonomic fibers have been associated with the oculomotor (cranial nerve III), facial (cranial nerve VII), glossopharyngeal (cranial nerve IX), and vagus (cranial nerve X) nerves in the elasmobranchs. The teleost autonomic system most closely resembles that of higher vertebrates.

Of note are the spinal ganglia that enter the cranium and have connections with the trigeminal (cranial nerve V)/facial (cranial nerve VII), glossopharyngeal (cranial nerve IX), and vagus (cranial nerve X) nerves. It is thought that innervation of the head and gills is associated with spinal ganglia. In the posterior region of the abdomen, the sympathetic chains fuse, and the vesicular nerves leave the chain to innervate the gut and urinogenital organs. Cranial autonomic fibers have been reported in the oculomotor (cranial nerve III) and vagus nerves (cranial nerve X), both of which reach the gut and swim bladder.

Although the number and nature of neurotransmitters are not as well resolved in fish as in mammals, compounds representative of those seen in higher vertebrates have been characterized. Assessments of catecholamine levels indicate that both adrenaline and noradrenaline are likely stored and released in adrenergic synapses. It is generally assumed within the vertebrates that acetylcholine is found in all postganglionic, parasympathetic neurons. In some teleosts, evidence for the presence of acetylcholine has been reported for the spleen, heart, and swim bladder (Abrahamsson et al., 1979; Balashov et al., 1981; Holmgren, 1977; Hsieh and Liao, 2002; Ishimatsu et al., 1986; Ovais et al., 1976; Thompson and O'Shea, 1997). Neurons containing 5-hydroxytryptamine which innervate the gut of several cyclostome and teleost species have been reported (Anderson, 1983; Anderson and Campbell, 1988; Anderson et al., 1991; Watson, 1979). Vagal neurons that innervate the gills in rainbow trout (Oncorhynchus mykiss) have also been reported to contain 5-hydroxytryptamine (Saltys et al., 2006; Sundin, 1995; Sundin et al., 1998). Using immunohistochemistry and radioimmunoassay techniques, a number of suspected neuropeptides have also been identified; for example, bombesin has been identified in autonomic nerves of the gut and circulatory system in several fish species (Bjenning and Holmgren, 1988; Bjenning et al., 1990; Cimini et al., 1985; Holmgren and Nilsson, 1983; Langer et al., 1979). In addition, neuropeptide Y, vasoactive intestinal polypeptide, and several somatostatins and tachykinins have been identified in fish (Donald, 1998; Nilsson and Holmgren, 1998). Nitric oxide has also been reported as a putative neurotransmitter in the autonomic nervous system (Donald, 1998).

Sensory Organ Systems

The sensory systems of fish can be divided into vision, auditory, mechanosensory, electroreceptive, and chemoreceptor. In the assessment of neurotoxicity, these sensory organ systems are viewed as readouts of a functional nervous system. The eyes of fish generally have the same structures as those noted in the vertebrates, including the anterior chamber, an iris, a lens, and a vitreous chamber that is lined by the retina. The retina is comprised of pigmented epithelium, which consists of photoreceptors (rod and cone cells), horizontal cells, bipolar cells, amacrine cells, and ganglion cells and nerve fibers that lead
to the optic nerve. The optic nerve is comprised of four different types of fibers, most of which are afferent and project to the contralateral side of the brain (the optic tectum). Horizontal cells facilitate information flow between photoreceptors, and amacrine cells facilitate lateral flow of information among ganglion cells. Bipolar cells direct excitatory or inhibitory responses vertically to the ganglion cells, which subsequently send information to the CNS through the optic nerve. A variety of rod and cone subtypes are observed in fish and suggest specialization in terms of discriminating wavelengths and intensity of light (Hawryshyn, 1998). Freshwater fish are generally found to have porphyropsin as their main photosensitive pigment, while marine fish typically have rhodopsin in their retina (Bond, 1996). As reviewed by Hawryshyn (1998) and Lasater (1990), glutamate is the putative neurotransmitter in the photoreceptors that mediates synaptic communication with bipolar cells, while GABA has been well-documented in horizontal and amacrine cells (reviewed in Hawryshyn, 1998; Lasater, 1990; Marc and Cameron, 2001). Glycine, dopamine, and acetylcholine have also been reported as neurotransmitters in the retina. A potential role of neuropeptides has yet to be elucidated.

The auditory, mechanosensory, and electrosensory systems include the inner ear, the lateral line (comprised of the neuromasts and canals), and the ampullary and tuberous organs of the electrosensory lateral line (Bond, 1996; Schellart and Wubbels, 1998). The ear functions contribute to balance and sound reception, while lateral line functions are related to sensing water displacement and pressure. The electrosensory lateral line is responsible for sensing electrical fields and signals. The inner ear of fish includes three otolith organs: the saccule, lagena, and utricle organs. The saccus and lagena are primarily responsible for sound reception. The sensory epithelium, termed the macula, is comprised of sensory hair cells that are the receptor cells. Auditory stimulus causes deflections of hair bundles, leading to a depolarization of hair cells and subsequent increase in the firing rate of afferent nerve fibers to the brain. The auditory nerve (vestibulocochlear nerve; cranial nerve VIII) innervates the macula. Those portions of the brain thought to integrate auditory inputs have been discussed previously. The mechanosensitive lateral line consists of neuromasts that are found over the entire body, either free on the skin or within pits, grooves, or canals. A neuromast consists of sensory hair cells with supporting cells, all of which are covered by a cupula. Some neuromasts are open to the water, via pores in the bone or scales. Water movement causes an impulse of fluid within the lateral line that in turn causes a deformation of the cupulae. This deformation of the hair cells results in a response in the nerve fiber innervating the neuromast. The number of innervating nerve fibers can range from a few to several hundred. The dorsal anterior lateral line nerve innervates the dorsal part of the head, the ventral lateral line nerve innervates the cheek and lower jaw, and the posterior lateral line nerve innervates the trunk and tail. The involvement of different portions of the brain in integrating responses to these inputs has been discussed previously. Of note are the projections of auditory and lateral line afferents to the Mauthner cells, which invoke the startle response.

Chemosensory systems in fishes are primarily divided into gustation and olfaction and are well developed in fish. Taste buds are comprised of 100 to 150 specialized epithelium cells, which include receptor cells, basal cells, and supporting cells. Taste buds are found primarily in the mouth, pharyngeal region, and gill arches; however, in some species they may be found on barbels, fins, and other parts of the body surface. Taste buds in the oropharyngeal cavity are innervated by the glossopharyngeal (cranial nerve IX) and vagus (cranial nerve X) nerves, while the facial nerve (cranial nerve VII) innervates taste buds on external surfaces. Receptor cells within the taste buds respond to specific compounds, such as amino acids. Many taste receptors are thought to be ion channels or proteins associated with ion channels. Binding of chemicals to the appropriate receptor elicits depolarization of the receptor cell. Through chemical synapses, the potential of the innervating nerve fiber may be modulated prior to propagation to the CNS (Bond, 1996; Sorensen and Caprio, 1998). The olfactory organs are found in pits on the anterior portion of the head. The epithelium within the olfactory chambers is arranged in a series of lamellae that form the olfactory rosette. Both sensory and nonsensory cells are found in the epithelium. The nonsensory cells serve to protect the sensory cells and transport chemical stimuli. Ciliated and microvillous receptor cells have been noted in fish. These cells are innervated by the olfactory nerve, which provides input to the olfactory bulb. The receptor cells are responsive to amino acids, bile acids, gonadal steroids, and prostaglandins (Byrd and Caprio, 1982; Caprio and Byrd, 1984; Chang and Caprio, 1996; Ohno et al., 1984; Sato and Suzuki, 2001). A variety of alcohols, amines, carboxylic acids, nucleotides, and hydrocarbons...
can also elicit olfactory responses (Kolesnikov and Kosolapov, 1993; Rolen et al., 2003). Specific receptor proteins in sensitive cells are thought to be responsible for initial olfactory responses. These receptors initiate second-messenger-mediated signal transduction pathways that open ion channels and lead to the production of action potentials (Bond, 1996; Sorenson and Caprio, 1998).

### Basic Mechanisms of Neurotoxicity

Before considering some mechanisms of neurotoxicity in adult fish, it is important to remember that neurotoxic compounds are also likely to have developmental effects. In particular, the potency and mode of action of neurotoxic compounds may be dramatically altered during maturation of the nervous system. To underscore the importance of this concept, compounds with adverse effects on the immature nervous system are categorized as developmental neurotoxicants.

It is widely accepted that the developing nervous system is particularly susceptible to some neurotoxic compounds (Costa et al., 2004; Mendola et al., 2002; Stein et al., 2002). This assertion is based largely on the fact that neurotoxicant exposures having little to no effect on the mature nervous system may produce significant and lasting effects on the developing nervous system. Several processes critical for proper nervous system structure and function, including neuronal specification, migration, and synaptogenesis, all occur *in ovo*. Furthermore, several neurotransmitters have developmental roles that are distinct from their synaptic roles (e.g., acetylcholine) (Lauder and Schambra, 1999). Disruptions in any of these processes or neurotransmitters may have unique effects during development that are not seen in the mature nervous system and are potential mechanisms of developmental neurotoxicity.

In the mature nervous system, neurotoxic mechanisms of action can be associated with a variety of unique anatomical and physiological characteristics of the nervous system (Anthony et al., 1996; Philbert et al., 2000). Many aspects of the mammalian nervous system contribute to its vulnerability: maintenance of the blood–brain or blood–nerve barrier, high energy requirements, high lipid environment, complex spatial geometry, and the need for rapid transmittal of information between cells. Because the structural and functional aspects of neurons are highly conserved, the framework proposed by Anthony and coworkers (1996) for understanding mechanisms of neurotoxicity, as well as the manifestations of toxic effects, is relevant and briefly reviewed.

In terms of xenobiotic exposure, the central and peripheral nervous system are unique in that tissue and cellular attributes minimize the transport of potentially toxic compounds to sites of action. The restricted interfaces between the blood and many components of the nervous system have been termed as barriers: the blood–brain barrier encloses the central nervous system, and the blood–nerve barrier encloses the peripheral nervous system. The basis for the blood–brain barrier is that the endothelial cells lining the vasculature of the brain have extremely tight intercellular junctions. As a consequence, to gain access, xenobiotics are required to diffuse through these cellular barriers. The lipid-rich myelin surrounding axons, which is critical for insulating the nerve and ensuring rapid propagation of action potentials, may provide a barrier to hydrophilic xenobiotics. In general, the more hydrophilic the compound, the less likely it will cross one of these barriers; however, some hydrophilic compounds may gain access by piggybacking on specific transporters that are present in these barriers. Oligodendrocytes and Schwann cells found in the central and peripheral nervous systems, respectively, are responsible for maintaining the myelin sheaths that exclude water and ions from the axons and for minimizing extracellular spaces. A wide variety of specialized proteins must be synthesized and complex lipid metabolism must be maintained to support the myelin environment. Neurons also need to maintain ion gradients to support neuronal transmission. These maintenance activities require a high aerobic metabolic rate. As a consequence, the nervous system requires high levels of oxygen and glucose to maintain homeostatic ion gradients and to restore ion gradients after depolarizations.

Finally, the unique spatial arrangement of the nervous system makes it vulnerable to toxic insults. The nervous system is comprised of relatively large cells as the result of evolutionary pressures to establish high-speed intracellular communication; consequently, axon volumes are typically much greater than those of the cell bodies and have a unique demand for macromolecule synthesis. The cell body of each neuron synthesizes everything that is needed by the rest of the cell (axons and dendrites); these
products are then transported throughout the cell to replenish cellular constituents. In addition, spent cellular constituents are transported retrogradely from the processes to the cell body. The retrograde transport also contains biochemical information related to the status of the environment in the distal portion of the neuron. Spatial arrangement of the nervous system also requires maintenance of the transmission of electrical pulses along the length of axons and across the synapses between neurons. Maintenance of both intracellular and extracellular communication is, of course, essential to the proper function of the nervous system.

In conclusion, the nervous system has a variety of unique structural characteristics that meet the need for rapid cellular communication. These unique structural characteristics are primarily associated with maintaining the integrity of the cell body and axon and the ability of the nerves to support propagation of action potentials and synaptic transmission. Xenobiotics capable of disrupting ion channels that are essential to maintaining and supporting proper ion balances or capable of disrupting chemical transmission of potentials across synapses are capable of causing neurotoxic effects. As discussed previously, the maintenance of aerobic respiration, high rates of protein and lipid synthesis, and extensive transport of synthetic products from the cell body to the axon are critical for maintaining structural and functional characteristics of the nervous system. Consequently, xenobiotics capable of disrupting neuron-specific synthetic and metabolic pathways, modifying the products of these reactions, or inhibiting or uncoupling aerobic metabolism can elicit adverse effects specific to the nervous system. It is interesting to note that some fish species have evolved specific metabolic strategies for anoxic conditions. Research suggests that anoxic-tolerant species may have decreased levels of excitatory neurotransmitters and increased levels of inhibitory transmitters in the brain that enable metabolic depression (Van Ginneken et al., 1996).

**Manifestations of Neurotoxicity in Fish**

Neurotoxic effects of chemicals are assessed by quantifying structural and functional responses at the subcellular to organismal levels of biological organization. Functional observations at the organismal and cellular levels can provide insights concerning potential sites and modes of action, while cellular and biochemical responses can provide insights on molecular mechanisms of action. Cellular and biochemical investigations can be used to identify neurotoxic potential, characterize the nature of neurological effects, and determine the mechanisms by which chemicals produce neurotoxic effects (Tilson, 1996). A significant challenge in developing investigative methods and associated bioassay techniques lies in linking neumorphological, neurochemical, and neurophysiological alterations with functional (i.e., behavioral) observations (NRC, 1992). Many chemically induced biochemical, physiological, or morphological perturbations have been reported in cellular and organismal systems, but consequent behavioral effects on organism have not been established. Because behavioral responses are an integration of biochemical, physiological, and morphological processes, linking behavioral observations to these types of observations can provide the needed bridge between subcellular and cellular responses and ecological consequences (Little, 1990). Examples where chemically induced biochemical, physiological, or morphological perturbations have been mechanistically linked to ecologically relevant behavioral responses in fish are limited.

**Structural Manifestations of Neurotoxicity in Fish**

As discussed previously, dynamic interactions between the neuronal cell body and the axon are critical to maintaining proper neuronal structure and function. Disruption of these interactions can result in a variety of pathologies. Neuronopathies result from toxicants capable of causing injury to the cell body followed by degeneration of the remaining cell processes. Neuronal loss is typically permanent and is manifested by global symptoms or dysfunctions consistent with the specific nervous tissue target; for example, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes neuronopathies in mammals through its cytotoxic dihydropyridium ion metabolite. Similar observations have been reported in fish.
Toxic Responses of the Fish Nervous System

Axonopathies are associated with toxicants whose primary site of action is the axon, causing degeneration of the axon along with surrounding myelin. Because the nerve cell body is not affected, recovery and regeneration of the axon are possible. The effects of neurotoxicants capable of eliciting axonopathies are sometimes described as causing chemical transections of the nerve (e.g., acrylamide, carbon disulfide, some diketones, and certain classes of organophosphorus esters). Likewise, compounds disrupting microtubules (such as colchicine) will perturb axonal transport and thereby cause pathology. Myelinopathies are associated with compounds capable of causing intramyelinic edema, such as hexachlorophene (Kinoshita et al., 2000; Yoshikawa, 2001). Hexachlorophene-induced myelinopathies have also been reported in non-mammals (Reier et al., 1978). Other compounds are capable of causing the selective destruction of the myelin, resulting in neuronal demyelination. These responses may be due to effects on the myelin itself or due to effects on the myelinating cells.

Physiological Manifestations of Neurotoxicity in Fish

Electrophysiology

Electrophysiological techniques measure electrical potentials and the transmission of impulses in the nervous system. These techniques can be applied to in vitro or in vivo preparations and used to assess responses of the brain, spinal cord, components of the peripheral nervous system, or sensory systems to determine whether or not a neurotoxic response can be elicited by a xenobiotic. Electrophysiological techniques can be used to determine if a toxicant acts pre- or postsynaptically on specific portions of a fiber or on specific ion channels.

Measurements can be made from the surface of the organism, extracellularly or intracellularly (Baker and Lowndes, 1986; Eisenbrandt et al., 1994; Fox et al., 1982). Bahr (1972, 1973), for example, reported an in vivo method to record evoked electrical activity of the trunk lateral line in surgically prepared rainbow trout (Oncorhynchus mykiss) for periods of up to 48 hours. Evans and Hara (1985) described an in vivo rainbow trout model to quantify evoked electro-olfactograms, and Kreft and coworkers (1985) described a technique to assess electoretinograms from photostimulated in vitro eye preparations. Schafer and coworkers (1995) described an in vivo anesthetized catfish (Ameiurus nebulosus) preparation to measure spontaneous afferent activity from electroreceptor organs. Several whole-cell and membrane preparations have also been used to study ion channels and the role of specific neurotransmitters in channel function; for example, voltage clamp analysis has been used to study the role of glycine, GABA, and N-methyl-D-aspartate (NMDA) in isolated lamprey spinal cord neuron function and to identify calcium channel subtypes in lamprey sensory and motor neurons (Baev et al., 1992; El Manira and Bussieres, 1997; Moore et al., 1987).

To date, most electrophysiological techniques applied to fish involved in vitro models or surgically invasive approaches use restrained or anesthetized animals. Although these methods yield data regarding cellular and molecular mechanisms of neurotoxic action, disruption of the nervous system or sensory organs can make it difficult to study behavioral outcomes of perturbed neurological function. Two laboratories (Carlson et al., 1998; Featherstone et al., 1991, 1993) have described an approach relating electrophysiological responses to a defined behavioral endpoint. In these studies, the electrophysiological responses of Mauthner cells, motorneurons and interneurons, and white musculature to an invoked startle response were measured in vivo in unanesthetized, freely moving larval fish. These waveforms are easily triggered and recorded, yielding stereotypical and reproducible results.

Respiratory–Cardiovascular

The effects of toxicants on the central or peripheral nervous system can elicit a wide variety of integrated respiratory–cardiovascular responses. In turn, these responses can be evaluated to determine the extent to which they can be associated with specific mechanisms of neurotoxic action. For example, several studies have demonstrated that specific suites of respiratory–cardiovascular responses of spinally transected rainbow trout (Oncorhynchus mykiss) were sufficient to differentiate effects associated with industrial organic chemicals whose sites and mechanisms of action are distinct, including narcotics.
acetylcholinesterase inhibitors, pyrethroid insecticides, cyclodiene insecticides, and strychnine (Bradbury et al., 1991a; Bradbury and Coats, 1989; Bradbury et al., 1991b; McKim et al., 1987). More specifically, distinct suites of effects on cough frequency, ventilation frequency, ventilation volume, oxygen consumption, and arterial blood oxygen, carbon dioxide, pH, hemoglobin, and hematocrit were associated with the different compound classes studied. Furthermore, these respiratory–cardiovascular responses were consistent with the neurodepressant, stimulant, or convulsant mechanisms of action of the xenobiotics studied.

**Behavioral**

The behavior of a fish reflects the integrated output of the nervous system at the organismal level in response to stimuli perceived in the environment. The extent to which these chemically induced behavioral changes are ecologically relevant must be considered in terms of adverse effects on an organism’s ability to survive and reproduce. Behavioral endpoints can be categorized as individual or interindividual responses (Rand, 1985). Individual responses include undirected and directed locomotion and feeding. Undirected locomotion refers to the movement of an animal that is not related to the intentional placement of a stimulus. The direction of undirected locomotion is considered random. This spontaneous activity can be affected by neurotoxicants and described with a variety of qualitative descriptors (Heath, 1995). Although such observations may be difficult to interpret, Drummond and Russom (1990) demonstrated that a behavioral response checklist could be used to categorize toxicants with known modes of action within specific syndromes. Heath (1995) and Rand (1985) also summarized a variety of experimental approaches whereby fish locomotion responses can be quantitatively measured following exposure to a wide variety of organic and inorganic neurotoxicants; for example, undirected locomotion can be quantified by measuring water currents created by the movement of fish or by measuring voltage changes in the water caused by swimming fish. The use of photoelectric gates and video cameras to quantify swimming speed and exploratory activity has also been described.

Directed locomotion refers to movement in response to specific external stimuli. The ability of a fish to swim with or against a water current (negative and positive rheotaxis) is a commonly studied forced locomotive response. The directed movement of fish due to avoidance or attractiveness to xenobiotics or natural chemical stimuli has also been examined in some detail (reviewed in Hara et al., 1983; Heath, 1995). Chemosensory disruption can result in overt preference or avoidance to a xenobiotic. Avoidance of or preference to natural and xenobiotic chemical cues during or following xenobiotic exposure can also occur. Disruption in normal locomotion can be quantified by observing movements of an organism across chemical gradients. The locomotion of a fish in response to a xenobiotic is likely the result of interactions with the olfactory or gustatory receptors; however, avoidance can also be the result of irritation of mucous membranes. The ability of a xenobiotic to mask or counteract natural chemical signals used in migration, for example, may be the consequence of competition for receptor sites on sensory cell membranes. Alternatively, the xenobiotics may be directly toxic to receptor cells (Hara et al., 1983). As reviewed by Heath (1995), a wide variety of xenobiotics, including metals, petroleum constituents, detergents, and insecticides, have been observed to depress fish feeding rates. This depression may be associated with effects on olfactory, gustatory, visual, or lateral line receptors or effects on the ability of the central nervous system to integrate environmental stimuli.

Of the interindividual responses, territoriality, dominance, schooling, and predator–prey interactions have been studied most extensively (Heath, 1995; Rand, 1985). Predator–prey interactions have been examined in studies in which either the predator or the prey is exposed to the xenobiotic, as well as in studies where both sets of organisms are exposed. Endpoints in these studies can include prey survival rates, predation rates, or prey handling times. Although effects on predator–prey interactions have been observed for a variety of insecticides, herbicides, and metals, the mechanistic basis for these effects is largely unknown. The need to understand fully the basis of xenobiotic effects on predator–prey interactions has been well articulated by Sandheinrich and Atchison (1990). In this regard, Carlson and coworkers (1998) reported an attempt to relate the effects of a wide variety of organic xenobiotics on prey survival in terms of electrophysiological responses of the Mauthner-cell-mediated startle response, which initiates an escape behavior in response to predation.
Examples of Major Classes of Neurotoxicants

Narcotics

Research reported during the early 1980s in both the United States and The Netherlands established that the majority of industrial organic chemicals (excluding pesticides and pharmaceutical agents) elicit acute toxic effects in fish through a narcosis mechanism (Konemann, 1981; Veith et al., 1983). Narcosis can be defined as a reversible state of arrested activity of protoplasmic structures resulting from exposure to a xenobiotic. In the context of the intact organism, the terms narcosis and general anesthesia are commonly used interchangeably (Bradbury et al., 1989). Although typically described in the literature as a nonspecific mode of action, the actual mechanism of narcosis and anesthesia remains unknown and is an active area of research, as discussed in several reviews (Århem et al., 2003; Bradbury et al., 1989; Franks and Lieb, 1990, 1994; Franks and Lieb, 2004; Narahashi et al., 1998; Pryor, 1995).

Mechanisms of Narcotic Neurotoxicity

Both lipophilicity (Meyer–Overton rule) and thermodynamic activity (Ferguson’s rule) (Ferguson, 1939) have been demonstrated to be related to the narcotic potency of chemicals. Thus, varying aqueous concentrations of different compounds may be required to cause comparable effects, even though their thermodynamic activity at the site of action is proposed to be the same. There is, however, ample evidence of narcotics and anesthetics whose potency is not consistent with thermodynamic theory. These departures from thermodynamic consistency suggest that sometimes potency must be viewed as a more complex toxicological and physicochemical interaction between the xenobiotic and a site of action. As a result, investigators developing hypotheses to explain the molecular events of anesthesia/narcosis are increasingly acknowledging that their models must accommodate the likelihood of multiple sites and mechanisms of action (Bradbury et al., 1989; Franks and Lieb, 1990, 1994, 2004; Miller 2002; Narahashi et al., 1998; Pryor 1995). Within the last 10 years, it has become increasingly apparent that anesthesia-like compounds interact directly and specifically with proteins, most notably proteins that form ion channels (Århem et al., 2003; Franks and Lieb, 1990, 1994, 2004; Narahashi et al., 1998).

Franks and Lieb (1990, 1994, 2004) proposed that anesthesia is the result of direct interactions between xenobiotics and neuronal ion channels. This hypothesis evolved partly from x-ray diffraction experiments undertaken by these investigators showing insignificant changes in lipid membrane bilayers at physiologically relevant concentrations of narcotic xenobiotics (although see the study by Cantor, 1997). Subsequent studies with firefly luciferase established that inhibition of luciferase activity correlated with anesthetic potency and that the sites of action can have polar and nonpolar characteristics. These investigators also demonstrated that narcotics caused reversible inhibition of specific, spontaneously firing neurons in the giant snail. This inhibition was saturable and consistent with binding to a receptor site. While the specific proteins associated with narcosis have yet to be identified, an implication of this hypothesis is that classes of receptors, or receptor sites, with varying hydrophobic and hydrogen-bonding characteristics may be involved. Differential effects on target proteins, or proteins in different neuron classes, could provide an explanation for different narcosis/anesthetic effects observed at the cellular through organismal level (Bradbury et al., 1989).

In conclusion, hypotheses developed to date provide implicit or explicit basis for attributing narcosis to more than one site of action or mechanistic process. In general, all of the hypotheses attribute narcosis to neuronal dysfunction ultimately caused by changes in the properties of neuronal ion channels, which are beginning to be identified (reviewed in Århem et al., 2003).

Manifestations of Narcotic Neurotoxicity in Fish

With the development of initial acute toxicity datasets for industrial organic chemicals, it was established that the potency of narcotics in fish was dependent on the hydrophobicity of a xenobiotic (Konemann, 1981; Veith et al., 1983). Subsequent experimental studies and modeling efforts have led to general acceptance that the relationships between hydrophobicity and lethality represent the minimum, or
baseline, toxicity that a compound can elicit in the absence of a more specific mode of toxic action. With additional study it became clear that there are subclasses of narcotics, more potent than would be predicted from baseline narcosis, that could be classified on the basis of either acute potency or physiological and behavioral characteristics of the narcosis response. More specifically, narcosis induced by certain esters, phenols, and anilines (typically termed polar narcotics) seemed to be unique (Bradbury et al., 1989).

Behavioral and gross morphological signs of stress in fathead minnows (*Pimephales promelas*) associated with acutely lethal aqueous exposures to baseline narcotics include depressed locomotor activity with little or no response to outside stimuli. Body color also becomes darker as fish are increasingly intoxicated. Most fish die within 24 hours, but effects are reversible if fish are transferred to "clean" water prior to death. In contrast, acutely lethal concentrations of polar narcotics with log octanol-water partition coefficients below 2.7 elicit hyperactivity and usually overreaction to outside stimuli for 24 to 48 hours, with subsequent depression and death (Drummond and Russom, 1990). Medaka (*Oryzias latipes*) larvae exposed to phenol and 1-octanol at levels 2 to 3 times lower than acutely lethal concentrations were more susceptible to predation by bluegill (*Lepomis macrochirus*) than were unexposed fish (Carlson et al., 1998).

To evaluate further the symptomology of narcosis in fish, researchers (Bradbury et al., 1989; McKim et al., 1987) examined the respiratory-cardiovascular responses of spinally transected rainbow trout (*Oncorhynchus mykiss*) to baseline narcotics (1-octanol and MS-222) and polar narcotics (phenol, 2,4-dimethylphenol, aniline, 2-chloroaniline, and 4-chloroaniline). The responses of the trout exposed to these groups of compounds were distinct. The overall response to the baseline narcotics was a dramatic slowing of all respiratory-cardiovascular functions. While ventilation volume and oxygen consumption decreased, oxygen uptake efficiency increased as water flow over the gills slowed and the blood-to-water perfusion ratio increased. A rapid drop in heart rate (reflex bradycardia) was thought to be related to an increase in vagal tone caused by hypoxia. As respiration rate declined, total arterial blood oxygen and pH also decreased. The associated increase in hematocrit, caused by red blood cell swelling, is well documented during anesthesia and is associated with hypoxia. These effects are reversible, as demonstrated by experiments in which fish at the point of respiratory failure could be revived if clean water was perfused across the gills.

In general, the respiratory-cardiovascular symptoms associated with baseline, or nonpolar narcosis, are consistent with depressant anesthesia as described by Winters (1976). The most striking feature associated with exposure of rainbow trout (*Oncorhynchus mykiss*) to lethal aqueous concentrations of polar narcotics was the development of tremors and clonic seizures that were initiated by coughs (Bradbury et al., 1989). These tremors and seizures originated in the head and moved posteriorly to include the tail, even though the fish were spinally transected. These observations suggest that polar narcotics affect the spinal cord posterior to the transection, or perhaps the peripheral nervous system. In mammals, the primary site of phenol stimulation is thought to be the spinal cord (Deichmann and Keplinger, 1981). With increasing length of exposure, seizure intensity subsided, and the fish became unresponsive to outside stimuli. Consistent with the increased activity associated with seizures and muscular activity, oxygen uptake initially increased, yet ventilation volume and frequency eventually declined which is more consistent with a general depressant effect. Depressions of arterial blood oxygen, carbon dioxide, and pH and an associated increased hematocrit were consistent with a shift to anaerobic metabolism during seizures and subsequent respiratory failure. Fish could be revived by artificially irrigating the gills. The responses of fish to polar narcotic exposure are generally consistent with the description of cataleptic anesthesia (Winters, 1976).

Carlson and coworkers (1998) investigated the sublethal effects of 1-octanol and phenol on *in vivo* electrical impulses generated within the Mauthner cells and associated interneurons, motorneurons, and axial musculature during the startle response reflex in larval medaka (*Oryzias latipes*). With 1-octanol, electrical waveforms were depressed at exposure concentrations 5 times lower than the 48-hour LC50, and the ratios of startle responses to stimuli were significantly depressed. These observations are suggestive of a sensory deficit due to an anesthetic-like effect. Phenol caused a significant decrease in the motorneuron-to-muscle delay, consistent with the initial hyperactivity and sensitivity noted in exposures to polar narcotics and reports that phenol stimulates the mammalian spinal cord (Deichmann.
and Keplinger, 1981). These electrophysiological studies generally support other toxicodynamic and behavioral studies that suggest baseline and polar narcotics act through different mechanisms or sites of action.

**Cholinesterase Inhibitors**

Several neurotoxic compounds are known to target the cholinergic nervous system. By far the largest classes are the organophosphorus and carbamate compounds. Organophosphorus and carbamate compounds have been used extensively as insecticides, herbicides, and fungicides. Initial studies on the synthesis and activity of organophosphorus insecticides were undertaken in Germany during the 1930s. Agricultural use of insecticidal organophosphorus and carbamates increased in the 1960s as the use of organochlorine compounds declined. In the United States, use of organophosphorus insecticides peaked in the mid-1970s and has declined with increased use of pyrethroid and carbamate insecticides. Insecticidal carbamates were first introduced in 1954 and are still employed for agricultural and household applications (Abou-Donia, 1995; Metcalf, 1995).

Organophosphorus insecticides are organic phosphoric ester compounds with the general structure of \( \text{R}_1\text{P(=Y)(R}_2\text{)X} \), where \( Y \) is an oxygen or thio group, \( X \) is a leaving group (e.g., halide, phenoxy, or other group), and \( \text{R}_1 \) and \( \text{R}_2 \) are typically alkyl, alkoxy, aryl, or aryloxy substituents. Representative structures of organophosphorus insecticide classes are depicted in Figure 9.3. Carbamate insecticides are organic compounds that contain a \( \text{RNC(=O)O} \) moiety; examples of common \( N \)-methylcarbamate and \( N \)-methylcarbamoyl oximes are provided in Figure 9.4. While organophosphorus and carbamate insecticides are structurally diverse, their common neurotoxic mechanism is related to their ability to phosphorylate or carbamylate esterases.
Mechanisms of Cholinesterase Inhibitor Neurotoxicity

The neurotoxic mechanism for organophosphorus and carbamate insecticides is inhibition of acetylcholinesterase (AChE) in the CNS and PNS (Figure 9.5) (reviewed in Ecobichon, 1996; Taylor, 2001). AChE is responsible for hydrolyzing the neurotransmitter acetylcholine; inhibition of the esterase causes accumulation of acetylcholine in synapses and excessive stimulation of muscarinic and nicotinic receptors (Figure 9.5B). Increased levels of acetylcholine overstimulate nicotinic and muscarinic receptors in the CNS, PNS, and neuromuscular junctions, causing a wide range of signs of poisoning. These signs may include changes in body temperature, heart rate, blood pressure, muscle twitching, or tremors. Death, although rare, is usually due to cessation of respiration due to anticholinesterase effects in both the CNS and PNS. Although organophosphates and carbamates both target acetylcholinesterase, nicotine acts as a direct cholinergic agonist rather than by cholinesterase inhibition; nicotine directly binds nicotinic receptors to hyperstimulate cholinergic neurons.

Both organophosphorus and carbamate compounds inhibit AChE activity by acting as pseudo-substrates. Unlike the natural substrate, these compounds remain in the active site of the enzyme for much longer periods of time, thereby preventing the enzyme from hydrolyzing its natural substrate, acetylcholine (Aldridge and Reiner 1969). Inhibition of AChE activity by organophosphorus insecticides is primarily a function of the electrophilicity of the phosphorus atom, with increased electrophilicity (increased partial positive charge) associated with greater rates of bonding to the negatively charged oxygen in the serine hydroxyl group. Thus, for organophosphorus insecticides, the binding of the organophosphorus compound to AChE is reasonably fast. The hydrolysis of the organophosphorus esters, however, is very slow, leading to an accumulation of phosphorylated AChE. Phosphorylated AChE is, for all practical purposes, irreversibly inhibited due to the very low reversibility of the enzyme activity.

Recovery of active AChE activity is dependent on the synthesis of new enzyme. In some cases, phosphorylated enzymes undergo an aging reaction when an alkoxy or aryloxy R group is dealkylated or dearylated and results in a negatively charged monoalkyl or aryl enzyme. In such instances, the hydrolysis step is no longer possible, thus, the enzyme is irreversibly altered. Kinetic studies on the inhibition of AChE activity, including aging and recovery, have been reported in a variety of fish species (Carr et al., 1995; Johnson and Wallace, 1987; Straus and Chambers, 1995; Wallace and Herzberg, 1988). Typically, recovery of AChE activity in fish is slower than that observed in mammals.

Inhibition of AChE activity by carbamate insecticides is a function of the carbamylation of the enzyme. The kinetics of this inhibition are slightly different from that observed with organophosphorus insecticides. Hydrolysis of carbamates (i.e., decarbamylation), while significantly slower than that observed for acetylcholine, is much more rapid than that observed with most organophosphorus compounds; thus, although AChE inhibition elicited by an organophosphorus pesticide is, in effect, irreversible, carbamate inhibition of esterase activity may be reversed. Furthermore, aging of the carbamylated enzyme does not occur.
FIGURE 9.5 Role of acetylcholinesterase at synaptic junctions. (A) Typical cholinergic synapse. Acetylcholine is secreted by the presynaptic neuron into the synapse, where it binds to presynaptic and postsynaptic muscarinic and nicotinic receptors. Acetylcholinesterase breaks down acetylcholine into cholinesterase and acetylcholine. Cholinesterase is transposed to the presynaptic neuron, where acetyl coenzyme A converts it into acetylcholine. (B) Effect of acetylcholinesterase inhibitors on cholinergic synapses. Acetylcholine is secreted into the synapse but is not broken down by acetylcholinesterase, leading to an excess amount of acetylcholine in the synapse. ACh, acetylcholine; AChE, acetylcholinesterase; mAChR, muscarinic receptor; nAChR, nicotinic receptor; chol, cholinesterase; coA, acetyl coenzyme A; ace, acetate.

Interestingly, although anticholinesterase compounds all disrupt acetylcholine signaling, all anticholinesterase compounds do not produce identical responses to poisoning (reviewed in Pope, 1999). Acetylcholine signaling disruption is the first step in the acute neurotoxic response but is not sufficient to explain all manifestations of toxicity. Several secondary, non-acetylcholinesterase targets have been recognized (Casida and Quisad, 2004; Pope, 1999) and are thought to play instrumental roles in the ultimate effects of cholinergic neurotoxicants. Furthermore, disruption of acetylcholine signaling alone may not fully explain the developmental neurotoxicity of these compounds. Careful consideration should be given to the non-cholinergic targets of these compounds when assessing toxicity, particularly in the developing embryo. As shown in Figure 9.5A, the biochemical mechanisms of acetylcholine production, secretion, and transmission are known in great detail. For the purposes of this discussion, only acetylcholine pathway components targeted by cholinergic agonists are addressed.

Manifestations of Cholinesterase Inhibitor Neurotoxicity in Fish

Brief exposures to organophosphorus pesticides can produce long-lasting neurological effects because of the irreversibility of AChE inhibition; in fact, depression of AChE activity is considered a primary indicator of organophosphate pesticide exposure in fish. As a result, the extensive research in fish neurotoxicity has sought to correlate acute toxicity in adult fish with level of acetylcholinesterase inhibition (Heath, 1995; Murty and Ramani, 1992; Zinkl et al., 1991). Based on work in several different fish species and with several cholinergic poisons, it is generally accepted that 70 to 80% AChE inhibition is lethal.

Acute exposures to organophosphorus and carbamate insecticides, in a variety of species including goldfish (Carassius auratus), fathead minnows (Pimephales promelas), medaka (Oryzias latipes), and rainbow trout (Oncorhynchus mykiss), increased spontaneous locomotor activity with high incidences of
convulsions, spasms, tetany, scoliosis, lordosis, and hemorrhage in the vertebral column, presumably due to damage resulting from spasms (Bradbury et al., 1991a,b; Drummond and Russom, 1990; McKim et al., 1987; Rice et al., 1997; Saglio et al., 1996). Organophosphorus and carbamate insecticides have variable effects on cough rate. Chlorpyrifos (Bradbury et al., 1991a) and fenitrothion (Klaverkamp and Hobden, 1980) have been reported to increase cough response in rainbow trout, while malathion, carbaryl (McKim et al., 1987), and acephate (Klaverkamp and Hobden, 1980) did not. Increased cough rate, however, is not thought to be associated with AChE inhibition (Klaverkamp and Hobden, 1980). Spinally transected rainbow trout exposed to chlorpyrifos exhibited increased defection and bile loss from the anal opening, consistent with muscarinic effects of AChE inhibition (Bradbury et al., 1991a).

In spinally transected rainbow trout (Oncorhynchus mykiss) exposed to carbaryl, malathion (McKim et al., 1987), or chlorpyrifos (Bradbury et al., 1991a), decreased heart rate, decreased gill oxygen uptake efficiency, and increased ventilation volume were observed. Decreased heart rate has been attributed to inhibition of the heart by the vagus nerve (cranial nerve X) through cholinergic synapses. Decreased oxygen uptake by the gills and a compensatory increase in ventilation volume have been proposed to be caused by continuous stimulation of neuromuscular junctions associated with sphincters at the base of the efferent filamental arteries to secondary lamellae of the gill. The resulting vasoconstriction is thought to reduce blood flow to the lamellae, effectively reducing respiratory surface area and oxygen uptake efficiency (McKim et al., 1987; Pavlov, 1994).

Despite the wealth of acute toxicity data, relatively little is known about the developmental neurotoxicity of cholinergic agonists in fish. Existing data demonstrate that exposure either in ovo or as juvenile fish has detrimental consequences on learning and motor neuron development. Chlorpyrifos exposure produced hypoactivity in zebrafish (Danio rerio) hatching swimming behavior (Levin et al., 2004). In addition, developmental chlorpyrifos exposure of zebrafish embryos has long-term effects on learning. Adult zebrafish exposed to chlorpyrifos during development show reduced choice accuracy and spatial discrimination (Levin et al., 2003). Behavioral effects have been seen in juveniles of other fish species as well. At concentrations of carbaryl or chlorpyrifos up to 10 times lower than 48-hour LC50 values, larval medaka (Oryzias latipes) were more susceptible to predation, although a consistent dose–response relationship between carbaryl exposure and susceptibility to predation was not observed (Carlson et al., 1998). In vivo electrophysiological studies of sublethal chlorpyrifos and carbaryl effects on the Mauthner cell startle response in larval medaka (Carlson et al., 1998) demonstrated an effect on neuromuscular junctions, as evidenced by a dose-related increase in the ratio of startle response to stimuli. An increase in motor neuron to muscle delay with increased exposure concentration was also noted. Both responses are consistent with AChE inhibition in the neuromuscular junction.

Interestingly, evidence from zebrafish (Danio rerio) suggests that AChE inhibition may not be the sole mechanism of developmental neurotoxicity. Developmental exposure to nicotine causes morphological changes in zebrafish hatchlings and impairs the swimming behavior and escape response (Svoboda et al., 2002). Using an Isl1-GFP transgenic zebrafish strain, nicotine was shown to delay development of spinal neurons and cause disruptions in axonal pathfinding of secondary motor neurons by nicotinic receptor activation (Svoboda et al., 2002). The molecular mechanism of developmental neurotoxicity remains unidentified for the majority of cholinergic agonists.

Pyrethroid Insecticides

Directed synthesis has produced insecticides derived from the natural pyrethrin esters of pyrethrum flowers (e.g., Chrysanthemum cinerariifolium) (Shafer et al., 2005). These synthetic, pyrethroid insecticides have greater stability in light and air than the natural pyrethrin esters yet maintain critical stereochemical characteristics required for alignment with target receptors (Soderlund et al., 2002). The synthetic pyrethroids are divided into two classes based on the presence or absence of a cyano group on the alpha carbon of the 3-phenoxybenzyl alcohol moiety (Figure 9.6). Type II pyrethroids all contain an α-cyano side group, while type I pyrethroids do not. Because of their potent insecticidal activity, low mammalian and avian toxicity, and varying levels of environmental stability (Bradbury and Coats, 1989; Clark, 1995), synthetic pyrethroids represent nearly 23% of the U.S. dollar value of the world insecticide market (Soderlund et al., 2002).
Toxic Responses of the Fish Nervous System

Mechanisms of Pyrethroid Neurotoxicity

In both mammals and insects, the primary mechanism of acute synthetic pyrethroid neurotoxicity is disruption of voltage-sensitive sodium channels (VSSCs) (Clark, 1995; Narahashi, 1992; Shafer et al., 2005; Soderlund et al., 2002). Mammalian VSSCs consist of a single α subunit and two β subunits (Shafer et al., 2005). The α subunit forms the channel pore, and the β subunits modify channel properties and membrane location (Figure 9.7A). Pyrethroid insecticides bind the α subunit and disrupt sodium regulation (Figure 9.7B,C). Type I pyrethroid insecticides prolong VSSC opening, allowing more sodium to cross the membrane and leading to repetitive firing of action potentials. Conversely, type II pyrethroids delay VSSC inactivation, resulting in a depolarization-dependent block that prevents action potential generation (Shafer et al., 2005; Soderlund et al., 2002). Although the primary mechanism of acute pyrethroid neurotoxicity is disruption of VSSCs, evidence suggests that numerous secondary sites and mechanisms of action are possible (Soderlund et al., 2002); for example, deltamethrin and resmethrin are toxic to *Paramecium tetraurelia*, although this organism does not have VSSCs (Soderlund et al., 2002). In this case, the synthetic pyrethroids were acting through calcium channels in the cilia membrane of the *P. tetraurelia* (Soderlund et al., 2002). Synthetic pyrethroids have also been shown to affect voltage-gated potassium and chloride channels, as well as ligand-operated channels such as the GABA receptor–ionophore complex, the nicotinic acetylcholine receptor, and the peripheral-type benzodiazepine receptor (Shafer et al., 2005; Soderlund et al., 2002). These effects, however, are usually associated with physiologically unrealistic pyrethroid exposures or nonspecific interactions. Both type I and type II pyrethroids cause repetitive firing in synapses, neuromuscular junctions, and the central nervous system. As a consequence, pyrethroid intoxication has been associated with releases of acetylcholine, GABA, dopamine, and norepinephrine. The type II compounds tend to elicit greater neurotransmitter releases than type I compounds because of enhanced ability to depolarize sensory and presynaptic nerve endings (Clark, 1995). In addition, perturbation of intraterminal calcium homeostasis, ATP-activated calcium sequestration, and responses of protein phosphorylation associated with calcium-dependent neurotransmitter release have also been suggested as playing a role in pyrethroid mode of action. Some of these effects may further accentuate neurotransmitter release caused by repetitive firing (Clark, 1995).

Unfortunately, there is little consensus on which, if any, of the acute neurotoxicity mechanisms are applicable to pyrethroid developmental neurotoxicity (Shafer and Meyer, 2004). Some pyrethroids insecticides show significant age differences in acute toxicity, with younger animals usually being more sensitive. Several studies also demonstrate persistent changes in motor activity, learning, and sexual activity following developmental pyrethroid exposure. None of these effects has been associated with a putative neurotoxic mechanism.
Manifestations of Pyrethroid Neurotoxicity in Fish

Although synthetic pyrethroids have minimal mammalian and avian toxicity, they are very toxic to fish (Bradbury and Coats, 1989). Because of their high lipophilicity, the synthetic pyrethroids are readily absorbed through the gills (Baser et al., 2003; Polat et al., 2002). Fish lack at least one enzyme that metabolizes pyrethroids, meaning that metabolic turnover is particularly slow (Baser et al., 2003; Tilak et al., 2003). As a result, fish are particularly susceptible to pyrethroids entering the aquatic ecosystem. An extensive amount of research has focused on determining acute toxic dosage in adult fish (Baser et al., 2003; Das and Mukherjee, 2003; David et al., 2004; Polat et al., 2002; Rebach, 1999; Saha and Kaviraj, 2003; Tandon et al., 2005; Tilak et al., 2003; Tripathi and Verma, 2004).

As reviewed by Bradbury and Coats (1989), acute pyrethroid intoxication in small aquarium fish typically causes loss of schooling behavior, followed by hyperactivity, erratic swimming, violent whole-body seizures, and loss of buoyancy. Consistent with the steep dose–response relationships typically observed for pyrethroids, Carlson and coworkers (1998) failed to note significant effects on medaka (Oryzias latipes) susceptibility to predation or electrophysiological responses associated with the Mauthner-cell-mediated startle response at sublethal levels. Spinally transected rainbow trout (Oncorhynchus mykiss) exhibited an elevated cough rate shortly after exposure (Bradbury et al., 1991a). Increased cough rates were typically associated with an elevated secretion of mucus. Further intoxication resulted in increased hyperexcitability followed by tremors that progressed to seizures anterior to the site of the
transsection. During seizures, the opercula were flared and in a state of tetany. Prior to death, seizures subsided and fish became inactive. Similar responses have been noted in bluegill (*Lepomis macrochirus*) (Bradbury et al., 1987; Little et al., 1993). The stages of behavioral changes in fish are generally consistent with those observed in mammals (Bradbury and Coats, 1989); however, an insufficient number of compounds have been studied in fish to differentiate pyrethroid intoxication syndromes as has been done with mammals (Shafer et al., 2005; Soderlund et al., 2002). Because seizures are typically stimulus dependent in hypersensitized fish, it seems reasonable to assume that pyrethroid-induced coughs could trigger convulsions. The cough response itself could be a CNS-mediated component in the seizure syndrome, a side effect due to interactions with sensory receptors in the pharynx and gill arches, or direct irritation of gill tissue. Both fenvalerate and permethrin have been shown to cause gill damage consistent with irritation (Bradbury et al., 1987; Kumaraguru et al., 1982). As reviewed by Clark (1995), pyrethroids have also been reported to cause transient dermal tingling, itching, and burning in humans and irritation to the mucous lining of respiratory passages.

Several measures of metabolic activity have been measured in fish exposed to pyrethroids. In general, protein levels and various dehydrogenases were reduced in response to pyrethroid exposure, while erythrocyte production was increased (Das and Mukherjee, 2003; Kumar et al., 1999; Tripathi and Verma, 2004). These data were interpreted as consequences of failed pyrethroid metabolism. Additionally, marked respiratory-cardiovascular effects observed in pyrethroid-intoxicated rainbow trout (*Oncorhynchus mykiss*) are consistent with increased muscular activity associated with seizures (Bradbury et al., 1991a). Increased ventilation volume was associated with moderate declines in oxygen uptake efficiency and therefore nearly constant oxygen consumption. Arterial blood oxygen levels were initially elevated but then declined dramatically, as did carbon dioxide levels and pH. Overall, these responses suggest a shift to anaerobic metabolism. These shifts in respiratory-cardiovascular and blood-chemistry parameters have also been reported in mammals and are also generally attributed to increased muscular activity (Bradbury and Coats, 1989).

Unlike studies of acute toxicity, few studies have addressed the developmental neurotoxicity of synthetic pyrethroids in fish. Exposure to sublethal levels of permethrin *in ovo* caused a hatching delay in medaka (*Oryzias latipes*). Medaka hatchlings demonstrated hyperactivity, uncoordinated movement, and an inability to respond to stimuli. These hatchlings also failed to inflate their swimming bladder and had spinal curvatures (González-Doncel et al., 2003). Unfortunately, these effects have yet to be correlated with a putative neurotoxicity mechanism, so it is unclear how pyrethroids may be inducing developmental neurotoxicity.

**Organochlorine Insecticides**

The organochlorine insecticides are among the largest category of insecticides and are used worldwide for public health (e.g., mosquito control) and agricultural production (Figure 9.8). The organochlorine insecticides are comprised of four distinct structural classes, which tend to be associated with unique mechanisms of action: (1) chlorinated ethane derivatives, (2) cyclodiienes, (3) polychlorobornanes, and (4) lindane. Use of organochlorines has declined dramatically because of insecticide resistance and environmental concerns. Due to their persistence in the environment and their continued use in some parts of the world, organochlorine residues in sediments, soils, and biota are still observed (Woolley, 1995).

**Mechanisms of Organochlorine Insecticide Neurotoxicity**

As with synthetic pyrethroid insecticides, the neurotoxicity mechanism of chlorinated ethanes is the disruption of VSSCs. Studies with invertebrate and vertebrate preparations established that dichlorodiphenyltrichloroethane (DDT) prolongs the falling phase of the action potential, which typically produces repetitive firing. This repetitive neuron firing leads to the hyperexcitability and tremor noted in intoxicated insects, mammals, birds, and fish. Studies by Narahashi (1994) demonstrated that DDT caused voltage-sensitive channels to remain in the open state longer than normal and close slowly resulting in an increased overall open time. As reviewed by Woolley (1995), it appears that the type I
pyrethroids and DDT likely affect the same target site on the sodium channel. Hyperexcitability is also associated with increased releases of neurotransmitters throughout the mammalian nervous system which likely reflect secondary responses but could represent an additional neurotoxic mechanism.

In mammals, the cyclodiienes and polychlorobornanes cause anorexia, salivation, vomiting, and convulsions. During convulsions, insect and amphibian preparations exhibit excessive uptake of calcium into synaptosomes. This synaptosomal calcium uptake is associated with increased release of neurotransmitters. Subsequently, it was shown that cockroach strains resistant to cyclodiene insecticides, lindane, and toxaphene were also resistant to picrotoxin. Picrotoxin acts at one of the receptor sites in the GABA receptor-ionophore complex and is thought to block the channel. Normally, the GABA-gated chloride channel opens upon GABA binding, causing an increased flow of chloride, neuronal hypopolarization, and depression of excitability. Picrotoxin blockage of the GABA receptor eliminates normal chloride modulation of neuronal activity, causing hyperexcitability and, potentially, even convulsions. As these toxic effects are also induced by cyclodiienes and polychlorobornanes, it was hypothesized that organochlorines may act by blocking GABA receptors. Confirmatory studies with insect and mammalian preparations established that several cyclodiene insecticides, toxaphene, and lindane act specifically at the picrotoxin site of the GABA receptor. In addition, binding at the picrotoxin site of the GABA receptor tends to correlate with toxic potency. Furthermore, these compounds inhibit GABA-stimulated flows of chloride across membrane vesicles (see reviews by Coats, 1990; Woolley, 1995).

Lindane is the gamma isomer of hexachlorohexane; it is approximately 10 times more acutely toxic to the rat than the alpha isomer and roughly 100 times more potent than the beta and delta isomers. The gamma isomer is strongly excitatory in both insect and mammalian nervous systems, while the alpha, beta, and delta isomers are weakly active or have depressant effects. At acute doses, lindane increases neuronal activity and neurotransmitter release. In addition to convulsant effects, lindane also causes anorexia, diarrhea, and hyperthermia in the rat. As discussed above, lindane is thought to antagonize GABA receptors in the CNS and the gastrointestinal tract. Consistent with this hypothesis, toxic effects of lindane can be attenuated or eliminated by pretreatment with GABA receptor agonists (Woolley, 1995).

Although disruption of VSSCs and GABA receptors are well characterized mechanisms of acute organochlorine neurotoxicity, there is little evidence for the role of these mechanisms in developmental neurotoxicity. Lindane is known to inhibit mammalian embryonic development from the eight-cell stage up to the blastocyst stage (Scascitelli and Pacchierotti, 2003). Several potential mechanisms of developmental neurotoxicity have been proposed, including disruption of endocrine homeostasis and alterations in intercellular communication (Scascitelli and Pacchierotti, 2003).
Manifestations of Organochlorine Insecticide Neurotoxicity in Fish

Organochlorine exposure affects behavior in many fish species. In unrestrained fish, cyclodiene exposure caused hyperactivity in response to stimuli, followed by recurrent tremors, rapid pectoral fin movement, and convulsions (Carlson et al., 1998). In spinally transected rainbow trout (*Oncorhynchus mykiss*), endosulfan and endrin intoxication induced branchial tremors, increased cough rate, and increased pectoral fin movement, with eventual tetany and convulsions anterior to the site of the transection (Bradbury et al., 1991a). Hyperactivity and increased cough frequency have also been reported with DDT intoxication in a number of fish species (Heath, 1995; Murty, 1986). DDT-induced hyperactivity is thought to contribute to decreased schooling behavior (Murty, 1986). At sublethal concentrations of endosulfan, medaka (*Oryzias latipes*) are less susceptible to predation, presumably due to hyperactivity; however, at endosulfan concentrations approximating the LC20 level, medaka are more susceptible to predation than control fish (Carlson et al., 1998). Respiratory-cardiovascular responses of cyclodiene-intoxicated rainbow trout included increased cough rate and ventilation volume, with no change in oxygen uptake efficiency, resulting in an increase in oxygen consumption. In a consistent manner, arterial blood oxygen remained near control levels until near death, while arterial blood carbon dioxide and pH levels decreased only slightly. Overall, the seizure activity in the cyclodiene-exposed trout was not associated with a shift to anaerobic metabolism. These responses were similar to those elicited by uncouplers of oxidative phosphorylation and may suggest a secondary effect associated with inhibition of ATPase activity (Bradbury et al., 1991a). Increased oxygen consumption has also been reported in a number of species following exposure to DDT and methoxychlor (Murty, 1986). In vivo electrophysiological studies of sublethal endosulfan exposures in medaka demonstrated increased motorneuron amplitude peaks and significantly increased stimulus-response ratio on the Mauthner cell startle response. The hyper-responsiveness of the Mauthner cell to stimuli is consistent with cyclodiene acting at the picrotoxin site in the GABA receptor–chloride complex. Furthermore, GABA is an important afferent inhibitory neurotransmitter to the Mauthner cell (Carlson et al., 1998).

Ethanol

Ethanol is a well-known neurotoxicant in mammals. Women who consume large amounts of ethanol during pregnancy often give birth to children exhibiting phenotypic abnormalities, collectively referred to as the fetal alcohol syndrome (FAS). These anomalies include growth deficiency, cognitive impairment, and distinctive craniofacial features (Coles and Platzman, 1993). The developmental potency of alcohol consumption has led to intensive investigation into the mechanisms of ethanol neurotoxicity, with particular emphasis on developmental neurotoxicity.

Mechanisms of Ethanol Neurotoxicity

Although many mechanisms have been postulated for the toxic effects of ethanol to the adult nervous system, ethanol is thought to produce neurotoxic effects mainly through interaction with the glutamnergic system, binding to the NMDA receptor and possibly interfering with the normal interaction of glycine with that receptor (Tsai and Coyle, 1998). Ethanol is a well-known developmental neurotoxicant in humans and laboratory animals. The mechanism for the toxic actions of ethanol on the developing nervous system is, however, unknown (Goodlett et al., 2005). Many hypotheses exist, including interaction with neurotrophins (Kentroti, 1997), cell-adhesion molecules (Bearer, 2001), or specific receptors (Costa and Guizzetti, 2002); increased apoptosis (Olney et al., 2002a,b); or increased oxidative stress (Cohen-Kerem and Koren, 2003). Very few studies have delved into the mechanisms of the effects of ethanol on the developing fish; however, it has been suggested that prechordal plate migration may be perturbed in ethanol-treated zebrafish (*Danio rerio*) embryos (Blader and Strähle, 2000).

Manifestations of Ethanol Neurotoxicity in Fish

Ethanol affects the function of the adult fish nervous system (Dlugos and Rabin, 2003; Gerlai et al., 2000), producing hyperactivity or hypoactivity (depending on dose), aggression, and changes in the
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startle response. Studies of ethanol-induced neurotoxicity in zebrafish (Danio rerio) have shown that ethanol is a teratogen that produces developmental delay (Reimers et al., 2004), craniofacial abnormalities (Bilotta et al., 2004; Blader and Strähle, 2000; Carvan et al., 2004), changes in eye development (Bilotta et al., 2002, 2004), cell death (Carvan et al., 2004; Loucks and Carvan, 2004), and behavioral changes (Carvan et al., 2004). Interestingly, the occurrence or severity of these effects varies with the particular strain of zebrafish exposed (Loucks and Carvan, 2004).

Metals

The neurotoxicological effects of metal exposures must be considered in context of the chemical form of the metal, route and duration of exposure, and toxicokinetics. Effects observed in the field also require consideration of sediment and water quality characteristics associated with bioavailability (Dopp et al., 2004; Rüdel, 2003). Sources of neurotoxic metals in aquatic ecosystems include atmospheric deposition, point-source discharges, and non-point-source releases. Cadmium is primarily released through petroleum refining, fossil fuel combustion, and the use of cadmium in copper and nickel smelting (Wren et al., 1995). Mercury inputs are typically associated with atmospheric inputs from the combustion of fossil fuels and acid rain, point-source discharges from industry and gold-mining operations, and non-point-source discharges from agricultural use of organomercury seed treatments. Organotin and copper compounds have been input directly into aquatic systems as biocides in the shipping, fishing, and aquaculture industry (Blunden and Chapman, 1986; Rüdel, 2003). Globally, lead inputs include wet and dry atmospheric deposition, sewage treatment effluents, industrial and mining discharges, and non-point releases from mine tailings and highways (Gidlow, 2004; Pain, 1995). Aluminum loading to aquatic ecosystems is primarily associated with acidification and resulting releases from rocks, soils, and sediments (Lukiw and McLachlan, 1995).

Mechanisms of Metal Neurotoxicity

Generally, organismal-level effects elicited by metals are derived from multiple mechanisms. In addition to direct neurotoxic mechanisms, metals can damage respiratory surfaces and interfere with energy metabolism, osmoregulation, and endocrine function (Heath, 1995; Weber and Spieler, 1994). The extent to which metals are acting through neurotoxic mechanisms of action is a function of the metal species, dose, and route of exposure. Comprehensive reviews of primary scientific literature on metal toxicology (Chang, 1996) and neurotoxicology (Chang and Dyer, 1995) have been published, based primarily on mammalian model organisms. Below are brief overviews of neurotoxicity mechanisms for some selected metals whose neurotoxic mechanisms have been well studied.

A long environmental half-life and wide range of organ toxicity make cadmium one of the most toxic metals in the environment (Patrick, 2003). Several mechanisms have been suggested for the acute neurotoxicity of cadmium. Cadmium is thought to act as a calcium channel blocker or to bind calmodulin. As a result, cadmium disrupts neuromuscular junctions by blocking calcium-mediated neurotransmitter release (Beauvais et al., 2001). In the brain, cadmium inhibits magnesium and sodium/potassium ATPases, disrupting neurotransmitter uptake (Beauvais et al., 2001). In addition, cadmium effects on phospholipid metabolism in synaptic membranes, axonal transport, and basal adenylate cyclase activity have been reported. Cadmium-mediated disruption of cellular antioxidant defense mechanisms has also been proposed to increase neuronal damage through oxidative stress (Hastings, 1995).

Although all forms of mercury are neurotoxic to humans and animals, the most toxic forms are organic mercury compounds (Gochfeld, 2003). Organic mercury compounds are more lipophilic than elemental mercury or inorganic mercury compounds and therefore bioaccumulate in animal tissues (Shafer, 2000). Of the organic mercury compounds, methylmercury has been particularly well studied. Accumulation of methylmercury in the CNS and primary sensory tracts of mammals causes degeneration of visual cortices, dorsal root ganglia fibers, and the cerebellum. Numerous mechanisms have been proposed to explain the neurotoxicity of methylmercury in vertebrates, including disruption of the cell cycle and induction of apoptosis. Subcellularly, methylmercury is hypothesized to disrupt microtubule assembly and alter ion channel function. At the molecular level, cation homeostasis disruption and interference
with synaptic transmission have been hypothesized to mediate methylmercury neurotoxicity. Additionally, methylmercury is highly reactive with sulfhydryl groups (Shafer, 2000). Binding of methylmercury to sulfhydryl groups in critical proteins or macromolecules has been hypothesized to disrupt protein, DNA, and RNA metabolism; to perturb Ca\textsuperscript{2+} homeostasis; to induce oxidative damage; and to alter protein phosphorylation. Many of these mechanisms are interrelated and likely interact to produce methylmercury neurotoxicity (Chang and Verity, 1995).

Organic tin compounds are largely used as plastic stabilizers, although the major route of environmental exposure is from antifouling paints and pesticides (Rüdel, 2003). Organic tin compounds are hydrophobic and significantly more toxic than either elemental tin or inorganic tin compounds (Rüdel, 2003). Neurotoxic effects of organic tin compounds are thought to occur by a variety of interacting mechanisms; for example, triethyltin is hypothesized to uncouple oxidative phosphorylation, inhibit ATPase and phosphodiesterase activity, and initiate oxidative stress via ethane or ethylene metabolites, resulting in myelinic edema. Methyltin has been proposed to suppress mitochondrial respiration by inhibition of oxidative phosphorylation. The resulting hypoxic condition is thought to produce intracellular edema, glutamate release, and subsequent neuronal excitation. This excitation triggers neuron hyperstimulation and subsequent neuronal injury and death in the rat hippocampus (Chang and Dyer, 1995; Dopp et al., 2004).

Lead is a potent neurotoxicant in humans and animals, associated with impaired perception of spatial organization and increased distractibility (Bressler et al., 1999). A variety of mechanisms have been proposed for lead neurotoxicity, including vascular and neuroglia injury, oxidative stress, alterations in transcription, blockage of neurotransmitter release, and failure of neuronal migration (De Gennaro, 2002). A common component of these neurotoxic mechanisms is the ability of lead to mimic biologically relevant metals and cations, particularly calcium (Bressler et al., 1999; Costa et al., 2004; Marchetti, 2003). Lead mimicry of calcium has two primary consequences leading to neurotoxicity (Marchetti, 2003). Initially, lead competes with calcium for binding sites on calcium-regulated proteins such as calmodulin, synaptotagmin, and cadherin, disrupting gene expression, proteomic synaptic machinery, and neuronal migration (Bressler et al., 1999; De Gennaro, 2002; Marchetti, 2003; Prozialeck et al., 2002). Binding-site competition displaces calcium ions, subsequently exposing cells to higher levels of free calcium and potentially leading to oxidative stress (De Gennaro, 2002) and impaired myelinization (Chang, 1996). The second method by which lead mimicry of calcium induces neurotoxicity is the disruption of calcium transport. Disruption of chloride ion exchange causes hyperexcitation of neurons (Chang, 1996). Lead also blocks calcium ion flux across dopamine receptors, glutamate receptors, and voltage-sensitive calcium channels (VSCCs). This blockage affects neurotransmitter synthesis and release, ultimately leading to neuron hyperexcitability and damage (Chang, 1996; Chang and Verity, 1995; Cory-Slechta and Pounds, 1995; De Gennaro, 2002; Marchetti, 2003).

Aluminum is a potent neurotoxicant in both animal brain and cultured neurons (Savory et al., 2003). Acute aluminum neurotoxicity disrupts blood–glia–neuron membranes and alters neuronal structure and function (Lukiw and McLachlan, 1995). Aluminum is of particular interest because some of its neurotoxic effects resemble Alzheimer’s disease (Yokel, 2000). Similar to this late-onset neurodegeneration disease, acute aluminum neurotoxicity causes the formation and accumulation of insoluble \(\beta\)-amyloid, aggregation of hyperphosphorylated tau protein, and disruption of cortical cholinergic neurotransmission (Yokel, 2000). These effects arise from disruption of calcium binding (Yokel, 2000). Aluminum perturbation of calcium binding induces excessive calcium-ion influx in cholinergic neurons and mitochondria. This increase in mitochondria calcium influx causes apoptosis and may explain the significant loss of cholinergic neurons in Alzheimer’s disease (Savory et al., 2003; Szutowicz, 2001). Significant pathological differences exist between Alzheimer’s disease and aluminum neurotoxicity, making direct comparisons controversial (Yokel, 2000). Furthermore, several other molecular mechanisms have been proposed for aluminum neurotoxicity, including increased iron-induced oxidative stress, inhibition of \(\text{Mg}^{2+}\)-requiring enzymes, and modification of second-messenger systems (Lukiw and McLachlan, 1995; Yokel 2000).

Metals represent some of the most potent developmental neurotoxicants, particularly mercury and lead. Fetal methylmercury exposure in humans causes Minamata disease, characterized by cerebral palsy, mental retardation, and seizures (Davidson et al., 2004). Autopsies of Minamata patients reveal cerebral
and cortical brain lesions with peripheral neuropathy (Gochfeld, 2003). Furthermore, studies with model organisms suggest that low-level exposure to methylmercury may not manifest effects until much later in life (Davidson et al., 2004). Ethylmercury is thought to produce the same developmental effects as methylmercury and may be associated with the spectrum of autism, learning, and speech disorders (Davidson et al., 2004). The developing nervous system is also vulnerable to lead exposure. Children exposed to lead have poor coordination, behavioral problems, and reading disabilities (De Gennaro, 2002). Several aspects of the developing nervous system are sensitive to metal neurotoxicity. Increased absorption through an immature blood–brain barrier means that neurotoxicity may be induced at a much lower exposure level. Furthermore, sublethal metal exposure levels may alter key processes such as synapse formation, synapse refinement, and neurotransmitter release, causing effects that are manifested later in life (Marchetti, 2003). Although currently under investigation, the extent to which the acute metal neurotoxic mechanisms described above are involved in developmental neurotoxicity is not well understood.

### Manifestations of Metal Neurotoxicity in Fish

Numerous reviews have summarized the behavioral responses of fish to metal intoxication (Atchison et al., 1987; Heath, 1995; Weber and Spieler, 1994). In addition to direct neurotoxic mechanisms, alterations in avoidance or attraction responses, activity patterns, critical swimming speed, respiratory behavior, intraspecific social interactions, reproduction, feeding, and predator avoidance (Atchison et al., 1987) can be attributed to direct damage to respiratory surfaces and interference with energy metabolism, osmoregulation, and endocrine function (Heath, 1995; Weber and Spieler, 1994). Examples provided below attempt to link neurophysiological or behavioral responses to neuropathological or biochemical alterations. The extent to which the neurotoxic mechanisms discussed above are relevant in fish remains to be assessed (Weber and Spieler, 1994). Studies of chemoreception have quantified the extent to which metals attract or repel fish and the extent to which metals affect responses to endogenous chemical signals such as pheromones. Alterations in avoidance or attraction responses have been observed in response to a number of metals, including cadmium, copper, and mercury. In rainbow trout (*Oncorhynchus mykiss*), lake whitefish (*Coregonus clupeaformis*), Atlantic salmon (*Salmo salar*), and goldfish (*Carassius auratus*), copper induces avoidance behavior (Atchison et al., 1987). This avoidance behavior is attributed to the effects of copper on the olfactory bulb. Copper attenuates electrical responses of the olfactory bulb and receptor cells to excitatory compounds (Hara et al., 1976; Sutterlin and Sutterlin, 1970; Winberg et al., 1992). Furthermore, copper exposure causes degeneration of specific olfactory receptor cells (Brown et al., 1982; Julliard et al., 1993), likely through oxidative-stress-mediated apoptosis (Julliard et al., 1993, 1996). Interestingly, oxidative stress may be partly responsible for the observed neurological effects in Wilson’s disease, a genetic defect in copper metabolism leading to copper neurotoxicity in humans (Bondy, 1996). In fish, cadmium exposure has been correlated to changes in brain acetylcholinesterase activity, although these neurochemical changes have not been correlated with changes in swimming behavior in larval rainbow trout (Beauvais et al., 2001).

Although many metals elicit an avoidance response, mercuric chloride and methylmercury attract fish (Atchison et al., 1987; Heath, 1995). Exposure of mercuric chloride and methylmercury to the olfactory bulb and receptors of rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) depressed electrical responses (Baatrup et al., 1990; Hara et al., 1976; Sutterlin and Sutterlin, 1970). Methylmercury has also been found to preferentially accumulate in olfactory receptors and the olfactory nerve of Atlantic salmon following dietary exposure (Baatrup et al., 1990; Berntssen et al., 2003). Chronic dietary exposure reduced overall activity in the Atlantic salmon (*Salmo salar*) and caused preferential histopathological damage to the brain stem (Berntssen et al., 2003).

Exposure to tributyltin oxide causes a variety of locomotor effects in fish. Rainbow trout (*Oncorhynchus mykiss*) exposed to tributyltin oxide swim longer distances at higher velocities but with erratic swimming tracks, indicating a loss of orientation (Triebskorn et al., 1994). In addition, intoxicated trout had a depressed startle response and were unresponsive to external stimuli. Similar behavioral and locomotor responses have been noted in minnows (*Phoxinus phoxinus*) (Fent and Meier, 1992). Tributyltin
oxide induces cytopathological damage in the brain and eyes of several fish (Fent and Meier, 1992; Triebskorn et al., 1994; Wester and Canton, 1987). Pathological lesions observed in the optic tectum and eyes suggest that the optic system is a putative target of tributyltin (Fent and Meier, 1992; Triebskorn et al., 1994; Wester and Canton, 1987). In support of this hypothesis, ultrastructural alterations have been found in the optic tectum of tributyltin-oxide-exposed rainbow trout (Triebskorn et al., 1994).

Lead exposure caused a general increase in locomotor activity in mirror carp (Cyprinus carpio) (Rehman, 2003). In zebrafish (Danio rerio) and fathead minnows (Pimephales promelas), lead exposure reduced feeding ability, as evidenced by feeding miscues and increased prey-handling times (Nyman, 1981; Weber et al., 1991). This reduction in feeding ability was attributed to psychomotor coordination, based on correlations between increased brain lead levels, increased serotonin and norepinephrine concentrations, and decreased feeding ability (Weber et al., 1991). Lead exposure induced similar increases in brain serotonin and decreases in GABA in walking catfish (Clarias batrachus) (Katti and Sathyanesan, 1986). Yet, although lead increased brain serotonin and norepinephrine levels, lead exposure did not increase dopamine levels in fathead minnows (Weber et al., 1991).

The effects of aluminum on fish behavior have not been reported. Long-term exposure of rainbow trout (Oncorhynchus mykiss) to aluminum caused aluminum accumulation in the cerebrovascular endothelium and throughout the telencephalon (Exley, 1996). This finding suggests that aluminum is capable of crossing the blood-brain barrier in fish as well as in mammals (Lukiw and McLachlan, 1995; Szutowicz, 2001). Also, in the telencephalon of aluminum-exposed rainbow trout were dense extracellular deposits of aluminum apparently surrounded by a protein matrix, suggesting similar neuropathological responses in fish and mammals.

The effects of a number of metals on prey susceptibility, schooling behavior, and aggression have been studied across a wide array of species (Heath, 1995; Weber and Spieler, 1994). Although many of these adverse effects are hypothesized to reflect alterations in sensory receptors or the structure and function of the PNS and CNS, direct cause-and-effect relationships have not been established. Even less evidence is available for developmental neurotoxicity of metals in fish.

**Neurotoxins**

Through evolutionary pressure, plants and algae have developed a number of strategies to avoid consumption and maximize competitive advantages in accumulating resources. It has been estimated that approximately 7000 terrestrial plant species are toxic to animals, with perhaps one half of these species producing compounds targeting the nervous system. In some instances, plant neurotoxins have been exploited to facilitate fish harvesting (Bhatt, 1991). Several aquatic organisms produce fish neurotoxins and are of increasing concern in many areas (Burkholder et al., 1992). The following discussion provides an overview of the neurotoxicity of several specific neurotoxins and illustrates how these compounds are used to assess fish brain function.

**Mechanisms of Neurotoxin Toxicity**

Neurotoxins derived from aquatic organisms produce toxicity by a variety of mechanisms. Toxins derived from cyanobacteria, dinoflagellates, and coral act on membrane sodium channels directly or on membranes to create ion permeabilities. Such toxins are classified in terms of their ability to activate, stabilize, or occlude the sodium channel (see review by Strichartz and Castle, 1990). Toxins that activate sodium channels cause long-lasting sodium ion influx and correspondingly prolonged depolarization of excitable membranes, preventing complete inactivation. Failure of membrane repolarization also causes periods of rapid, spontaneous impulse firing (Strichartz and Castle, 1990). Brevetoxins and ciguatoxin, derived from the marine dinoflagellates Ptychodiscus brevis and Gambierdiscus toxicus, respectively, are among the most studied sodium-channel activator toxins. Toxins isolated from P. brevis bind VSSCs and cause activation at resting membrane potentials. Competitive binding studies have established that the most toxic brevetoxins bind preferentially at the active site within the sodium channel (Trainer et al., 1990). Frelin and coworkers (1990) have also substantiated that ciguatoxin acts as a sodium-channel activator and binds to the same active site as the brevetoxins.
Toxins stabilize sodium channels by inhibiting inactivation of the sodium current during depolarization, thus keeping the sodium channel in the open state. This sodium channel stabilization causes a prolonged period of calcium influx through VSCCs, causing increased calcium-mediated secretions and contractions (Strichartz and Castle, 1990). Toxins that stabilize sodium channels bind different sites than toxins that activate sodium channels; consequently, sodium-channel-stabilizer toxins can synergize with sodium-channel-activator toxins, causing larger membrane depolarizations at lower doses. Small peptides produced by anemones and larger proteins produced by mollusks in the family Conidae are examples of sodium-channel-stabilizer toxins. Sodium-channel-occluder toxins are small organic cations that are high-affinity, but reversible, blockers of the channel (Strichartz and Castle, 1990). The subsequent inhibition of sodium conductance renders excitable membranes inactive and halts impulse propagation. Tetrodotoxins and saxitoxins are classic examples of sodium-channel-occluding toxins. Tetrotoxin is produced by some fish in the order Tetraodontiformes and the Costa Rican frog Atelopus (Ritchie and Greene, 1985). Saxitoxins are produced by marine dinoflagellates in the genera Gonyaulax and freshwater cyanobacteria in the genera Anabaena and Aphanizomenon (Carmichael, 1997). Some sodium-channel-occluding toxins discriminate between sodium-channel types; for example, the μ-conotoxins, from the mollusk Conus geographus potently block muscle sodium channels but only weakly inhibit sodium currents in neuronal and cardiac sodium channels.

Some toxins create ion permeabilities without affecting ion channels. Palytoxin, derived from coral, irreversibly increases cation permeability, perhaps by converting the sodium/potassium pump to a passive channel (Strichartz and Castle, 1990). Other aquatic neurotoxins have mechanisms that do not center on ion regulation; for example, cyanobacteria of the genera Anabaena and Oscillatoria produce anatoxins that disrupt acetylcholine function at neuromuscular junctions. Anatoxin-a acts as an acetylcholine mimic, binding nicotinic acetylcholine receptors at vertebrate muscle endplates, and is reported to be eight times more potent than acetylcholine. Furthermore, anatoxin-a is resistant to acetylcholinesterase hydrolysis, causing an overstimulation of muscle cells to the point of fatigue. The similarly named anatoxin-a(s) causes the same symptoms of neurotoxicity as anatoxin-a but by a different mechanism. Anatoxin-a(s) is a naturally occurring organophosphate that inhibits acetylcholinesterase activity in a manner similar to the organophosphate insecticides discussed previously.

In addition to aquatic toxins, terrestrial plant toxins can affect the CNS of vertebrates, including fish. Notable examples are the pyrethrins, derived from Chrysanthemum cinerariifolium, whose structures were subsequently modified synthetically to develop the pyrethroid insecticides, as summarized previously. Certain plant toxins have also been exploited by several cultures as an aid in harvesting; for example, piscicidal plants derived from plants of the Garhwall hills of India are of great ethnobiological importance. Bhatt (1991) has described how a flavonoid derived from Engelhardia colebrookiana (Lindley) causes degeneration of neurons and neural tracts in the medulla oblongata of freshwater fish. Strychnine, an alkaloid derived from Strychnos nux-vomica, has long been known as a central nervous system stimulant in animals. Strychnine selectively antagonizes GABA in the brain and glycine in the spinal cord (Dorling et al., 1995) and has been exploited in fish neurotoxicology studies to elucidate the role of these inhibitory neurotransmitters.

**Manifestations of Neurotoxin Toxicity in Fish**

Brevetoxins and ciguatera toxins elicit similar effects in fish, consistent with their identical mechanisms of neurotoxicity summarized previously. Red tides caused by Pychodiscus brevis brevotoxins are associated with massive fish kills. Exposure of ciguatera toxins to coney (Epinephelus fulvus), schoolmaster (Lutjanus apodus), mahogany snapper (Lutjanus mahogoni), largemouth bass (Micropterus salmoides), blueheads (Thalassoma bifasciatum) (Davin et al., 1986, 1988), and western mosquitofish (Gambusia affinis) (Lewis 1992) caused skin color variations, rapid opercular movement, inactivity, loss of equilibrium, erratic swimming, jerky feeding movements, loss of orientation, and death. Exposure to another toxic dinoflagellate, Pfiesteria piscicida, has been reported to induce sudden sporadic movement, disorientation, lethargy, and apparent suffocation followed by death in 11 species of fish, including striped bass (Morone saxatilis), southern flounder (Paralichthys lethostigma), Atlantic menhaden (Brevoortia tyrannus), and American eel (Anguilla rostrata) (Burkholder et al., 1992; Glasgow et al., 1995).
There are few reports of other aquatic neurotoxin effects in fish. Accumulations of saxitoxins, the toxins responsible for paralytic shellfish poisoning, and anatoxins in fish tissues have been documented primarily because they are monitored within the context of human health protection. Toxic endpoints of saxitoxins and anatoxins in fish, however, are rarely assessed. In mammals, including humans, tetrodotoxins and saxitoxins block vasomotor nerve function, ultimately causing death by paralysis of respiratory muscles (Ritchie and Greene, 1985). In laboratory studies, anatoxin-a and anatoxin-a(s) elicit acetylcholine and acetylcholinesterase inhibition responses in electric rays (Torpedo californica) and in electric eels (Electrophorus electricus) (Hyde and Carmichael, 1991; Mahmood and Carmichael, 1987; Swanson et al., 1991). Although anatoxin poisonings have been reported in livestock, domestic animals, and wild birds (Carmichael, 1997), effects on fish in the field remain largely undocumented.

Spinally transected rainbow trout (Oncorhynchus mykiss) exposed to strychnine (Bradbury et al., 1991b) rapidly exhibit increased cough rate as well as whole-body spasms that included tail arching. These spasms were similar to those elicited by polar narcotics. The strychnine-exposed rainbow trout, however, exhibited an elevated response to outside stimuli that was not observed with trout exposed to narcotics, acetylcholinesterase inhibitors, or cyclodiene and pyrethroid insecticides. Similar responses to strychnine exposure were also observed in sharks (Baldrige, 1969), medaka (Oryzias latipes) (Carlson et al., 1998; Rice et al., 1997), and fathead minnows (Pimephales promelas) (Drummond and Russom, 1990). Under acute exposure conditions to nonlethal concentrations of strychnine, medaka are less susceptible to bluegill (Lepomis macrochirus) predation, presumably due to heightened responses to outside stimuli (Carlson et al., 1998). Rainbow trout exposed to lethal strychnine concentrations had decreased ventilation frequency and ventilation volume, causing increased oxygen uptake efficiency and oxygen consumption (Bradbury et al., 1991b); consequently, arterial blood oxygen level decreased throughout these strychnine exposures and was associated with a drop in arterial blood pH and an increase in hematocrit. These respiratory–cardiovascular responses are consistent with anaerobic metabolism during strychnine-induced seizures. Decreased respiration and hypoxia have similarly been noted in mammals exposed to strychnine. In mammals, periods of impaired respiration during convulsions are thought to cause hypoxia, leading to medullary paralysis and death (Klaassen, 1996; Slater, 1965). Studies of the Mauthner cell startle response in strychnine-exposed medaka showed an increase in reflex response to stimuli. An associated delay between the Mauthner cell and motoneuron action potentials and minimal decline in the time between the motoneuron action potential and excitation of the tail musculature were noted (Carlson et al., 1998). These results in medaka are consistent with glycine antagonism and blockage of spinal cord motor cell inhibitory pathways, both of which are documented responses to strychnine exposure in the Mauthner cell soma and dendrites of several other fish species (Furukawa et al., 1964; Legendre and Korn, 1994).

Summary

The mechanisms and effects of neurotoxicants in fish have been studied with a wide variety of in vitro and in vivo methods. In addition to advancing understanding of fundamental neurobiology, this research helps develop techniques to assess adverse effects of neurotoxicants for ecological risk assessments. Of special note are those investigations that link structural and functional alterations of the nervous system at the subcellular or cellular level to physiological and behavioral effects. Research integrating information across levels of biological organization will further mechanistic understanding of neurotoxicants, allowing for extrapolation across species and chemicals and development of approaches to identifying ecologically relevant neurotoxic responses. The National Research Council (NRC, 1992) has observed that one of the greatest challenges in developing methods for neurotoxicology is associating neuromorphological, neurochemical, and neurophysiological alterations with behavioral changes. The in vitro and in vivo fish models described here are uniquely suited for characterizing chemical-induced neurotoxicity and understanding the biochemical, physiological, and morphological mechanisms underlying behavioral alterations.
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References


The Toxicology of Fishes


