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NEUROENDOCRINE DISRUPTION: MORE THAN HORMONES ARE UPSET

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Only a small proportion of the published research on endocrine-disrupting chemicals (EDC) directly examined effects on neuroendocrine processes. There is an expanding body of evidence that anthropogenic chemicals exert effects on neuroendocrine systems and that these changes might impact peripheral organ systems and physiological processes. Neuroendocrine disruption extends the concept of endocrine disruption to include the full breadth of integrative physiology (i.e., more than hormones are upset). Pollutants may also disrupt numerous other neurochemical pathways to affect an animal's capacity to reproduce, develop and grow, or deal with stress and other challenges. Several examples are presented in this review, from both vertebrates and invertebrates, illustrating that diverse environmental pollutants including pharmaceuticals, organochlorine pesticides, and industrial contaminants have the potential to disrupt neuroendocrine control mechanisms. While most investigations on EDC are carried out with vertebrate models, an attempt is also made to highlight the importance of research on invertebrate neuroendocrine disruption. The neurophysiology of many invertebrates is well described and many of their neurotransmitters are similar or identical to those in vertebrates; therefore, lessons learned from one group of organisms may help us understand potential adverse effects in others. This review argues for the adoption of systems biology and integrative physiology to address the effects of EDC. Effects of pulp and paper mill effluents on fish reproduction are a good example of where relatively narrow hypothesis testing strategies (e.g., whether or not pollutants are sex steroid mimics) have only partially solved a major problem in environmental biology. It is clear that a global, integrative physiological approach, including improved understanding of neuroendocrine control mechanisms, is warranted to fully understand the impacts of pulp and paper mill effluents. Neuroendocrine disruptors are defined as pollutants in the environment that are capable of acting as agonists/antagonists or modulators of the synthesis and/or metabolism of neuropeptides, neurotransmitters, or neurohormones, which subsequently alter diverse physiological, behavioral, or hormonal processes to affect an animal's capacity to reproduce, develop and grow, or deal with stress and other challenges. By adopting a definition of neuroendocrine disruption that encompasses both direct physiological targets and their indirect downstream effects, from the level of the individual to the ecosystem, a more comprehensive picture of the consequences of environmentally relevant EDC exposure may emerge.

Following the first WWF Wingspread Conference in 1991 and the publication of Theo Colborn's book *Our Stolen Future* in 1996, there has been increasing public concern about how natural or synthetic compounds interact with the hormonal systems

of humans and wildlife. In the last two decades the scientific community has continued to explore the presence and effects of endocrine-disrupting chemicals (EDC) in the environment (Colborn et al. 1993; Vos et al. 2000; Porte et al. 2006; Hotchkiss et al. 2008).

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The U.S. Environmental Protection Agency (EPA) defines endocrine disruptors as chemicals that either mimic or block the effects of hormones at the target receptor/tissue or by directly stimulating or inhibiting production of hormones by the endocrine system (U.S. EPA 2007). It is our intention to define “neuroendocrine disruption” for the broader community interested in endocrine disruption and ecotoxicology in order to describe how environmental pollutants may impact brain functions as they relate to hormonal systems. To our knowledge it is the first such attempt, and will no doubt require extensive debate and refinement in the coming years. Indeed, the purpose of the first symposium on Neuroendocrine Effects of Endocrine Disruptors (NEED) is to present existing data and begin the debate on the emerging concept of neuroendocrine disruption. We realize that this term may be too general for some but perfect for others. It succinctly encompasses our view of how pollutants disrupt development and physiological functions in animals.

The field of neuroendocrinology has expanded considerably since the first dedicated meetings in the early 1970s. One definition consisting of elements from various mission statements of journals and societies could serve well in this discussion of neuroendocrine disruption. Neuroendocrinology is the study of the interplay between the endocrine and nervous systems that control all bodily processes in vertebrates and invertebrates, and its expanding interface with the regulation of behavioral, cognitive, developmental, immunological, degenerative, and metabolic processes. Therefore, neuroendocrine disruption from an environmental perspective comprises all these elements and how they are affected by biologically active pollutants of diverse origins.

There is an expanding body of evidence that industrial, agricultural, and pharmaceutical chemicals exert effects on vertebrate and invertebrate neuroendocrine systems (Tables 1–4). One part of a definition might be that neuroendocrine disruptors exert their

effects as agonists/antagonists of neuropeptides, neurotransmitters, or neurohormones, thereby affecting hormonal systems. There is also evidence that some environmental pollutants disrupt the synthesis or metabolism of neurotransmitters that regulate hormone release. These changes result in an altered neurophysiological state, which subsequently influences many downstream systems under control of the neuroendocrine brain. Neuroendocrine systems integrate internal (e.g., hormones, metabolic signals) and external (e.g., pheromones, temperature, photoperiod) stimuli to allow physiological and behavioral adaptation to the environment. Therefore, neuroendocrine disruption extends the concept of endocrine disruption to include the full breadth of integrative physiology—that is, neuroendocrine disruption is more than just hormones. It is possible that pollutants disrupt numerous other neurochemical pathways, upsetting diverse physiological and behavioral processes to affect an animal’s capacity to reproduce, grow, or deal with stress and other challenges.

Much in the way that endocrine disruption is different from classical toxicology, neuroendocrine disruption is distinguishable from neurotoxicology. Neurotoxicologists study chemical insults and mechanisms underlying subsequent neuronal cell death, which eventually lead to the failure of key regulatory systems and death of exposed individuals. Rather, the consequences of disrupting the complex neurohormonal brain–pituitary–target organ communication systems are within the domain of neuroendocrine disruption.

It is difficult to pinpoint the first use of the phrase “neuroendocrine disruption.” However, studies of pollutants on the brain–pituitary complex most certainly predate 1991 (Singh and Singh 1980; Smith 1983), when the term “endocrine disruption” was introduced (Gore 2010). It appears that serious consideration of the hypothalamus as a main EDC target was likely developing in the mid to late 1990s, since some of the first papers specifically addressing this issue in fish, frogs, turtles, and mammals

TABLE 1. In Vivo Neuroendocrine Disruption Observed in Vertebrates by Pharmaceuticals and Personal Care Products Released in Municipal Effluents and Detected in the Environment

Disruptor	Species	Effect	Treatment
Ethinylestradiol	<i>Xenopus tropicalis</i>	Decreased ER- α expression in brain, skewed female sex ratio with females lacking oviduct	1–100 nM waterborne (Pettersson et al. 2006)
	<i>Danio rerio</i>	Differential expression changes in liver and tel measured by microarray	10 ng/L waterborne, males (Martiniuk et al. 2007)
	<i>Carassius auratus</i>	Increased CYP19B expression in hyp and tel	10 ng/L waterborne, males (Martiniuk et al. 2006)
	<i>Oryzias latipes</i>	Decreased GnRH-R in brain	5000 ng/L waterborne, males (Zhang et al. 2008a)
	<i>Oreochromis niloticus</i>	Decreased AR- α in brain, impaired sexual behavior	5 ng/L waterborne, males (Zhang et al. 2008a)
Fadrozole	<i>C. auratus</i>	Decreased IGF-1 expression in female brain	125 μ g/g b.w. dietary (Shved et al. 2007)
		Normal IGF-1 expression patterns disrupted in males and females, normal ER- α expression patterns disrupted in males and suppressed in females	5 and 25 ng/L waterborne (Shved et al. 2008)
Fadrozole	<i>C. auratus</i>	Differential gene expression in hyp and tel measured by microarray. Downregulation of CYP19B in hyp and tel	50 μ g/L waterborne, females (Zhang et al. 2009)
Clotrimazole	<i>X. tropicalis</i>	Decreased CYP19 activity in brain of developing tadpoles	375 nM waterborne (Gyllenhammar et al. 2009)
Ketoconazole SSRIs	<i>O. latipes</i> <i>vertebrates</i>	Decreased GnRH-R expression in brain Neuroendocrine disruption	3–300 μ g/L females (Zhang et al. 2008b) (Mennigen et al. 2011)

Note. Hyp, hypothalamus, Tel, telencephalon, GnRH-R, GnRH receptor, IGF-1, insulin-like growth factor 1, ER- α , estrogen receptor alpha, AR- α , androgen receptor alpha, CYP19B, aromatase B, and SSRI, selective serotonin reuptake inhibitor.

were being published around that time and early in the new millenium (Van Der Kraak et al. 1992; Cooper et al. 1999; Khan and Thomas 2001; Trudeau et al. 2002; Crump et al. 2002). One could find the term “neuroendocrine disruption” in the titles of five papers. Two were on molluscs (Gagne et al. 2007a; 2007b), one on the antidepressant mianserin that was phased out of use in most markets (van der Ven et al. 2006), a review on sexual maturation (Bourguignon et al. 2010), and an editorial by Gore and Patisaul (2010). In none of these papers was “neuroendocrine disruption” conceptualized or a definition proposed. These publications are recent, which highlights the novelty and importance of this emerging issue. Around the same time as the first of these publications, a definition was posted on our website (www.teamendo.ca/Community/Our+Lab+Members/1064.aspx), and Gore (2008) and Zoeller (2008) began debating and discussing neuroendocrine targets of EDC in

2008. Regardless, the historical foundations leading any discussion of neuroendocrine effects of EDC are elegantly presented by Gore and Patisaul (2010). In the same issue of *Frontiers in Neuroendocrinology* there are articles covering the effects of EDC on reproductive health, neuroendocrine function, energy balance, and other topics in mammalian models and humans. Here a broader view is taken, and disparate data are examined from numerous invertebrate and vertebrate model systems.

Human activities have introduced neuroendocrine disruptors to the air, water, and soil globally through extensive use of pharmaceuticals and pesticides and through industrial activities that create and/or emit neuroactive byproducts. This is not an exhaustive review, but several examples are used to illustrate key points to provide the framework for future debate on the concept of neuroendocrine disruption.

TABLE 2. In Vivo Neuroendocrine Disruption Observed in Vertebrates by Chlorinated Pesticides

Disruptor	Species	Effect	Treatment
Atrazine	<i>Rattus norvegicus</i>	Loss of brain-stimulated release of prolactin, inhibition of LH and FSH	200 mg/kg/d dietary, females (Goldman et al. 1999)
	<i>Coturnix japonica</i>	Increased GnRH, nonsignificant positive trend with increased exposure	0.5, 5, 50 µg injections to eggs (Ottinger et al. 2009)
Methoxychlor	<i>R. norvegicus</i>	Disrupted prolactin secretory patterns via inhibition of DA in hyp, decreased circulating LH	25 mg/kg/d dietary, females (Lafuente et al. 2000)
	<i>C. japonica</i>	Increased NA and disrupted 5-HT levels in different parts of hyp	25 mg/kg/d dietary, females (Lafuente et al. 2008)
		Increased GnRH in 5 ppm treated males, 0.5 ppm treated females	0.5 and 5 ppm dietary (Ottinger et al. 2005)
	<i>Mus domesticus</i>	Reduced NA and adrenalin in affected individuals	0.5 and 5 ppm dietary, females (Ottinger et al. 2009)
Vinclozolin	<i>Oryctolagus cuniculus</i>	Female offspring displayed typical male exploratory behavior, decreased D1-like receptor density in nucleus accumbens and olfactory tubercle.	20 µg/kg/d dietary to mothers (Panzica et al. 2007)
		Fewer GnRH neurons and increased calbindin neurons in reproductive brain centres of offspring	10 mg/kg/d dietary to mothers (Bisenius et al. 2006)
	<i>C. japonica</i>	Decreased FSH and impaired sexual behavior and loss of pituitary GnRH sensitivity in male pups	7.2 and 72 mg/kg/d dietary to mothers (Veeramachaneni et al. 2006)
Prochloraz	<i>Oryzias latipes</i>	Disruption to GnRH levels	25, 50, and 100 ppm injection to eggs (McGary et al. 2001)
		Decreased GnRH, GnRH-R, and CYP19 in brain, reduced fecundity	3–300 µg/L waterborne to females (Zhang et al. 2008b)
Dieldrin	<i>Micropterus salmoides</i>	Transcriptomic and proteomic changes in hyp	10 mg/kg injections (Martyniuk et al. 2010a)
		Decreased ER-β expression in hyp	2.95 ppm dietary (Martyniuk et al. 2010b)

Note. GnRH, gonadotropin-releasing hormone, Hyp, hypothalamus, Tel, telencephalon, GnRH-R, GnRH receptor, DA, dopamine, 5-HT, serotonin, LH, luteinizing hormone, FSH, follicle-stimulating hormone, NA, noradrenaline, and ER-β, estrogen receptor beta.

NEUROENDOCRINE CONTROL AND ITS DISRUPTION BY ENVIRONMENTAL CONTAMINANTS IN VERTEBRATES

Many of the peripheral endocrine glands including thyroid, adrenal, and gonads are directly under the control of the pituitary gland. Chemical messengers such as releasing hormones and neurotransmitters from the hypothalamic regions in the brain send signals to secretory cells within specific regions of the anterior and intermediate pituitary to stimulate the release of numerous trophic hormones such as thyrotropic hormone, adrenocorticotrophic hormone, gonadotropins, prolactin, or growth hormone. Many of these chemical messengers such as the neurotransmitter serotonin or the catecholamines play a role not only as chemical messengers in the brain, but as

hormones themselves in the peripheral tissues. The neurohypophyseal neuropeptides oxytocin and vasopressin and their homologues are produced in the hypothalamus and released by nerve terminals situated in the posterior pituitary. These secretions from the pituitary control hormone release from endocrine glands, and these hormones then exert their influence on target tissues to elicit specific effects. Inputs at each level of endocrine control in this system may be endogenous as in the case of homeostasis via feedback mechanisms or exogenous in the case of perceptible changes in environmental factors, such as photoperiod, temperature, or population stresses. Signals from pollutants also affect an organism at all four levels of the endocrine system from brain, pituitary, endocrine gland, and/or target tissue to result in behavioral and physiological

TABLE 3. In Vivo Neuroendocrine Disruption Observed in Vertebrates by Industrial Contaminants

Disruptor	Species	Effect	Treatment
Cadmium	<i>R. norvegicus</i>	Disruption of DA, 5-HT, and NA in different brain regions, and disruption of FSH, LH, ACTH, prolactin, and TSH, depending on route, dose, and time of exposure	(Lafuente et al. 2000a; Lafuente et al. 2000b; Lafuente et al. 2001a; Lafuente et al. 2001b; Lafuente et al. 2003)
	<i>M. salmoides</i>	Increased GnRH in whole brain	67 ng/kg Injection, males (Martiniuk et al. 2009)
Cadmium and lead	<i>R. norvegicus</i>	Decreased DA and 5-HT from cadmium recovered when treated with both lead and cadmium at the same time. NA decreased by lead, cadmium, and lead and cadmium. Decreased LH and FSH from cadmium and cadmium and lead exposures.	0.05 mg/kg injection, females (Pillai et al. 2003)
Methylmercury	<i>Neovison vison fish</i>	Disruption of GABA levels	
		Neuroendocrine control of reproduction, neurotransmitter systems	0.1–2 ppm dietary, males (Basu et al. 2010) (Castoldi et al. 2001; Johansson et al. 2007; Crump and Trudeau 2009)
PCBs	<i>R. norvegicus</i>	Disruption to DA, glutamate, GABA, 5-HT and others.	(Fonnum and Mariussen 2009)
Pulp and paper mill effluents	<i>C. auratus</i>	Disruption of DA, GABA, glutamate systems	<i>In vitro</i> (Basu et al. 2009)
	<i>Pimephales promelas</i>	Differential gene expression in hypothalamus measured by microarray	100% effluent exposure, females (Popesku et al. 2010)

Note. DA, dopamine, 5-HT, serotonin, NA, noradrenaline, FSH, follicle-stimulating hormone, LH, luteinizing hormone, ACTH, adrenocorticotropic hormone, TSH, thyroid-stimulating hormone, GABA, gamma-aminobutyric acid.

changes, some of which will most certainly be maladaptive.

The hypothalamus–pituitary–gonad (HPG) axis tightly regulates vertebrate reproduction through the production of the gonadotropins, and much of the endocrine disruption research to date focuses on reproductive upsets, such as gonadal maturation and gametogenesis, sexual differentiation and behavior, or sex steroid mimics. Other regulatory axes, namely, the hypothalamus–pituitary–thyroid (HPT) and hypothalamus–pituitary–adrenal (HPA) axes, also contribute to reproductive regulation at various levels (including transcriptional, receptors, hormonal, or cellular) of “cross-talk.” For example, hormones from the HPA axis modulate the activity of the HPG axis and vice versa (Dobson et al. 2003). The cross-talk or reciprocal regulation of the HPT and HPG axes during development is another good example (Hogan et al. 2007). Conceptually, the importance of this integrative endocrine communication is that a pollutant that mimics or disrupts a

specific reproductive neuroendocrine pathway is also likely capable of affecting a stress- or thyroid-dependent neuroendocrine pathway. Therefore, cross-talk at all levels of a neuroendocrine axis needs to be taken into consideration when interpreting the recognized phenomenon of endocrine disruption.

Pharmaceuticals and Personal Care Products

Human and veterinary pharmaceutical usage results in the release of compounds to aquatic environments that are deliberately engineered to alter physiological states. Pharmaceuticals are eliminated from the body either in their original form or as by-products of the metabolic system. Drugs and their metabolites are ultimately flushed down the drain when eliminated from the body or disposed of improperly and end up in aquatic systems receiving municipal effluent. This exposes fish and other aquatic wildlife to pharmacologically active agents.

TABLE 4. Evidence of Neuroendocrine Disruption in Invertebrates

Disruptor	Species	Effect	Treatment
Cadmium	<i>Limnaea palustris</i>	Disrupted calcium currents in nerve collar neurons	1 mg/L waterborne (Szucs et al. 1994)
	<i>L. stagnalis</i>	Inhibition GABA-activated chloride currents via increased calcium levels in nerve collar cells	In vitro, 50 μ M cell perfusion (Molnár et al. 2004)
	<i>Uca pugilator</i>	Blocking of NA-stimulated release of light-adapting hormone Inhibition of PDH synthesis	10 mg/L waterborne (Reddy et al. 1997a) 8.5 mg/kg injection (Reddy and Fingerman 1995)
	<i>Procambarus clarkii</i>	Increased release of GIH Acetylcholinesterase inhibition	1 mg/L waterborne (Rodríguez et al. 2000) 5 ppm waterborne (Devi and Fingerman 1995)
	<i>Chasmagnathus granulata</i>	Inhibited GIH release	0.5 mg/L waterborne (Medesani et al., 2004)
Municipal effluents	<i>Ellipectio complanata</i>	Decreased 5-HT and DA, increased MAO activity in nerve ganglia Decreased MAO and 5-HT transporter activity, increased DAT activity in nerve ganglia Increased DA, 5-HT, increased DAT, MAO, and COX activity, decreased 5-HT transporter activity Decreased GABA, decreased GAD and MAO activity, increased 5-HT, DA, increased 5-HT transporter, DAT, and acetylcholinesterase activity; effects not mitigated by ozone treatment	Injections and exposure to plume (Gagné and Blais, 2003) Exposure to plume (Gagné and Blais, 2007) Direct exposure to aeration lagoon (Gagné and Blais, 2007) Direct exposure to primary and ozone-treated effluents (Gagné et al., 2007)
	<i>U. pugilator</i>	Suppressed NA release from neural tissue, inhibiting PDH release from sinus gland	Injection of Aroclor 1242 (Hanumante et al. 1981)
	<i>U. pugilator</i>	Suppressed NA release from neural tissue, inhibiting PDH release from sinus gland	Injection (Staub and Fingerman 1984)
	<i>P. clarkii</i>	Suppression of GSH release	10 mg/L waterborne (Sarojini et al. 1994)
Copper	<i>Palaemon elegans</i>	CHH release and hyperglycemia, triggered by 5-HT stimulation	5 mg/L waterborne (Lorenzon et al., 2004; Lorenzon et al., 2005)
	<i>C. granulata</i>	Inhibited GIH release	0.1 mg/L waterborne (Medesani et al., 2004)
Mercury	<i>P. elegans</i>	CHH release and hyperglycemia	5 mg/L waterborne (Lorenzon et al., 2004)
	<i>P. clarkii</i>	Inhibition of 5-HT stimulated release of GSH Acetylcholinesterase inhibition	0.5 mg/kg injection (Reddy et al. 1997b) 0.2 ppm waterborne (Devi and Fingerman 1995)
Lead	<i>P. clarkii</i>	Acetylcholinesterase inhibition	100 ppm waterborne (Devi and Fingerman 1995)
Organophosphates and organocarbamates	crustaceans	Acetylcholinesterase inhibition	(Rapetto et al. 1988; Surendranath et al. 1990; Reddy et al. 1990)
Azadirachtin	insects	Blocked release of neurosecretory material, disruption of acetylcholine, GABA, and increased 5-HT	(Mordue and Blackwell 1993)
	<i>Labidura riparia</i>	Disruption of allatostatins, which inhibit JH synthesis	0.5, 1, 2, and 3 μ g injection (Sayah et al. 1998)

Note. DA, dopamine, 5-HT, serotonin, NA, noradrenaline, FSH, follicle-stimulating hormone, LH, luteinizing hormone, ACTH, adrenocorticotropic hormone, TSH, thyroid-stimulating hormone, GABA, gamma-aminobutyric acid, PDH, pigment-dispersing hormone, GIH, gonad-inhibiting hormone, GSH, gonad-stimulating hormone, MAO, monoamine oxidase, DAT, dopamine transporter, COX, cyclooxygenase, CHH, crustacean hyperglycemic hormone, and JH, juvenile hormone.

Ethinylestradiol (EE2) is widely used for birth control and has been detected at appreciable levels (1–800 ng/L) in municipal effluents and waters receiving these effluents (Desbrow et al. 1998; Ternes et al. 1999; Kolpin et al. 2002). EE2 is neuroactive at environmentally relevant levels, being able to bind to and activate estrogen-response-elements (ERE) in the brains of vertebrates such as the frog, *Xenopus laevis*, and goldfish, *Carassius auratus* (Trudeau et al. 2005). Furthermore, environmentally relevant concentrations of EE2 induced disruption of neuroendocrine functions in adult and developing fish and frogs.

Waterborne EE2 exposures to newly hatched *X. tropicalis* tadpoles through to metamorphosis skewed the adult sex ratio toward female individuals at concentrations as low as 1 nM, producing defects in reproductive tissues of females. Decreased estrogen receptor alpha (ER α) expression occurred in the brain of the juveniles with these defects, suggesting that EE2 may interfere with the development of the reproductive system via reorganizations in the brain (Pettersson et al. 2006).

While environmentally relevant exposures of EE2 to male goldfish were shown to increase aromatase B expression (CYP19B) in the hypothalamus and telencephalon (Martyniuk et al. 2006a), microarray analysis performed with zebrafish telencephalon did not confirm this result, although differential expression of numerous other genes was observed in the liver and telencephalon (Martyniuk et al. 2007). Through microarray analysis of the brains of EE2-exposed male Japanese medaka (*Oryzias latipes*), significantly decreased expression of gonadotropin-releasing hormone (GnRH) receptor 1 (GnRH-R1) occurred at 5000 ng/L, which is a much higher concentration than one might find in the environment. Decreased androgen receptor alpha (AR α) mRNA was observed at the more meaningful (in the context of environmental effects) concentration of 5 ng/L, and these males exhibited impaired sexual behavior (Zhang et al. 2008a). Changes in AR α in the brain of fish exposed to environmentally relevant levels of EE2 may have implications on processes such

as steroidal feedback in the reproductive axis or disrupted spawning due to impaired reproductive behaviour.

The importance of the timing of exposure to neuroendocrine-disrupting chemicals is highlighted in studies where dietary (125 μ g/g) (Shved et al. 2007) and waterborne (5 and 25 ng/L) (Shved et al. 2008) exposures of EE2 suppressed insulin-like growth factor 1 (IGF-1) in the brains of female tilapia (*Oreochromis niloticus*), but only at certain stages of development. IGF-1 and IGF-1 receptors are highly expressed in extrahepatic tissues (such as the brain) during ontogeny, suggesting it plays an important role during tissue differentiation, growth, and development (Perrot et al. 1999). In the waterborne exposure, ER α mRNA was determined, being elevated in male brains at 30 d postfertilization (DPF) but suppressed by the end of the experiment (100 DPF). In females, ER α was decreased over the course of the EE2 exposure (Shved et al. 2008).

Microarray analysis of the hypothalamic tissues of mature, prespawning female goldfish exposed to 50 μ g/L of fadrozole, a potent aromatase inhibitor that suppresses serum estradiol (E2) and is used in the treatment of breast cancer in Japan, resulted in differential expression of many estrogen-responsive genes in the hypothalamus and telencephalon, including the decrease of aromatase B mRNA in both tissues (Zhang et al. 2009). The importance of aromatase B in the brain is now well known in fish (Diotel et al. 2010). Disruptions in brain aromatase may be indicative of disruption of neurogenesis, as it is only expressed in radial glial cells that give rise to neurons in fish brains (Diotel et al. 2010).

Clotrimazole is a chlorinated imidazole used by humans and animals as an antifungal treatment. Clotrimazole is present in municipal effluents and detectable downstream from waste water treatment plants at levels from <1 to 34 ng/L, and exposure of clotrimazole in the micrograms per liter range decreased aromatase activity in the brains of developing *X. tropicalis* during gonadal differentiation (Berg et al. 2009; Gyllenhammar et al. 2009). Ketoconazole, another imidazole-type

fungicide, is used in personal care products such as antidandruff shampoos and antifungal skin ointments. Waterborne ketoconazole (3–300 $\mu\text{l/L}$) has an inhibitory effect on fecundity in female Japanese medaka and decreased whole-brain GnRH receptor expression (Zhang et al. 2008b). This result is hard to interpret, because the role of GnRH receptors in the brain is not well known. However, functional assessments of pituitary sensitivity to GnRH-induced LH release are required to test the hypothesis that changes in receptors following environmentally relevant antifungal exposures are physiologically meaningful.

Selective serotonin reuptake inhibitors (SSRI) are used as antidepressants for their ability to inhibit serotonin reuptake by presynaptic cells, increasing serotonin (5-HT) concentrations in the synaptic cleft. In fish and mammals, there is evidence that 5-HT is important for neuroendocrine control of growth, feeding, reproduction, and stress. It is clear that SSRI are now considered serious aquatic environmental contaminants with neuroendocrine effects that disrupt feeding and reproduction (Mennigen et al. 2008; 2009; Oakes et al. 2010). However, more detail is provided in the review in this issue by Mennigen et al. (2011).

Studies on pharmaceuticals and personal care products as environmental neuroendocrine disruptors in the aquatic environment are still relatively new (Table 1). Popesku et al. (2008) started to analyze the effects of model pharmacological agents on hypothalamic function in fish. However, the neuroendocrine-disrupting potential of pharmaceuticals and personal care products released in municipal waste waters needs to be thoroughly examined, as many of these products are specifically designed to affect neurochemicals and enzymes that regulate brain function. As the effects of neuroactive compounds are investigated in model species and mechanisms of action are proposed for environmentally relevant concentrations, studies need to extend to field investigations to determine whether the effects are indeed observed in wild populations, as is the case with EE2 (Kidd et al. 2007). It is also noteworthy that neuroactive

pharmaceuticals are only part of the problem. Neuroactive illicit drugs such as cocaine, ecstasy, or amphetamines and their metabolites are now found in aquatic environments (Zuccato and Castiglioni 2009; Metcalfe et al. 2010) and are likely to affect key neurotransmitter systems, regulative neuroendocrine function, and behavior in aquatic vertebrates as in humans.

Organochlorine (OC) Pesticides

The heavy use of pesticides in agriculture often results in chemicals being washed into aquatic systems and the soil by rain or entering the air by volatilization. Pesticides and their residues on plants destined for human or animal consumption expose us to toxic or neuroactive chemicals daily, while other, more persistent chemicals may end up in humans and wildlife through the process of biomagnification up food webs. These chemicals are designed with the express purpose of producing toxicity in pests or disrupting an organism's normal physiological state. Some examples of a certain class of pesticides, the organochlorines (OC) (Table 2), which have been notorious since the early days of the "green revolution" for their endocrine-disruptive effects, are presented.

Atrazine is a chlor-s-triazine herbicide that is extensively used globally despite being banned in the European Union. Goldman et al. (1999) reviewed the adverse effects of atrazine, specifically the inhibition of the rat luteinizing hormone (LH) preovulatory surge. Prolactin release from the pituitary was also suppressed by atrazine, and Goldman et al. (1999) indicated that these disruptive effects occur at the level of the brain rather than the pituitary. Ottinger et al. (2009) demonstrated the neuroendocrine effects of atrazine in birds, highlighting atrazine's potential to affect GnRH in the Japanese quail, *Coturnix japonica*.

Methoxychlor is an OC pesticide that was developed shortly after the ban of DDT (dichlorodiphenyltrichloroethane) and has largely replaced its use in agriculture. Methoxychlor has widely reported estrogenic

effects (Cummings 1997), and one study detected its presence in 1.2% of food samples in the United States (Duggan et al. 1983). Methoxychlor fed to female rats (25 mg/kg/d) was also shown to disrupt prolactin secretion through the inhibition of dopamine (DA), a potent inhibitor of prolactin (Lafuente et al. 2000a). Circulating LH was also lower in methoxychlor-treated rats (Lafuente et al. 2000a). Methoxychlor also increased noradrenaline (NA) and 5-HT in the anterior hypothalamus while decreasing 5-HT in the posterior hypothalamus in male rats fed at 25 mg/kg/d, providing further insight toward mechanisms by which methoxychlor may disrupt LH- and prolactin-mediated control of reproductive endpoints (Lafuente et al. 2008). Ottinger et al. (2005) showed that environmentally relevant dietary exposures of methoxychlor to adult Japanese quail enhanced gonadotropin-releasing hormone (GnRH) in the hypothalamus of 5 ppm treated males and 0.5 ppm treated females. When hypothalamic GnRH was measured in the young adult offspring of the treated parents, the female offspring of the 0.5 ppm treated group also had elevated GnRH, demonstrating the ability of endocrine disruptors to produce multigenerational changes to normal brain physiology. Ottinger et al. (2009) postulated that increased hypothalamic GnRH is due to inhibition of release of GnRH from this region as both NA and adrenaline, two catecholamines that stimulate GnRH release, were found to be reduced in the treated quail.

Methoxychlor was also found to alter the behavior of female mice, producing a defeminized or masculinized behavior upon prenatal exposure (Panzica et al. 2007). Panzica et al. (2007) measured D1-like receptor density in the nucleus accumbens and olfactory tubercle of prenatally exposed mice and attributed the altered behaviors to changes in brain reward pathways targeted by amphetamine, possibly involving monoaminergic circuits. Changes to neuroendocrine function or disruption of the endocrine axes might often manifest in behavioral changes. Panzica et al. (2007) extensively explored the neurological and behavioral

effects of other xenoestrogens, and data on behavioral effects of endocrine disruptors are published in Palanza et al. (1999) and Ottinger et al. (2008).

Vinclozolin is a chlorinated dicarboximide fungicide that is used on food crops, golf courses and turf, and ornamental plants. When vinclozolin was fed (10 mg/kg/d) to female Dutch-belted rabbits (*Oryctolagus cuniculus*), the offspring had significantly fewer neurons containing GnRH in the anterior hypothalamus/preoptic area (AH/POA) and organum vasculosum of the lamina terminalis regions of the brain when compared to controls (Bisenius et al. 2006). A rise in calbindin-positive neurons was observed in the AH/POA in both male and female offspring. Calbindin plays an important role as a biomarker for sexual dimorphism, with females having more of these neurons than males in the sexually dimorphic region of the POA (Sickel and McCarthy 2000; Bisenius et al. 2006). Veeramachaneni et al. (2006) reported decreased follicle-stimulating hormone (FSH) secretion and inhibited sexual behavior in adult male rabbits born from females that were treated orally with vinclozolin (7.2 and 72 mg/kg/d) between gestation day 15 and postnatal week 4. These male offspring were less sensitive to GnRH-induced increases in both circulating FSH and testosterone (T). Injection of Japanese quail eggs with vinclozolin (25, 50, or 100 ppm) also resulted in disruption of GnRH and reproductive behavior, but these effects were only seen in males, with differential effects observed depending on the brain region and the dose used (McGary et al. 2001). However, in the same experiment (McGary et al. 2001), circulating sex steroids were unchanged in both male and female treatment groups.

Prochloraz is a chlorinated imidazole-type fungicide used in agriculture. Prochloraz residues were found in straw used as live-stock fodder and detected in the milk and meat from goats that ingested this prochloraz-containing fodder (Campbell 1983). Prochloraz exerts inhibitory effects on reproduction, such as reducing fecundity and decreasing GnRH, GnRH receptors, and aromatase expression in

the brains of female Japanese medaka exposed to high (3–300 $\mu\text{g/L}$) waterborne concentrations (Zhang et al. 2008b).

Dieldrin is a cyclodiene pesticide that antagonizes the GABA-A type receptor and blocks Cl^- entry into neurons. Given the importance of GABA in the stimulatory control of LH release in fish (Trudeau et al. 1997), Martyniuk et al. (2010a) determined global transcriptomic and proteomic responses to dieldrin in the large mouth bass (*Micropterus salmoides*) hypothalamus. Martyniuk et al. (2010a) showed acute responses and concluded that impaired reproduction following dieldrin exposure may result in dieldrin-mediated neurotoxicity, upsets in LH, and potentially altered steroid receptor signalling. In another study Martyniuk et al. (2010b) fed bass with food contaminated with dieldrin. The mRNA levels for ER-beta mRNA levels were significantly lower in the hypothalamus after dieldrin feeding. Cluster and pathway analyses showed that genes and proteins involved in human neuropathologies such as Alzheimer's disease, inflammation, DNA damage, and ischemia were also affected by dietary dieldrin, as in the previous acute exposure study (Martyniuk et al. 2010a; 2010b).

Industrial Contaminants

Industrial processes, such as mining or the burning of fossil fuels, release naturally occurring toxic and neuroactive substances (Table 3) to the environment at levels much higher than what most life is able to adapt to. Toxic and endocrine-disrupting by-products created through the processing of materials and manufacturing of goods are released from smokestacks to the air and effluent pipes to water.

Cadmium (Cd) is an industrially important metal, being primarily released to the environment through the burning of fossil fuels and municipal wastes. Cadmium is a reproductive endocrine disruptor (U.S. EPA 2000) capable of bioaccumulating into the milligram per kilogram body weight range in both aquatic and terrestrial organisms (Frazier 1979;

Wijnhoven et al. 2007). Cadmium is known to affect neurotransmitter levels in the brains of exposed organisms (Lafuente et al. 2001a). In a series of experiments, Cd exposure to rats was shown to affect DA, 5-HT, and NA in different brain regions, with the severity and direction of the impact depending on route, dose, and timing of exposure (Lafuente et al. 2000b; 2000c; 2001a; 2001b; 2003). Endpoints controlled by these neurochemicals (FSH, LH, adrenocorticotrophic hormone [ACTH], prolactin, and thyroid-stimulating hormone [TSH]) were also differentially impacted depending on the difference between experimental designs, stressing the fact that predictions on how this metal, or any other neuroactive chemical, may impact wild populations depends on the route by which the population is exposed. Cadmium is also capable of upregulating GnRH expression in the whole brain of injected largemouth bass (Martyniuk et al. 2009).

When an organism is exposed to more than one contaminant at a time, which is most certainly the case in wild populations, the effects observed have the potential to be additive, synergistic, or antagonistic. Experiments performed by Pillai et al. (2003) determined the combined effects of intraperitoneal (ip) injections of either 0.5 mg/kg body weight of lead (Pb) and Cd alone or in combination in the hypothalamus of female adult rats. Pillai et al. (2003) found that Pb + Cd treatment abated reductions of 5-HT observed in the hypothalamus of Cd-only-treated rats. Dopamine levels were also decreased in Cd-treated, but not in Pb- or Pb + Cd-treated groups. Lead and Cd alone and in combination led to reduced NA. Decreased levels of both LH and FSH were observed in the Cd-treated group, while neither LH nor FSH was markedly changed in the Pb-exposed group. When Pb was administered with Cd, the effects were not significantly different from the Cd alone effect on LH and FSH. Pillai et al. (2003) compared and contrasted the effects of Pb and Cd seen in their experiments with those that also showed disruption of 5-HT, NA, and DA and the downstream effects on LH and FSH secretion.

Mercury (Hg) and its organic form, methylmercury (MeHg), are well known for their neurotoxicity (Ratcliffe et al. 1996). The effects of mercury on neuroendocrine control of reproduction in fish were reviewed by Crump and Trudeau (2009) and effects on neurotransmitter systems were reviewed by Castoldi et al. (2001) and Johansson et al. (2007). While most studies occur in the laboratory setting using model animals with high-dose and acute exposures, chronic neuroendocrine disruption of the gamma-aminobutyric acid (GABA) system at environmentally relevant dietary concentrations (0.1–2 ppm for 3 mo) of MeHg was noted in the fish-eating captive juvenile male American mink (*Neovison vison*), supporting the notion that neuroendocrine disruption may indeed be occurring in wild populations of fish-eating animals (Basu et al. 2010).

Polychlorinated biphenyls (PCB) and brominated flame retardants also exert neurotoxic and neuroendocrine effects. The effects of PCB in the brains of rats are widespread, altering DA turnover and the uptake of glutamate and GABA, inhibiting GABA signalling, and decreasing 5-HT levels. Fonnum and Mariussen (2009) reviewed the neuroendocrine effects of PCB and brominated flame retardants.

NEUROENDOCRINE DISRUPTION IN INVERTEBRATES

While most investigations on EDC focused on effects in vertebrates, the importance of the impacts of EDC on invertebrates should not be overlooked. Invertebrates represent 95% of animal species and play an invaluable role in any ecosystem. Furthermore, the endocrinology of many invertebrates is well understood, and their sensitivity, size, ease of handling, fast generation time, and/or more facilitative regulatory restrictions make them convenient sentinels for monitoring ecosystem health. A few examples of neuroendocrine disruption in invertebrates are provided in Table 4. More extensive reviews on EDC in invertebrates are found in deFur (2004), Lagadic et al. (2007), and LeBlanc (2007).

Molluscs

In the review of endocrine disruption by Lagadic et al. (2007), data demonstrated how Cd was capable of affecting embryo development, numbers of egg masses, number of eggs per egg mass, and egg hatching in different species of the genus *Lymnaea*, an aquatic pulmonate gastropod. Szücs et al. (1994) showed that chronic exposure to waterborne Cd (1 mg/L) increased calcium currents in nerve collar neurons of *L. stagnalis*. Collar cells were found to stimulate spermatogenesis in gastropods (Takeda 1982). An in vitro study in *L. stagnalis* by Molnár et al. (2004) also suggested that Cd inhibited GABA-activated chloride currents by increasing intracellular calcium levels in collar cells. These are plausible mechanisms by which Cd may disrupt neurotransmitter control of reproduction in gastropods.

The effects of municipal effluents on the reproductive axis in the freshwater mussel, *Elliptio complanata*, were assessed by Gagné and Blaise (2003). In mussels, the gonads are surrounded with nerve ganglia that project into the gonad and control spawning and gonadal maturation through DA, 5-HT, and arachidonic acid cyclooxygenase (COX) activity (Gagné and Blaise 2003; Gagné et al. 2007a). Briefly, COX synthesizes prostaglandin and induces 5-HT release, which stimulates spawning, and 5-HT stimulates, while DA inhibits, gonadal maturation. 5-HT also plays an important role in sex determination, specifically in the differentiation of male sex organs. Extracts of municipal effluents exerted an estrogen-like response upon injection or direct exposure to a municipal effluent plume (Gagné and Blaise 2003): Effluent exposure and E2 injection decreased 5-HT and DA levels in nerve tissues. Different effluents produced either an elevation, such as observed upon E2 injections (Gagné and Blaise 2003), or a decrease in monoamine oxidase (MAO) activity (Gagné et al. 2007a). Gagné et al. (2007a) also measured 5-HT and DA transporters, demonstrating that exposure to the plume decreased 5-HT transport and increased DA transport. Gagne et al. (2007a) concluded that these effluents exerted an

overall stimulatory effect on reproduction, yet this hypothesis was not directly tested. When the mussels were put directly in the aeration lagoons that were used to treat the effluents, DA and 5-HT levels, as well as DA transport, MAO activity, and COX activity levels, were increased, while 5-HT transport levels were decreased. Data suggested that conflicting results might be produced by higher levels and diversity of neuroactive pharmaceuticals present in the lagoon versus the plume, where advanced degradation and dilution may occur. Ozone treatment of effluents was unable to mitigate effects produced by primary effluents (reduced GABA levels and glutamic acid decarboxylase [GAD] and MAO activity, and elevated 5-HT, DA, and 5-HT transporter, DA transporter, and acetylcholinesterase activity), demonstrating that certain treatments of effluents may be ineffective at removing neuroactive chemicals from municipal wastes (Gagné et al. 2007b).

Crustaceans

Neuroendocrine disruption was also described in crustaceans (Fingerman et al. 1998). Injections of the PCB mixture Aroclor 1242 (Hanumante et al. 1981) and the polycyclic aromatic hydrocarbon (PAH) naphthalene (Staub and Fingerman 1984) are capable of inhibiting NA-stimulated release of pigment-dispersing hormone (PDH) in the fiddler crab, *Uca pugilator*. The PDH octadecapeptide is released from the sinus gland and modulates color change in crustaceans. Waterborne exposures of Cd (10 mg/L) block the stimulatory action of NA on light-adapting hormone release, reducing the eye's ability to adapt to light conditions (Reddy et al. 1997a). There is also evidence that injected Cd (8.5 mg/kg body weight injection) inhibits PDH synthesis in the fiddler crab (Reddy and Fingerman 1995).

Fingerman et al. (1998) summarized experiments on the effects of Cd, DDT, sumithion (an organophosphate insecticide), and naphthalene on hyperglycemia in different crustaceans. Evidence indicated modulation of

hyperglycemia was attributed to changes in production and release of crustacean hyperglycemic hormone (CHH). The CHH family of polypeptide neurohormones plays important roles in carbohydrate and lipid metabolism (Zarubin et al. 2009). In the shrimp *Palaemon elegans*, lethal levels (5 mg/L) of both copper (Cu)- and Hg-contaminated seawaters induced a rapid release of CHH from the sinus gland in the eyestalk and a subsequent hyperglycemic response (Lorenzon et al. 2004). At sublethal levels, 0.1 mg/L Cu and Hg produced a slower release of CHH, with recovery occurring after 3 h. The sublethal intermediate level of 0.5 mg/L Hg was capable of eliciting an even stronger release of CHH than the lethal dose of 5 mg/L. In a follow-up study, Lorenzon et al. (2005) determined that CHH release by Cu was triggered by release of 5-HT in the eyestalk, which subsequently stimulated CHH secretion.

In the crayfish *Procambarus clarkii*, the stimulatory effect of 5-HT, which triggers gonad-stimulating hormone (GSH) release from the brain and thoracic ganglia (Sarojini et al. 1995), on in vivo ovarian maturation was inhibited by 0.5 mg/kg body weight injections of Hg (Reddy et al. 1997b). Ovarian maturation was also inhibited in *P. clarkii* by waterborne exposure of naphthalene at 10 mg/L (Sarojini et al. 1994). The inhibitory effect of naphthalene on ovarian maturation may have been via inhibition of GSH release from neural tissues (Sarojini et al. 1994). Waterborne Cd (1 mg/L) was also found to enhance secretion of gonad-inhibiting hormone (GIH) from the eyestalks of the fiddler crab, *U. pugilator*, which led to inhibition of gonadal growth either through suppression of GSH release and/or direct inhibition of the oocytes (Rodríguez et al. 2000). Conversely, in the crab *Chasmagnathus granulata*, both waterborne Cu (0.1 mg/L) and Cd (0.5 mg/L) were found to inhibit GIH release (Medesani et al. 2004), highlighting the possible existence of species-specific response to Cd exposure.

Acetylcholinesterase (AChE) activity in neural tissues was shown to be inhibited by Hg, Cd, and Pb in *P. clarkii* (Devi and Fingerman 1995).

AChE inhibition was also observed in crustaceans after exposure to organophosphates (Repetto et al. 1988; Surendranath et al. 1990) and organocarbamates (Rao et al. 1991), pesticides specifically designed to inhibit AChE in insect pests. The inhibitory effects on AChE activity in the prawn *Metapenaeus monoceros* by the organocarbamate carbaryl were still evident after a 10-d recovery period where the prawns were kept in clean water. Inhibition of AChE was stronger in prawns exposed to commercial-grade versus technical-grade carbaryl, indicating emulsifiers or other ingredients in the pesticide mix increased the penetrability of carbaryl in tissues and cells (Reddy et al. 1990).

Insects

Many insecticidal products are manufactured with the express purpose of interfering with the hormonal systems in pest insects. The intended use and modes of actions of these pesticides are beyond the scope of this review, although the potential exists for neuroendocrine disruption in nontarget insects. One example of a potential neuroendocrine disruptor in nontarget insects is azadirachtin (AZA), an insecticidal limonoid isolated from the seeds of the neem tree, *Azadirachta indica*, which has antifeeding, growth-regulating, and sterilizing properties in insects. AZA is capable of blocking the release of neurosecretory material from the corpus cardiaca, interacting with the acetylcholinergic and GABAergic systems in the brain–ring gland complexes, and elevating levels of 5-HT in the brain, subesophageal ganglion, and corpus cardiaca in some insects (Mordue and Blackwell 1993). AZA was also shown to inhibit vitellogenesis in the earwig *Labidura riparia*, by altering the distribution of the neuropeptide allatostatins (Sayah et al. 1998). These allatostatins inhibit juvenile hormone (JH) synthesis in the corpora allata. Juvenile hormone controls growth and development of immature insects and vitellogenesis in adults. In this study, treatment of earwigs with JH counteracted the inhibitory effects of AZA on earwig vitellogenesis.

THE IMPORTANCE OF AN INTEGRATIVE, SYSTEMS APPROACH TO UNDERSTAND ENDOCRINE DISRUPTION

Endocrine disruption research, especially in vertebrate models, has been largely biased toward the hypothesis that pollutants may or may not be estrogenic EDC. Estrogen receptor binding, in vitro transcriptional reporter gene assays, and vitellogenin production are all indicative of essentially one pathway. Only a small proportion of the hundreds of published studies on EDC in the last decade or so reflect neuroendocrine disruption processes. One case where relatively narrow hypothesis testing strategies have only partially solved a major problem in environmental biology is that of the effects of pulp and paper mill effluent (PPME) on fish reproduction. This serves as a good example where the broader approach of systems biology and integrative physiology is warranted.

Since 1994, the Canadian Fisheries Act requires pulp and paper mills to conduct environmental effects monitoring (EEM) to determine whether PPME affect fish, and thereby determine whether regulations adequately protect the environment on a site-specific basis. Many studies show that PPME rapidly inhibit egg production in fish, and that life-cycle exposure alters sex ratio and induces intersex animals (males with eggs in their testes) (Hewitt et al. 2006; McMaster et al. 2006; Parrott et al. 2006; Hewitt et al. 2008). An important pattern to emerge from these studies is that despite significant reductions in acute effluent toxicity, suspended solids, and released chlorinated organics, PPME still reduces gonad size and inhibits reproduction in fish. The minimal reproductive improvements observed over numerous cycles of EEM (Hewitt et al. 2008) generated a cause-and-effect hypothesis that EDC in effluents are functioning as steroid mimics or interfere with steroid signaling. Bioassays have been based largely on assessments of gonadal function via in vivo and in vitro measurements of biologically active sex steroids in response to effluent exposures,

and studies have assessed whether fish tissue, effluent, and wood extracts contain ligands for fish sex steroid receptors (Hewitt et al. 2006; 2008). Pioneering work by MacLatchy and Van Der Kraak (1995) indicated that the phytoestrogen β -sitosterol is one possible anti-reproductive chemical. These studies are all-important, and there is strong evidence that compounds in effluents are acting as steroid mimics or interfering with steroid signaling, but they have yet to fully explain the observed reproductive effects downstream from Canadian mills. It is clear from national surveys that other pathways may be implicated in reproductive disruption, as metabolic disruption was also noted in affected populations (Hewitt et al. 2006; 2008).

The observation that PPME rapidly and reversibly inhibit spawning in the fathead minnow (Kovacs et al. 2007) was our first important clue that disruption of neuroendocrine processes may be involved. Given that ovulation and sperm release are essentially triggered by neural events (i.e., it is a neuroendocrine cascade), it was postulated that PPME contain neuroactive chemicals. Only one early study attempted to assess the neuroendocrine status of fish exposed to effluents. Van Der Kraak et al. (1992) demonstrated that in a white sucker, *Catostomus commersonii*, population downstream from a bleached kraft mill, LH levels were suppressed and pituitary LH responsiveness to GnRH was reduced. Changes in the neuroendocrine control of gonadotropin secretion may provide one mechanism by which pulp mill effluents exert adverse effects.

The first study to explore the neuroactivity potential of pulp mill effluent was published by Basu et al. (2009). It is well known that the catecholamine DA is a potent inhibitor of GnRH and LH release in multiple teleost species (Dufour et al. 2005). This inhibition is so potent that it is necessary to co-inject DA receptor antagonists and GnRH agonists to induce ovulation in many fish species. Another key neurotransmitter system controlling LH release is the amino acid gamma-aminobutyric acid (GABA). GABA is synthesized from glutamic acid by the enzyme

glutamic acid decarboxylase. By inhibition of DA and stimulation of GnRH, GABA stimulates LH release in goldfish (Trudeau 1997; Popesku et al. 2008). Basu et al. (2009) found that PPME contain compounds that interact with key enzymes and receptors that regulate the GABAergic, glutamatergic, and DAergic modulation of GnRH and LH in fish. Further validation of the neuroendocrine disruption hypothesis for the effects of PPME on fish was obtained using microarray analysis coupled with comparative genomic approaches. Expression profiling of the female fathead minnow hypothalamus revealed distinct effects of various PPME (Popesku et al. 2010). Comparison of these effects in the fathead minnow with the effects of specific DA agonists injected into female goldfish indicated that PPME, to some extent, may be acting on DA-sensitive pathways, thereby inhibiting reproduction. PPME in receiving waters, as with all situations of environmental contamination, represent complex mixtures of diverse substances with endocrine-disrupting activities. It is likely that there are effects through a multitude of mechanisms and several levels of the neuroendocrine system. Therefore, it is essential that a more comprehensive approach to the problem of neuroendocrine disruption be taken.

CONCLUSIONS AND FUTURE DIRECTION

We used several examples to illustrate that diverse environmental pollutants have the potential to disrupt neuroendocrine control mechanisms. One major observation is that negative effects of diverse pollutants on reproductive processes are common. This has been used by some sectors as an argument against the specificity of effects of EDC, as they may represent a nonspecific stress response. This critique has little scientific basis. Rather, reproductive effects reflect the complexity of neuroendocrine control mechanisms, and chemicals inhibit reproduction specifically through multiple highly complex pathways. For example, SSRI disturb neuroendocrine control of

reproduction through the central 5-HT system (Mennigen et al. 2008; 2011), but EE2 specifically alters neuroendocrine control of reproduction via activation of multiple nuclear estrogen receptors (ER) in numerous estrogen-sensitive neuronal systems, in addition to specific ER-mediated effects in gonads and liver (Martyniuk et al. 2006b; Zhang and Trudeau 2006). The multitude of neuropeptides, neurotransmitters, enzymes, hormones, and receptors that may be involved in physiological responses to neuroendocrine disruptors is staggering, and perhaps this has dissuaded some from exploring the neuroendocrine effects of endocrine disruptors. Another important challenge is separating observed effects on the neuroendocrine system as a whole from direct actions of EDC on the central nervous system. For example, one may observe an effect on a receptor or peptide in the hypothalamus, but is this change a direct result of exposure to a pollutant or the consequence of effects of EDC action on a peripheral hormone involved in a feedback loop? This reveals the major challenge, and one of the most compelling reasons to adopt the methods of systems biology and integrative physiology to address the effects of EDC. Another major challenge is the testing of the neuroendocrine disruption hypothesis at the scale of the ecosystem. This will require a concerted effort between experts in vertebrate and invertebrate neuroendocrinology, ecotoxicology, and ecology. For example, many assays for neurotransmitter/neuropeptide receptor binding or for neurotransmitter enzyme synthesis and degradation are well established and could be more broadly used for screening environmental samples obtained from areas where animals, including humans, are being exposed to pollutants.

Neuroendocrine disruptors are defined as pollutants in the environment that are capable of acting as agonists/antagonists or altering the synthesis and/or metabolism of neuropeptides, neurotransmitters, or neurohormones, which subsequently alter diverse physiological, behavioral, or hormonal processes to affect an animal's capacity to reproduce, develop and

grow, or deal with stress and other challenges. By adopting a definition of neuroendocrine disruption that encompasses both direct physiological targets and their indirect downstream effects, from the level of the individual to the ecosystem, a comprehensive picture of the consequences of EDC exposure may emerge.

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