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The goldfish (*Carassius auratus*) as a model for neuroendocrine signaling

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A R T I C L E I N F O

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We would like to dedicate this article to the memory of Professor Richard E. Peter, a true pioneer in neuroendocrine signaling. He passed away suddenly and prematurely in March 2007. We respected his vision and benefited from his friendship and encouragement.

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Brain
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A B S T R A C T

Goldfish (*Carassius auratus*) are excellent model organisms for the neuroendocrine signaling and the regulation of reproduction in vertebrates. Goldfish also serve as useful model organisms in numerous other fields. In contrast to mammals, teleost fish do not have a median eminence; the anterior pituitary is innervated by numerous neuronal cell types and thus, pituitary hormone release is directly regulated. Here we briefly describe the neuroendocrine control of luteinizing hormone. Stimulation by gonadotropin-releasing hormone and a multitude of classical neurotransmitters and neuropeptides is opposed by the potent inhibitory actions of dopamine. The stimulatory actions of γ-aminobutyric acid and serotonin are also discussed. We will focus on the development of a cDNA microarray composed of carp and goldfish sequences which has allowed us to examine neurotransmitter-regulated gene expression in the neuroendocrine brain and to investigate potential genomic interactions between these key neurotransmitter systems. We observed that isotocin (fish homologue of oxytocin) and activins are regulated by multiple neurotransmitters, which is discussed in light of their roles in reproduction in other species. We have also found that many novel and uncharacterized goldfish expressed sequence tags in the brain are also regulated by neurotransmitters. Their sites of production and whether they play a role in neuroendocrine signaling and control of reproduction remain to be determined. The transcriptomic tools developed to study reproduction could also be used to advance our understanding of neuroendocrine–immune interactions and the relationship between growth and food intake in fish.

1. Introduction

Teleosts represent more than half of all vertebrate species and are adapted to a wide range of marine and freshwater habitats (Nelson, 2006). Numerous characteristics of the goldfish (*Carassius auratus*; reviewed in Trudeau, 1997) make this species an excellent model for understanding neuroendocrine signaling and the regulation of reproduction in vertebrates, including commercially important teleost fish.

In temperate climates in the northern hemisphere, goldfish reproduce annually in April and May. This reproductive cycle is primarily regulated through the release of luteinizing hormone (LH; previously called gonadotropin-II or GtH-II) which is structurally and functionally similar to mammalian LH (Blazquez et al., 1998a,b). LH is important because it is an essential regulator of annual gonadal growth cycles, sex steroid and sex pheromone synthesis, and sperm production in males or ovulation in females during the breeding season. Failure of an environmental or endocrine signal to activate the neural LH release mechanisms at spawning may lead to reduced fertility. LH release is regulated by the stimulatory and inhibitory actions of multiple forms of gonadotropin-releasing hormone (GnRH) and dopamine (DA), respectively (Blazquez et al., 1998a,b; Peter et al., 1986; Omeljaniuk et al., 1987). GnRH and LH release are regulated by interactions between a multitude of classical neurotransmitters and neuropeptides (Trudeau, 1997) (Fig. 1). In teleosts, the gonadotrophs, as well as other endocrine cells in the anterior pituitary, are directly innervated (Ball, 1981). Interestingly, the teleost pituitary is highly regionalized, with gonadotrophs being clustered in the proximal pars distalis in association with somatotrophs (Ball, 1981), which allows for the precise determination of the preoptic telencephalic and hypothalamic origins of hypophysiotropic inputs to the pituitary using tract-tracing methods (Anglade et al., 1993). This is in contrast to mammals and other tetrapods, which have a hypothalamic–hypophyseal blood portal system, and for which multiple neurotransmitter and neuropeptidergic inputs converge

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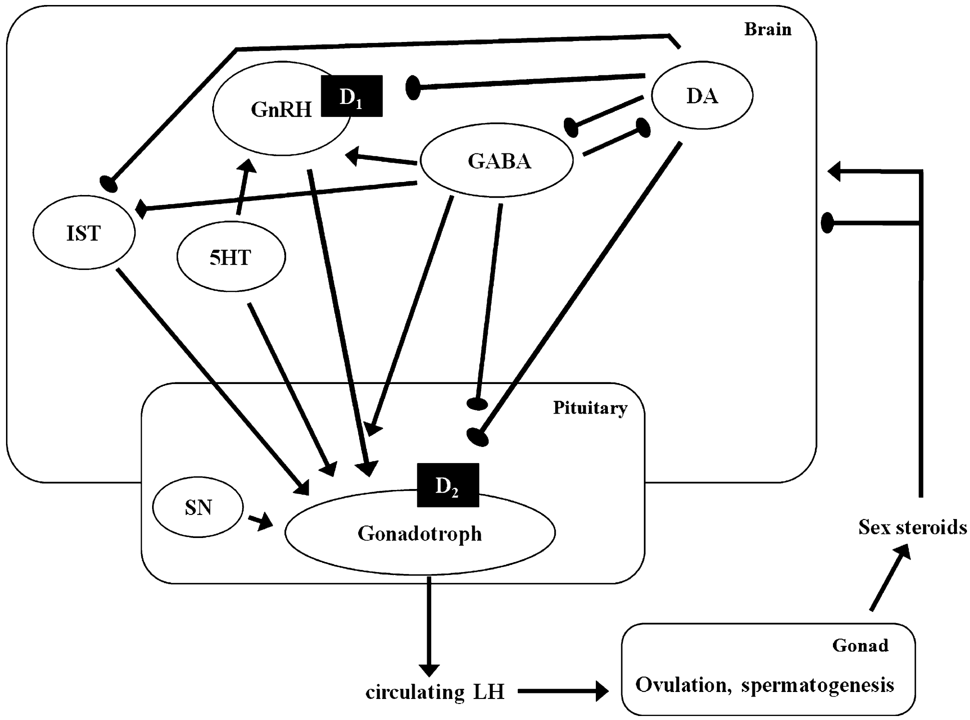

Fig. 1. Schematic representation of the reproductive neuroendocrine axis in the brain of the goldfish. Arrows indicate stimulation. Bulbous arrows indicate inhibition. The diamond-tipped arrow indicates a speculated pathway. See text for abbreviations.

at the median eminence. It is thus very difficult to determine the origins of the hypophysiotropic neurons in tetrapod models. Recent work has taken advantage of the regionalized distribution of cells in the fish pituitary to demonstrate a unique reciprocal paracrine relationship between gonadotrophs and somatotrophs that is mediated by LH and growth hormone (GH) (Wong et al., 2006).

The interactions between the neuropeptide GnRH, the catecholamine DA and amino acid γ-aminobutyric acid (GABA) (Fig. 1) form the central core of our understanding of the integrated control of LH in fish, which has been compared previously to parallel systems in the rat (Trudeau, 1997). The multiplicity of peptides and receptors and the central role of GnRH has been reviewed rather extensively (Guilgur et al., 2006; Klausen et al., 2002; Lethimonier et al., 2004), and will not be covered here. Numerous other neuropeptides and neurotransmitters are involved (Trudeau, 1997; Trudeau et al., 2000), and with the exception of the indoleamine serotonin (5HT), our understanding of their roles in fish is rather limited. Here we will review the roles of DA, GABA and 5HT, and advance the hypothesis that modulation of these systems is at the foundation of complex control of LH release, and thus reproduction in a vertebrate. Recent advances in transcriptomic analysis reveal that neurotransmitters not only control pituitary hormone release, but have rapid and profound receptor-mediated effects to regulate gene expression in the neuroendocrine brain.

2. The goldfish model serves multiple disciplines

The availability of model organisms with unique characteristics, a wide range of research reagents, and tools drive research discoveries in the biological sciences. The common goldfish has served this purpose for more than 3 decades. As a member of one of the largest vertebrate families, the *Cyprinidae*, goldfish are related to important ecological and genetic models, for example fathead minnows and zebrafish, and to economically important cultured carp species. Perhaps the most significant scientific advances resulting from research on goldfish are largely related to neuroendocrine signaling and how the brain regulates growth, feeding, reproduction, pituitary and gonadal physiology, sex pheromones and behaviour, and stress response (Bernier and Peter, 2001; Chang et al., 2000; Martyniuk et al., 2006; Nero et al., 2006; Trudeau et al., 2005; Volkoff et al., 2005; Wagg and Lee, 2005; Zheng and Stacey, 1997) (and references therein). Indeed, basic discoveries on reproductive neuroendocrine signaling in goldfish by R.E. Peter's group led to the development of an internationally successful commercial spawning kit named OVAPRIM® and marketed to the aquaculture industry by Syndel Inc. (www.syndel.com) in Vancouver, BC.

Goldfish also serve as useful model organisms in the fields of cell biology, immunology, toxicology, endocrine disruption, molecular evolution and comparative genomics, neurobiology, olfaction, learning and memory, vision, and taste (Bretaud et al., 2002; Gomez et al., 2006; Hanington et al., 2006; Huesa et al., 2005; Lee et al., 1997; Luo et al., 2006; Nakamachi et al., 2006; Preuss et al., 2006; Szczerbik et al., 2006; Yamaguchi et al., 2006).

3. Dopamine (DA) is the key inhibitor of LH release

The catecholamines, dopamine (DA), norepinephrine (NE), and epinephrine (E) are all produced from tyrosine in a sequential manner. DA is synthesized from the hydroxylation and subsequent decarboxylation of tyrosine through the action of the enzymes tyrosine hydroxylase (TH) and DOPA decarboxylase (DDC; also known as aromatic amino acid decarboxylase, AAADC), respectively. DA can then be converted into NE through further hydroxylation by dopamine β-hydroxylase (DBH), and subsequently, into E through the methylation of NE via phenylethanolamine *N*-methyltransferase (PNMT). TH catalyzes the rate-limiting step in the process (Levitt et al., 1965) and is regulated by a variety of different mechanisms (reviewed by Kumer and Vrana, 1996). It was recently shown that goldfish possess olfactory sensitivity to dopamine and epinephrine as well as their 3-*O*-methoxy derivatives, metadrenaline and 3-*O*-methoxytyramine (Hubbard et al., 2003), raising the intriguing possibility that catecholamines and/or their metabolites may be used in external chemical communication. DA is the only identified inhibitor of LH release in goldfish and is well studied and will be addressed further. In contrast, the role of NE and E are less clear and will not be discussed.

Dopamine is involved in four main pathways in the vertebrate CNS: the mesolimbic (pleasure and reward, addiction), the mesocortical (learning and memory), the nigrostriatal (movement, and hence Parkinson's disease), and the tuberoinfundibular pathways. Neuroanatomical features of the catecholaminergic systems in goldfish and zebrafish are well described (Kaslin and Panula, 2001; Ma, 2003; Northcutt, 2006; Rink and Wullimann, 2001; Smeets and Gonzalez, 2000; Wullimann and Mueller, 2004; Wullimann and Rink, 2002; Ikenaga et al., 2006; Butler and Hodos, 2005). The area dorsali telencephali, pars dorsalis has been identified as being the striatum based on the presence of TH and lack of DBH immunoreactivity (Hornby and Piekut, 1990). There is still considerable debate to whether fish possess a defined nigrostriatal pathway. Based on hodologic and anatomical studies in the Senegal bichir (Reiner and Northcutt, 1992) and zebrafish (Rink and Wullimann, 2001), the periventricular posterior tuberculum (TPp) is a good candidate of the ventral tegmental area and substantia nigra of tetrapods. However, based on functional and neurochemical studies by dopamine depletion via 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; a selective dopaminergic neurotoxin) in goldfish (Goping et al., 1995; Poli et al., 1990; Pollard et al., 1992, 1996), it appears that the telencephalic nucleus pars medialis is also a good candidate as the fish homolog of the tetrapod substantia nigra. A more recent study in the zebrafish (Kaslin and Panula, 2001) provides support to both tuberal and intrinsic telencephalic nigrostriatal hypotheses; however, the authors of the study are more inclined towards the tuberal hypothesis based on their results. The lack of information of a definitive substantia nigra in fish is a disadvantage not only to the goldfish model, but to all fish models, for the study of diseases involving movement disorders such as Parkinson's disease.

While goldfish have been used to investigate the role of DA in motivation, memory, and learning (Mattioli et al., 1995, 1997; Zeller et al., 1976; Medalha and Mattioli, 2007), most research involving DA and fish has been in the area of reproduction and growth. However, the role of DA in reproduction is not limited to fish, as it is implicated in the reproduction of amphibians, birds, mammals including humans, and even crustaceans (Vidal et al., 2004).

In goldfish, the gonadotrophs located in the proximal pars distalis (Kah, 1986) are directly innervated by GnRH neurons originating in the anteroventral preoptic area and latero-basal hypothalamus (Ball, 1981; Peter and Paulencu, 1980; Kah et al., 1987). A surge of LH is responsible for ovulation (Stacey et al., 1979) and it is clear that ovulation is due in part to the disinhibition of DA (Anglade et al., 1991; Chang and Peter, 1983). Two main TH-immunoreactive (TH-ir) tracts were traced from preoptic cells to the infundibulum in the goldfish (Hornby et al., 1987). DA has been shown to inhibit LH release directly from the goldfish pituitary (Omeljaniuk et al., 1987) as well as GnRH-stimulated LH release (De Leeuw et al., 1989). Significantly, it is the only identified inhibitor of LH in this species (Trudeau, 1997; Peter et al., 1986). Several intracellular signaling pathways are involved in LH synthesis and release in gonadotrophs, and DA may act to inhibit several points in these signal transduction cascades (Van der Kraak et al., 1998), including inhibition of GnRH-induced Ca^{2+} mobilization, cAMP generation, and protein kinase C-dependent mechanisms (Chang et al., 1993; Chang and Jobin, 1994). DA has also been shown to down-regulate goldfish pituitary GnRH receptors (De Leeuw et al., 1989) and to inhibit the stimulatory actions of GABA (Fig. 1; for review see Trudeau, 1997; Trudeau et al., 2000). Levels of mRNA for the GABA synthetic enzyme glutamic acid decarboxylase 67 (GAD_{67}) have been shown to be up-regulated in goldfish telencephalon and optic tectum (Hibbert et al., 2004) by dopaminergic loss following MPTP injections, suggesting that DA inhibits the production of GABA. Furthermore, the tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine (αMPT), has also been shown to significantly deplete DA levels in the goldfish brain and pituitary (Trudeau et al., 1993a,b,c; Chang et al., 1985) and to potentiate the response of LH to GnRH (Peter et al., 1986). DA has recently been found to be implicated in maintaining the European eel, *Anguilla anguilla*, in a pre-pubertal state for many years (Vidal et al., 2004).

Table 1
Comparison of the D_1 and D_2 receptor classes in the goldfish (derived from reference Callier et al., 2003)

DA receptor class	D_1	D_2
Activity	Stimulate AC[a]	Inhibit AC; modulate VGCC[b] and VGKC[c]
Subtypes	D_{1A}/D_1, D_{1B}/D_5, D_{1C}	D_2, D_3, D_4
Third cytoplasmic loop	Short	Long
C-terminal	Long	Short
G protein	G_s/G_{olf} class of Gα proteins	G_i/G_o class of Gα proteins
Genomic DNA	Intronless	Several large introns

[a] AC = adenylate cyclase.
[b] VGCC = voltage-gated calcium channel.
[c] VGKC = voltage-gated potassium channel.

In order for DA to be such a potent inhibitor of LH secretion and reproduction, we propose that it must inhibit multiple LH–stimulatory systems. Various pharmacological manipulations reducing DA function also potentiate GABA-mediated LH release (Trudeau et al., 1993a,b,c). DA depletion increases GABA synthesis (Trudeau et al., 1993a,b,c) supporting our working hypothesis of reciprocal DAergic and GABAergic inhibition (Fig. 1) as an element in the control of LH release (see also Section 4). Moreover, this inhibition of DA synthesis by GABA is at least partially mediated by rapid effects to decrease the expression of TH (Martyniuk et al., 2007a,b). There are numerous other neurohormones that stimulate LH release in goldfish (Trudeau, 1997; Trudeau et al., 2000). Dopaminergic inhibition of these multiple other stimulatory signaling pathways is suspected but remains to be fully substantiated experimentally. There is, however, one example that serves to illustrate this point. Secretogranin-II (SGII) is a large secretory vesicle protein and well-characterized marker of the regulated secretory pathway that undergoes processing by prohormone convertases to produce the bioactive peptide secretoneurin (SN). SN is a 34 amino acid peptide that stimulates LH release in goldfish pretreated with a DA receptor antagonist (Blazquez et al., 1998a,b; Zhao et al., 2006), very much a corollary to the situation with GnRH-stimulated LH release at some periods of the reproductive cycle (Trudeau, 1997; Peter et al., 1986).

Dopamine exerts its effects via seven-transmembrane domain, G-protein-coupled receptors, which are separated into the D_1 and D_2 receptor classes (Kebabian and Calne, 1979). The two classes differ both structurally and functionally (Table 1; reviewed in Missale et al., 1998; Callier et al., 2003), as well as pharmacologically (reviewed in Seeman and Van Tol, 1994). The current hypothesis is that D_1 and D_2 receptors arose through convergent evolution and independently evolved the ability to bind DA (Callier et al., 2003).

Generally, the D_1 class of receptors stimulate cAMP production whereas the D_2 class of the receptors inhibit cAMP production (Kebabian and Calne, 1979), and also modulate the activity of voltage-gated calcium and potassium channels. The actions of dopamine receptors are achieved via the coupling of a G-protein.

The various G-proteins coupled to dopamine receptors have been reviewed (Sidhu and Niznik, 2000).

The D_1 class can be subdivided into the D_{1A}/D_1, D_{1B}/D_5, and D_{1C} receptor subtypes in fish (reviewed in Le Crom et al., 2003). The D_2 receptor class can be further categorized into the D_2, D_3, and D_4 subtypes. In mammals, two alternately spliced D_2 receptors have been identified as having short (D_{2S}) or long (D_{2L}) third cytoplasmic loops, differing by 29 amino acids (Monsma et al., 1989). There is currently no evidence that fish or amphibians contain an alternatively spliced variant of the D_2 receptor (Hirano et al., 1998; Levavi-Sivan et al., 2005; Macrae and Brenner, 1995; Martens et al., 1993; Vacher et al., 2003), suggesting this post-transcriptional modification occurred recently in the vertebrate lineage (Macrae and Brenner, 1995). Partial DNA sequences for DA receptors, DA transporter (DAT) as well as for the biosynthetic enzymes for DA and NE have been identified in goldfish (Table 2).

Many factors contribute to the development of DAergic neurons. This has been most studied in the zebrafish, and include regulation by numerous transcription factors and signaling pathways including the Orthopedia homeodomain (Ryu et al., 2007), neurogenin1 (Jeong et al., 2006), pax6 (Wullimann and Rink, 2001), tof/fezl (Levkowitz et al., 2003), as well as Shh, FGF8, Nodal/TGF, and retinoic acid (reviewed in Ryu et al., 2006). It was recently found that the D_2 receptors are expressed developmentally before the D_1 receptors in zebrafish (Li et al., 2007). In what has been termed D_1/D_2 synergism (LaHoste et al., 1993), some effects of dopamine action are observed, in mammals, only if both D_1 and D_2 receptors are stimulated concurrently (reviewed in Dziedzicka-Wasylewska, 2004). Further experimentation in fish is needed to establish if this occurs in lower vertebrates and whether this receptor synergism has a role to play in the control of reproduction.

Plasma membrane transporters modulate the action of neurotransmitters by neurotransmitter reuptake by the presynaptic axon and surrounding glial processes (Kimmel and Joyce, 2003). The DA receptors and transporter are the primary targets in the treatment of schizophrenia and Parkinson's disease, and, therefore have a rich assortment of effective pharmacological agents (for review see Missale et al., 1998; Seeman and Van Tol, 1994; Civelli et al., 1993; Chen and Reith, 2000; Uhl, 2003). Domperidone, a selective D_2 receptor antagonist, has been shown to decrease DA in the goldfish pituitary without affecting DA levels in the hypothalamus or telencephalon, as it does not cross the blood–brain barrier (Sloley et al., 1991). Quinpirole, a selective D_2 receptor agonist, has been shown to reduce the mRNA level of the tilapia GnRH receptor (Levavi-Sivan et al., 2004). Quinpirole and SKF 38393, a selective D_1 receptor agonist, which are both able to cross the blood–brain barrier, were used separately to examine their effects on gene expression in the hypothalamus of goldfish, compared with other neurotransmitter systems (Table 3; Fig. 4).

Table 2
Partial coding sequences for neurotransmitter receptors, transporters, biosynthetic and degradation enzymes recently cloned from the goldfish by our laboratory

System	Gene	Accession number
Dopamine	D_{1B}	EF377327
	D_{1C}	EF396233
	D_2	EF382625
	D_3	EF382624
	D_4	EF640988
	DAT	EF371919
	TH	AY644727
	DDC	EF371918
	DBH	EF396232
Serotonin	$5HTR_{1A}$	EF493019
	$5HTR_{2B}$	EF493017
	$5HTR_{2C}$	EF493018
	$5HTR_3$	EF490968
	$5HTR_5$	EF493015
	$5HTR_7$	EF493016
	SERT	EF490971
	TRPH1	EF490969
	TRPH2	EU003451
	MAO	EF490970
GABA	$GABA_{A\alpha1}$	AY640225
	$GABA_{A\beta2}$	AY640229
	$GABA_{A\beta4}$	AY635467
	$GABA_{A\gamma1}$	AY640226
	$GABA_{A\gamma2}$	AY640227
	$GABA_{B1}$	AY640228
	GAT1	AY640223
	GAT3	AY640224
	GABA-T	DQ287923
Glu	NR2A	EF645246
	NR2B	EF645245
Kisspeptin	KISS1R	EU622877

Note: the D_{1A} receptor had previously been cloned from the goldfish retina (Frail et al., 1993).

Studies using the goldfish have demonstrated that intraperitoneal (i.p.) administration of MPTP causes a selective depletion of DA and NE, without altering the serotonergic system (Goping et al., 1995; Poli et al., 1990; Pollard et al., 1992, 1996; Hibbert et al., 2004) and induces a parkinsonian syndrome which parallels that of mammals (Pollard et al., 1996). To act as a neurotoxin, MPTP must first be converted to MPP^+, its oxidized congener, by astrocytes, which is then selectively taken up by dopaminergic neurons (Snyder et al., 1986; Javitch and Snyder, 1984). MPP^+ accumulates in the mitochondria where it inhibits complex I of the respiratory chain leading to anoxia, anaerobic respiration, and cell death (Vyas et al., 1986; Singer et al., 1987). A light and electron microscopic study on the effect of MPTP on DAergic neurons in the goldfish brain showed progressive irregularities occurring on the contours of the

Table 3
Descriptions of the experiments used in the cluster analysis

Experiment	Mechanism	Date	GSI[a] (%)	Description	Exposure	Sex
MPTP + αMPT	DA depletion	Early-May	4.7 ± 0.6	i.p.-injected 50 μg/g MPTP on Day 0, i.p.-injected 240 μg/g αMPT on Day 5	24 h after final injection	Female
SKF 38393	D_1 agonist	Mid-May	4.5 ± 1.3	i.p-injected 40 μg/g	5 h	Female
Quinpirole	D_2 agonist	Mid-May	4.5 ± 1.3	i.p-injected 2 μg/g	5 h	Female
Fluoxetine	SSRI[b]	Mid-December	N.D.[c]	i.p.-injected 5 μg/g twice a week for 17 days, for a total of five injections	24 h after final injection	Female
Muscimol	$GABA_A$ agonist	Late-August	N.D.[d]	i.p.-injected with 1 μg/g	6 h	Female
Baclofen	$GABA_B$ agonist	Early-September	N.D.[d]	i.p.-injected with 10 μg/g	6 h	Female

[a] Gonadosomatic index.
[b] Selective serotonin reuptake inhibitor.
[c] Not determined; expected GSI for mid-December is approximately 2.5% (Kobayashi et al., 1986).
[d] Not determined; expected GSI for this time of year is approximately 2% (Kobayashi et al., 1986). A separate experiment conducted in mid-September confirmed a GSI for female goldfish of 2.1 (±0.1)% (unpublished).

neuronal nuclei and progressive granularization of nucleoplasm, suggesting an apoptotic mechanism of cell damage (Goping et al., 1995). These results led Pollard et al. (1993) to propose the goldfish as a model for drug discovery in Parkinson's disease research. While MPTP is an irreversible neurotoxin in mammals, including primates and humans (Burns et al., 1983; Heikkila et al., 1984; Langston et al., 1983), goldfish appear to completely recover to basal DA and NE levels after 6 weeks (Poli et al., 1992). The study was unable to determine whether catecholamine recovery was due to regenerative processes or by metabolic compensation of the surviving neurons. Nevertheless, these results are suggestive of a degree of plasticity in DAergic systems in the adult fish brain as is the case in the fish optic system (Braisted and Raymond, 1992; Matsukawa et al., 2004). We have used a similar MPTP-treated goldfish model, in combination with αMPT, to explore the effects of DA depletion on LH release and regulation of hypothalamic gene expression (see Section 7, Table 3 and Fig. 4).

Dopamine neurons may also mediate steroid negative feedback on LH release. The inhibitory action of DA on LH varies during the seasonal reproductive cycle in goldfish (Trudeau et al., 1993a,b,c). Reducing the DAergic inhibition of LH, for example, by blocking DA action through specific DA antagonists, has been shown to significantly enhance LH secretion in the goldfish, particularly in late ovarian recrudescence (Peter et al., 1986; Sloley et al., 1991; Sokolowska et al., 1985; Omeljaniuk et al., 1989) suggesting that the tone of DA inhibition increases in parallel with gonadal development and increased steroid secretion (reviewed by Trudeau, 1997). Indeed, testosterone and estradiol increase pituitary dopamine turnover rates, and the serum LH response to DA antagonist injections relative to sexually regressed fish without supplemental steroids (Trudeau et al., 1993a,b,c). While this has yet to be demonstrated conclusively, this is likely a direct effect of the sex steroids on DA neurons. A neuroanatomical study of the trout demonstrated that TH-positive DA neurons express the estrogen receptor-α (ERα) protein (Linard et al., 1996) whereas trout GnRH neurons do not express the ERα (Navas et al., 1995). In marked contrast, the positive feedback actions of the sex steroids do not appear to involve changes in DA function, but rather are a result of increased pituitary sensitivity to GnRH, and increased GABAergic activity (Trudeau et al., 1993a,b,c). The co-activation of both the positive and negative sex steroid feedback mechanisms during the seasonal reproductive cycle allows for dynamic and sensitive control of LH release and thus gonadal development and function (Trudeau, 1997; Blazquez et al., 1998a,b).

Clearly, DA serves a key inhibitory role in reproduction through multiple mechanisms, and is intricately controlled through the actions of other neurotransmitters (i.e. GABA) and sex steroids.

4. GABA plays multiple roles to stimulate LH release

GABA is considered to be the major inhibitory amino acid neurotransmitter in the vertebrate CNS. It is largely produced from precursor glutamate in a single step reaction by glutamic acid decarboxylase (isoforms GAD65 and GAD67) and is degraded by the enzyme GABA transaminase (GABA-T) into succinic semialdehyde. The molecular evolution of the GAD gene family has been described previously (Lariviere et al., 2002) and demonstrates that teleost fish, as in mammals, have both major GAD isoforms present, in addition to a novel GAD ortholog we have termed GAD3.

GABA stimulates LH release by stimulating GnRH and by inhibiting DA neurons in the telencephalon preoptic–hypothalamic (TEL-POA–HYP) region of the goldfish brain (Trudeau et al., 2000). This has been shown by increasing GABA levels with the irreversible inhibitor of GABA-T, γ-vinyl gamma (GVG), intraventricular injection of GABA, and intraperitoneal injections of GABA agonists. GABA-mediated LH release in likely an important physiological signal, since we have shown that blood testosterone levels in males also increases after GABAergic manipulations. Our previous differential display analysis indicated that pituitary SGII was up-regulated following GVG treatment (Blazquez et al., 1998a,b). Accompanying increases in pituitary SGII mRNA following GVG are increases in LHβ subunits mRNA levels (Trudeau et al., 2000). Thus, activation of endogenous GABAergic pathways leads to activation of secretion and transcription in the pituitary. Perhaps this is an example of 'stimulus-transcription coupling' as proposed by O'Connor's group who have observed this process *in vivo* in neuroendocrine cells in the mouse adrenal gland and brain (Mahata et al., 2003). Increases in water temperature stimulate spawning and increase both GABA synthesis and LH release (Fraser et al., 2002). We developed a novel method to study GABA mRNA levels and GABA synthesis rates in the same sample and determined that there are season-dependent sex differences in the effects of sex steroids on GABA synthesis (Bosma et al., 2001; Lariviere et al., 2005). Therefore, the GABAergic system transduces both external environmental and internal hormonal feedback signals to exert control over LH release.

The effects of GABA are mediated largely by two major membrane-bound GABA receptor classes. The pentameric ionotropic $GABA_A$ receptors conduct Cl^- and are composed of different subunits ($\alpha_{1\text{-}6}$, $\beta_{1\text{-}4}$, $\gamma_{1\text{-}3}$, δ, ε, θ, and π) of which the subunit stoichiometry influences receptor kinetics (reviewed in Mathers, 1991; Sieghart, 2006). In contrast to the ionotropic $GABA_A$ receptors, the dimeric G-protein coupled $GABA_B$ receptors are slower acting and responsible for prolonged GABAergic signaling through K^+ and Ca^{2+} channels (Bormann, 2000). Phylogenetic analysis of the $GABA_A$ receptor subunit family shows that there are fish homologs to mammalian $GABA_A$ receptor subunits (Martyniuk et al., 2007a,b). Due to genome duplication events in the teleost lineage, there appears to be multiple copies of $GABA_A$ receptor subunits and it is presently unclear whether these represent orthologous or paralogous pairs. It will be interesting to determine how or if the duplicated isoforms in fish alter $GABA_A$ receptor function and kinetics, and how they would contribute to neuroendocrine signaling. Genome searches also indicate the presence of $GABA_B$ receptors in fish. Patch-clamp electrophysiological studies indicate that both receptor subtypes are active in neuroendocrine cells in the goldfish telencephalon and hypothalamus (Trudeau et al., 2000). Moreover, injection of the $GABA_A$ agonist muscimol or the $GABA_B$ agonist baclofen, both stimulate LH release within 30 min (Martyniuk et al., 2007a,b).

The classical view of neurochemical communication is that the release of neurotransmitters at the synapse regulates the activity of post-synaptic neurons through specific, multiple membrane receptors and the resulting depolarization or hyperpolarizations affect intracellular signaling pathways to modulate the release of stored neurotransmitter on a millisecond scale. We have shown that GABA can regulate the expression of its own receptor subunits in brain over a 24 h period (Martyniuk et al., 2005). In goldfish, a single intraperitoneal injection of GVG increases levels of GABA approximately 3–4-fold in the neuroendocrine brain after 4 h and remains elevated after 24 h (Trudeau et al., 1993a,b,c). The significant elevation of GABA in the hypothalamus down-regulates the chicken and fish specific $GABA_A$ receptor β4 subunit mRNA while in the telencephalon, GABA decreases $GABA_A$ receptor β2 subunit mRNA approximately twofold after 24 h (Martyniuk et al., 2005). Thus, GABA appears to modulate genes involved in GABAergic synaptic transmission that we speculate would alter GABA receptor binding kinetics in fish.

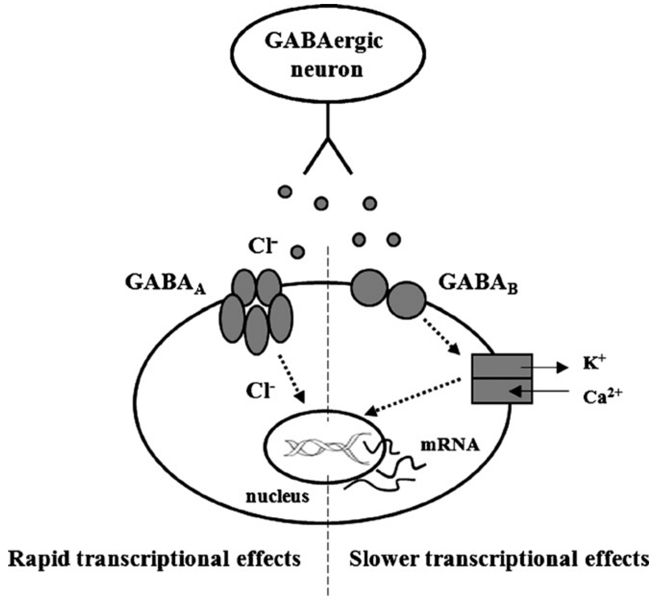

Fig. 2. Hypothesized post-synaptic dual effect of GABAergic signaling on gene expression in a neuron.

GABA appears to also regulate gene transcription in the vertebrate brain through its post-synaptic receptors as demonstrated with both *in vitro* and *in vivo* experiments. Female goldfish injected i.p. with muscimol showed a decrease in telencephalon GAD_{65} but not GAD_{67} abundance after 6 h. In contrast baclofen (10 μg/g body weight) significantly reduced GAD_{67} but not GAD_{65} levels in the hypothalamus (Martyniuk et al., 2007a,b). Both agonists reduced GABA-T expression. These data indicate that there are feedback mechanisms to finely regulate GABA levels in the neuroendocrine brain. Moreover, in the goldfish there are tissue differences in the expression levels of genes that are mediated through specific GABA receptors with differing signaling pathways (Fig. 2).

Using microarray analysis, Ghorbel et al. (2005) profiled the genomic effects of GABA in embryonic day 18-rat hippocampal neurons incubated with baclofen for 2 h. The authors demonstrated that cell signaling proteins, such as growth factors (e.g. brain derived neurotrophic factor), G-protein coupled receptors (e.g. β2 adrenergic receptor), and signaling molecules (e.g. MAP2K4) were modulated after baclofen stimulation. Other molecular pathways mediated by $GABA_B$ receptor activation included endocytosis, transcription and translation, intracellular transportation. The aforementioned studies demonstrate that GABA has multiple downstream genomic effects on, for example, transcription/translation, cell signaling, and neuroendocrine function. There is structural and electrophysiological evidence for kinetic and temporal differences between the $GABA_A$ and $GABA_B$ signaling pathways (Bormann, 2000) and it is hypothesized that temporal differences may also occur at the level of transcriptional regulation of gene expression (Fig. 2). Renier et al. (2007) have recently reported that GABA receptor modulators such as benzodiazepines, barbiturates, and baclofen have conserved effects in zebrafish when compared to mammals, raising the possibility that fish can be excellent pharmacogenomic models for the study of neurotransmitter and drug interactions in the vertebrate CNS. Despite such evidence that GABA regulates gene transcription in fish and mammals, defining the extent of this regulation is generally lacking. To address this, our group has studied the differential effects of GABA receptor agonists on gene expression in the hypothalamus that is detailed in Section 7.

Through its stimulatory action on GnRH neurons and inhibitory actions on the DA system, as well as its other functions outlined in this section, GABA plays multiple roles in the stimulation of LH release.

5. Serotonin stimulates LH release

The neurotransmitter serotonin (5HT) is synthesized from the amino acid tryptophan through decarboxylation and a rate-limiting hydroxylation step controlled by the enzyme tryptophan hydroxylase (Grahame-Smith, 1967). There are two isoforms of the enzyme in both mammals and fish, with tryptophan hydroxylase 2 being more abundantly expressed in the CNS (Bellipanni et al., 2002; Sakowski et al., 2006) and controlling brain 5HT synthesis (Zhang et al., 2004). Serotonin is degraded by monoamine oxidase, of which only one form exists in teleost fish (Anichtchik et al., 2006). In mammals, 5HT receptors are generally classified into seven subfamilies, based on molecular and pharmacological properties (Hoyer et al., 2002). Second messenger signaling is G-protein-dependent, with the exception of the $5HT_3$ receptor, which is ionotropic and controls Na^+ channels. Its activity has been shown, in human embryonic kidney cell culture, to be modulated by the ratio of subunits A and B in the pentameric ion channel (Hapfelmeier et al., 2003). Serotonin receptors belonging to family 1 and possibly 5 interact with G_i proteins, whereas 5HT receptors 4,6,7 interact with G_s proteins and 2 G_q proteins, respectively (Raymond et al., 2001). We have identified some of the corresponding partial coding sequences of the enzymes, transporter and receptors in goldfish (Table 2) that show a relatively high sequence similarity to other vertebrates, providing additional evidence for a conservation of the serotonergic system that has been suggested elsewhere (Hen, 1993).

Several 5HT receptor subtypes have recently been identified to be involved in GnRH and LH release. For example, 5HT receptor subtypes 2C, 4 and 7 have been shown to mediate stimulatory effects of 5HT on GnRH release from the immortalized mouse neuronal cell line GT1–7, while 5HT receptor 1A has been shown to have inhibitory effects on GnRH release in the same system (Wada et al., 2006). Stimulation occurs through the adenylate cyclase (Wada et al., 2006) and the phospholipase C (PLC) (Wada et al., 2006; Kim et al., 2006) pathways. Activation of the ionotropic receptor $5HT_{3A}$ has been shown to increase LHβ mRNA expression in a rat pituitary *in vitro* (Quirk and Siegel, 2005). This represents a possible mechanism for rapid modulation at the level of the pituitary.

Only a $5HT_2$-like receptor is known to mediate stimulatory effects on LH release in goldfish (Somoza and Peter, 1991) and Atlantic croaker (Khan and Thomas, 1992) at either the GnRH cell body or nerve terminal (Yu et al., 1991). This also appears to be the case for the red seabream (Senthilkumaran et al., 2001). Based on the selectivity of the $5HT_2$ antagonist ketanserin used in these studies, it is possible that this receptor has properties that are similar to the mammalian $5HT_{2A}$ and $5HT_{2C}$ type, as ketanserin has low nanomolar affinities for both the $5HT_{2C}$ and the $5HT_{2A}$ receptor (Fiorella et al., 1995). Interestingly, an increase in ketanserin binding has been observed in the hypothalamus of sexually mature rainbow trout when compared to juveniles, suggesting an involvement of $5HT_2$-type receptors in reproduction (Agrawal and Omeljaniuk, 2000). This correlates well with our own analysis of seasonal gene expression of the goldfish brain $5HT_{2C}$ receptor, where the highest levels were detected in the reproductive phase (unpublished data). Additionally, 5HT inhibits GH release in goldfish (Somoza and Peter, 1991). Changes in brain receptor levels may therefore contribute to the seasonal program of reproduction followed by growth (Marchant and Peter, 1986; Tecott and Abdallah, 2003).

Little more is known about the mechanism of action of 5HT to stimulate LH release in fish; however, our initial gene expression analysis of the effects of the selective serotonin reuptake inhibitor, fluoxetine, suggests that other pathways are likely to be involved (see Section 7).

6. Kisspeptin/GPR54 in fish

The recent discovery and characterization of the kisspeptin (kiss1) system in mammals (see Kauffman et al., 2007 for review) led to the cloning of kiss1 in zebrafish (van Aerle et al., 2008) and medaka (Kanda et al., 2008). Potential kiss1 gene loci were also identified in tetraodon, fugu, and sea lamprey (van Aerle et al., 2008). The kiss1 sequence is conserved only in its active decapeptide site (80–100%) (van Aerle et al., 2008), while other parts of the gene is poorly conserved (32%) (Kanda et al., 2008). This poor conservation is also reflected in the organization of the zebrafish kisspeptin gene which has 2 exons, whereas medaka possesses 3 exons (van Aerle et al., 2008; Kanda et al., 2008). In both fish species, kisspeptin was found to be mainly expressed in brain, intestine and testis, with no expression in the ovary. Further investigation of kisspeptin expression distribution in the brain of medaka revealed two distinct populations in the nucleus ventral tuberis (NVT) and nucleus posterioris periventricularis (Npp) paralleling findings in mammals (Kauffman et al., 2007). The expression of kiss1 in the NVT is sexually dimorphic, favouring male fish. Injection of mammalian kisspeptin in the fathead minnow resulted in changes of ERα, aromatase, GnRH3 and the kisspeptin receptor (Kiss1r; also known as G-protein-coupled receptor 54; GPR54) (Filby et al., 2008).

Kiss1r is more highly conserved than its ligand and has been cloned in several fish (Mohamed et al., 2007; Nocillado et al., 2007), including goldfish (Table 2). It is expressed in brain and ovary in fathead minnow (Filby et al., 2008) and in tilapia its expression level is negatively regulated by continuous light exposure (Martinez-Chavez et al., 2008). Tilapia GnRH1, GnRH2 and GnRH3 neurons express GPR54 (Parhar et al., 2004). Studies on the role of kisspeptin, however, are generally lacking in fish and its relationship to known inhibitory (DA) and stimulatory (GABA, 5-HT) pathways outlined in this review warrants investigation.

7. Determining potential genomic interactions and pathways regulated by DA, GABA and 5HT

From multiple microarray experiments, we collected raw data (Table 3) and performed unsupervised, average-linkage hierarchical clustering (Eisen et al., 1998) to investigate the interactions of different neurotransmitter systems on gene expression in the female goldfish hypothalamus (Fig. 4; see caption for method). Clustering was performed in two ways. First, by clustering genes across all of the treatments, co-expressed groups can be delineated, many of whom share common functional properties or participate in coherent biological pathways or processes. Secondly, by clustering between treatments it is possible to use the extraordinarily rich datasets from the array to identify which treatments are most closely related to other treatments in terms of the overall gene expression profile. Thus, it may be possible to identify shared regulatory sequences by bioinformatic methods such as Gibbs sampler (Xia, 2007) in the sets of co-upregulated or co-downregulated genes.

Somewhat surprisingly, the quinpirole, fluoxetine, and baclofen experiments clustered together, suggesting similarities in the effects of these drugs on gene expression in the hypothalamus. However, these three pharmaceuticals all target G-protein-coupled receptors either directly (quinpirole and baclofen) or indirectly (fluoxetine). An additional unexpected result was that the muscimol

Table 4
Isotocin mRNA expression following various neurotransmitter system modulations in the hypothalamus of female goldfish as determined using cDNA microarray

Experiment	Effect on mRNA
MPTP + αMPT (DA depletion)	Up
SKF 38393 (D_1 agonist)	Up
Quinpirole (D_2 agonist)	Down
Fluoxetine (SSRI)	Down
Muscimol ($GABA_A$ agonist)	Up
Baclofen ($GABA_B$ agonist)	Down

experiment clustered with the catecholamine depletion experiment (MPTP + αMPT). We speculate that this is indicative of the inhibitory effects that GABA has on both DA (Fig. 1) and NE systems in goldfish brain (Trudeau et al., 1993a,b,c). The D_1 agonist experiment (SKF 38393) did not cluster with any of the other experiments, reflecting a distinct pharmacological profile and differential distribution of D_1 versus D_2 receptors in the brain. These data demonstrate that the carp-goldfish microarray (Martyniuk et al., 2006) coupled with cluster analysis can distinguish between pharmacological manipulations. There are genes both commonly and differentially regulated by these treatments (Figs. 5 and 6).

While it is not our intention to describe in detail all the results of the cluster analysis, there are several interesting transcripts that warrant further discussion here. Modulation of the DA, GABA and 5HT systems all lead to effects on isotocin gene expression (Table 4). This is intriguing since isotocin is a reproductive neuropeptide and the fish homolog of mammalian oxytocin (see Section 8).

As GABA has multiple roles in the stimulation of LH, the effects of baclofen also warrants further analysis. Firstly, using PCR analysis, we previously demonstrated that baclofen but not muscimol stimulated the expression of activin βa in goldfish hypothalamus (Martyniuk et al., 2007a,b). This was confirmed here in our microarray experiment. Gene Ontology (GO) classification of the response to baclofen was possible because there were sufficient known genes (128 in total) that could be identified with confidence (Table 5). Our data suggests that within the period of the reproductive cycle examined, baclofen regulated a larger number of transcripts than muscimol, perhaps reflecting the mode of action of these GABA agonists. It is clear that activation of the $GABA_B$ receptor leads to changes in the expression of genes involved in multiple processes. Activin, for example, falls under both the hormone activity and receptor binding GO categories. In addition, the genes regulated by GABA agonists grouped by GO term for cellular components reveals similarities and differences in types of transcripts regulated (Fig. 3). Hypothalamic transcripts that code for proteins largely located in the extracellular region are both induced and decreased after GABA agonist treatment. Some examples include granulin (decreased by baclofen), isotocin (induced by muscimol) and lipid mobilization and transport proteins such as apolipoprotein *ApoA4* (increased) and *ApoE* (decreased) by baclofen in the hypothalamus. Interestingly, hypothalamic genes coding for proteins involved in transcription factor complexes were induced by baclofen but not muscimol. Examples include heat shock factor 2 and myocyte-specific enhancer factor 2A, two genes that are involved in transcription anti-termination as categorized further by GO biological process categories. In addition, genes encoding proteins located in the nucleus such as histone 2A and a non-histone protein high mobility group box 1 are regulated by baclofen. We hypothesize that baclofen may have prolonged downstream effects on the transcriptome by increasing the expression of the molecular machinery involved in regulating transcription. However, additional studies are needed to ascertain whether prolonged transcriptional effects are detectable after treatments with pharmacological agents specific for neurotransmitter receptors. Isotocin

Table 5
Enrichment analysis of functional category for differentially expressed genes in the goldfish hypothalamus exposed to baclofen

GO Term_Biology Process_4	Count	%	*P*-value	Benjamini
Alcohol metabolism	7	5.4	0.008	0.99
Negative regulation of organismal physiological process	3	2.3	0.009	0.92
Lipid transport	4	3.1	0.009	0.83
Organelle organization and biogenesis	11	8.5	0.030	0.99
Organic acid metabolism	8	6.2	0.030	0.97
Macromolecule catabolism	7	5.4	0.032	0.95
Lipid metabolism	9	6.9	0.034	0.94
GO Term _Molecular Function_3				
Hormone activity	5	3.8	0.007	0.90
Coenzyme binding	4	3.1	0.022	0.98
Receptor binding	10	7.7	0.024	0.94

DAVID programs (http://david.abcc.ncifcrf.gov/home.jsp) were used to screen GO terms for biological process at 4th level and GO terms for molecular function at 3rd level. 128 genes totally were analyzed. Only categories over three genes, scoring a statistically significant EASE score (<0.05) for over-representation are shown. Count = gene numbers; % = percentage of over-represented gene.

and activin will be discussed further in Sections 8 and 9, respectively.

8. Isotocin is a reproductive neuropeptide regulated by multiple neurotransmitter systems

Isotocin (IST) is a nine amino acid neuropeptide synthesized in magnocellular and parvocellular neurons of the preoptic area, which project widely in the brain, especially in the telencephalon, hypothalamus and to the neural lobe of the pituitary (Saito et al., 2004). It is the teleost homologue of mammalian oxytocin (OXT) and has roles in a variety of physiological functions, for example, ion homeostasis at the level of the gill (Guibbolini and Avella, 2003; Kleszczynska et al., 2006). Importantly, there is accumulating evidence for its role in fish reproduction. Pickford and Strecker (1977) first described the stimulating effect of injected IST on spawning in

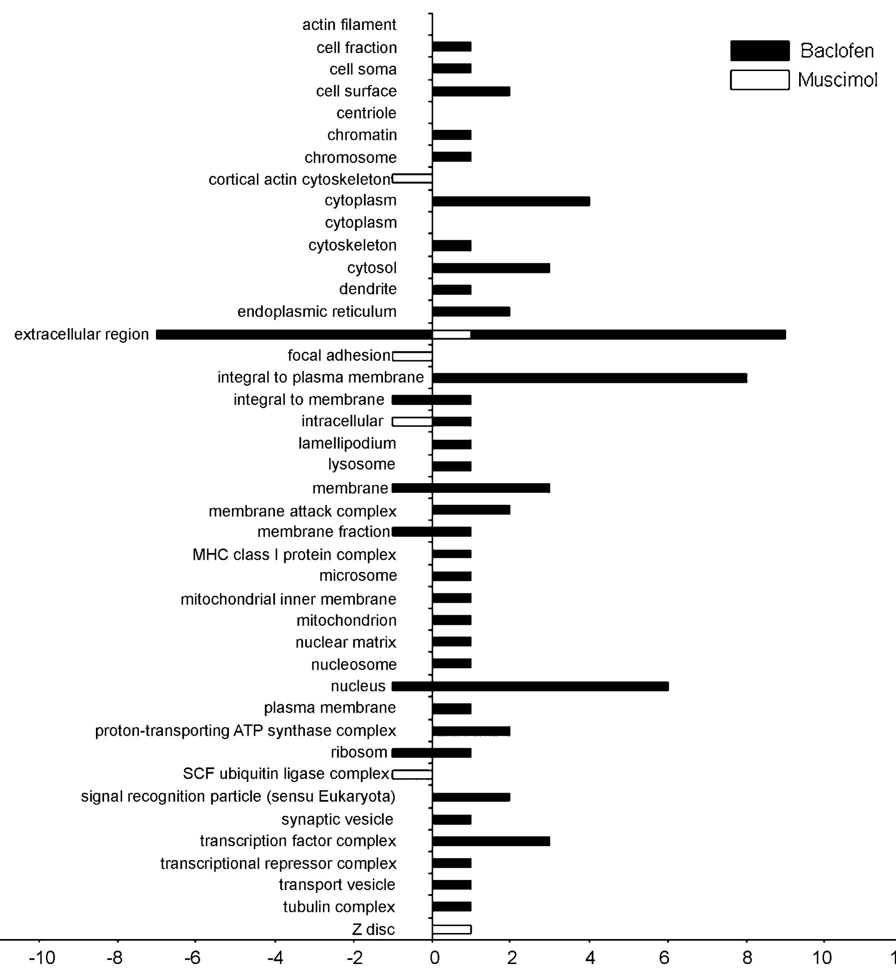

Fig. 3. Number of transcripts categorized into cellular component (GO terms) showing differential expression after baclofen and muscimol after microarray analysis in the hypothalamus. Note that transcripts that code for proteins found in extracellular regions are both induced and decreased after GABA agonist treatment whereas transcripts that code for proteins found in transcription factor complexes are largely induced by baclofen treatment. Transcripts that code for proteins with a role in signal recognition are regulated by baclofen alone.

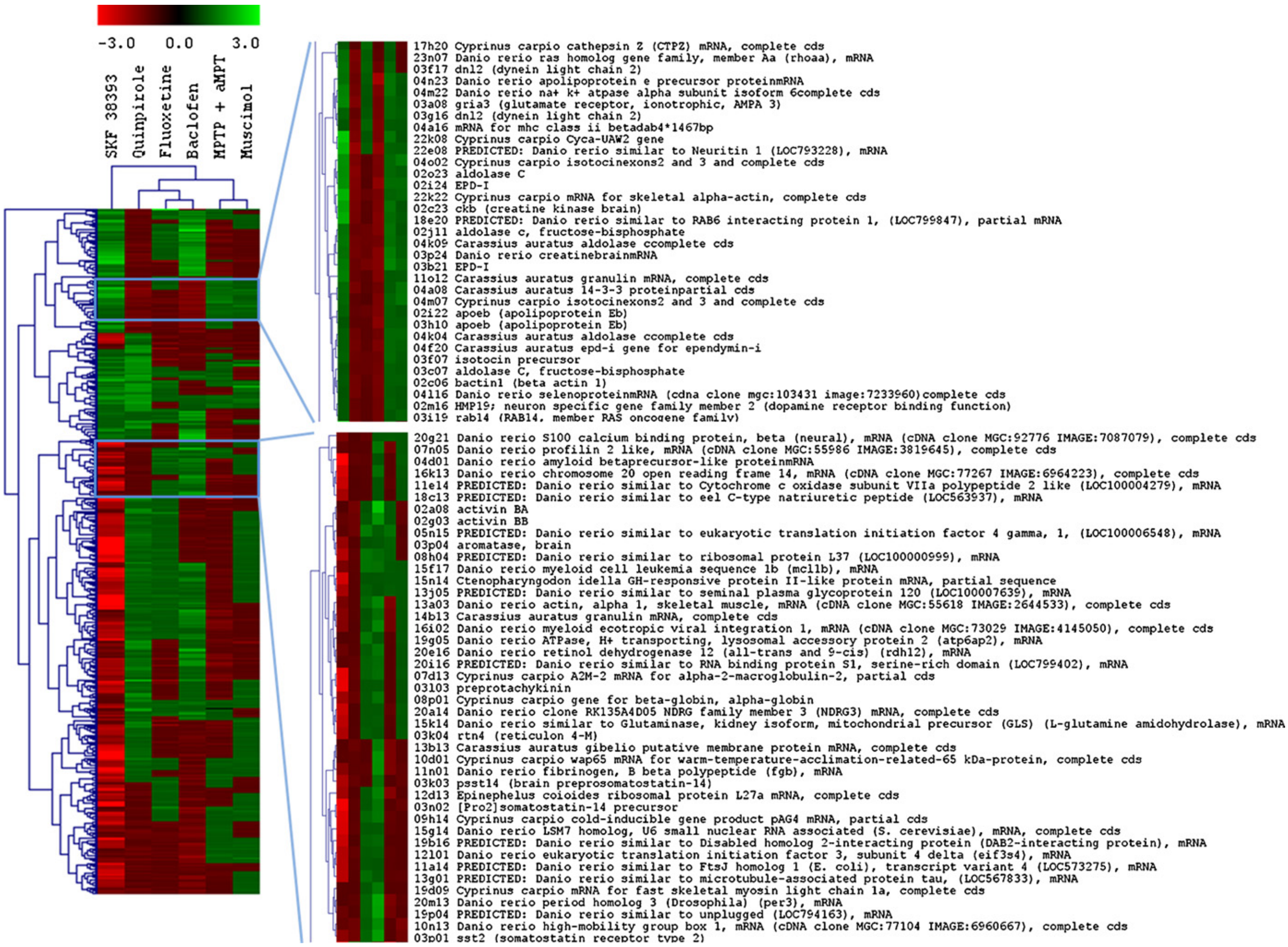

Fig. 4. Microarray data was retrieved from six different studies (Table 3) subjected to goldfish brain cDNA microarray. The microarrays of experiment were hybridized using the common reference design (Yang and Speed, 2002). For each study, signals were normalized using a locally weighted scatterplot-smoothing regression (Lowess; Yang et al., 2002) to remove intensity dependent noise. Modified t tests with q values (false-discovery rate) management were used for statistical analysis. The sets of cDNAs were ranked based on *q* values and FDR threshold for the identification of differentially expressed genes was set to 0.05 (Tusher et al., 2001). 1932 cDNA genes were selected with differential expression at least in one study (*q* values < 0.05 and fold-change > 1.5), and were further reduced in number by removing unclassified and/or unannotated ESTs (1415). The resultant 517 annotated genes were used to perform unsupervised, average-linkage hierarchical clustering (Eisen et al., 1998). The complete image is included in Supplementary Material.

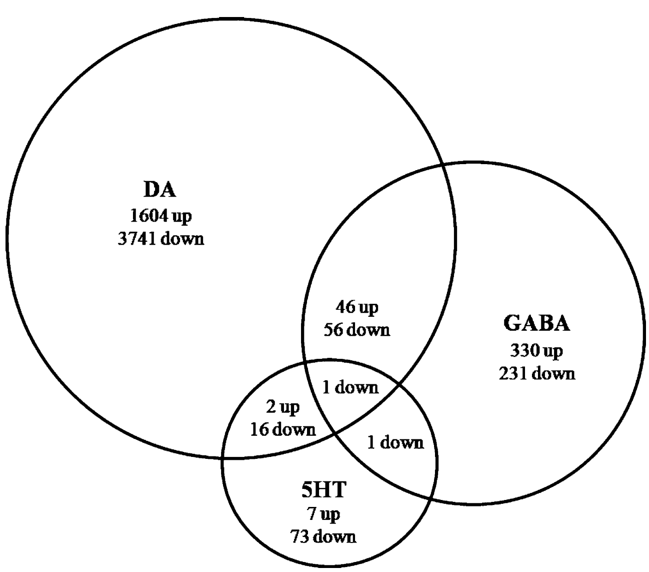

Fig. 5. Number of mRNAs commonly regulated.

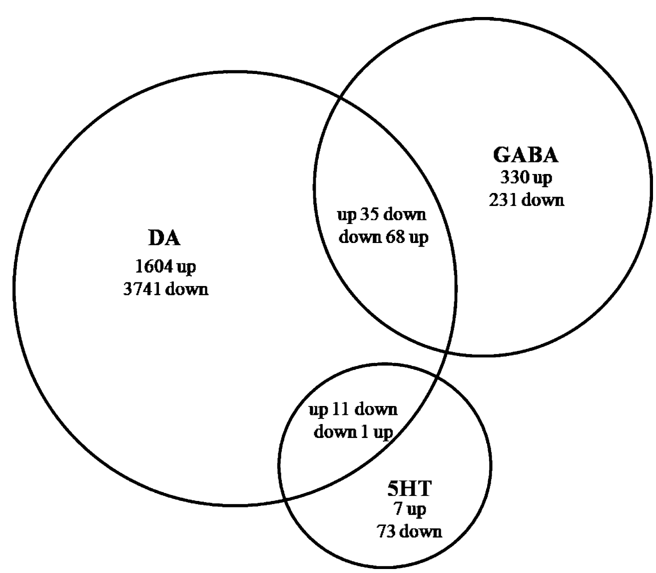

Fig. 6. Number of mRNAs differentially regulated.

killifish. Circulating IST levels have been found to vary seasonally in female three-spined sicklebacks, with the highest levels occurring in July, the period of reproduction for this long-day breeder (Gozdowska et al., 2006). A similar peak was found in brain IST mRNA expression in female masu salmon, in May, correlating with high estradiol levels (Ota et al., 1999). The IST gene has been shown to be estrogen-responsive, in that it possesses three estrogen response elements (ERE) in teleosts (Venkatesh and Brenner, 1995). The number of IST-immunorective neurons was found to be sexually dimorphic in medaka and decrease significantly after spawning in females (Ohya and Hayashi, 2006). Some preoptic neurons in the dwarf gourami, *Colisa lalia*, stained positively for both IST and GnRH (Maejima et al., 1994), further supporting a role of IST in reproduction. In rainbow trout, both chicken GnRH-II and salmon GnRH immunopositive nerve-fibers have been found to be in close proximity to IST neurons and application of either GnRH form generated synchronous Ca^{2+} pulses in IST neurons (Saito et al., 2004). Isotocin is also involved in sex-specific vocal courting behaviours in plainfin midshipman fish, *Porichthys notatus* (Goodson and Bass, 2000), and changes during socially induced sex-change in blue banded goby, *Lythrypnus dalli* (Black et al., 2004). Effects of OXT on LH (Evans, 1996; Rettori et al., 1997; Robinson et al., 1992) and steroid (Wuttke et al., 1998; Chandrasekher and Fortune, 1990; Fortune and Voss, 1993) release have been described for mammalian models *in vivo* and *in vitro*. Similarly, in rainbow trout, IST has been found to stimulate testosterone release at the level of the testis *in vitro* (Rodriguez and Specker, 1991). Furthermore, a stimulatory effect of mammalian OXT on sperm release has been shown in the African catfish (Viveiros et al., 2003). The IST receptor mRNA has been quantified in different tissues of the reproductive axis in the white sucker, *Catostomus commersoni*, for example, brain and ovary (Hausmann et al., 1995), further substantiating its status as a reproductive neuropeptide.

MPTP, in combination with αMPT, reduced DA and NE levels by 70% in telencephalon and by 80% and 87%, respectively, in the hypothalamus of goldfish, relative to saline-injected controls as determined by high-performance liquid chromatography (Popesku et al., unpublished). This was associated with a statistically significant ($p < 0.05$) 1.8-fold decrease and a 1.3-fold increase of IST in the telencephalon and hypothalamus, respectively, as determined by real-time RT-PCR (unpublished). This result in the hypothalamus was confirmed in our microarray experiment (Fig. 4, Table 4). The differences in the IST response in these tissues may possibly be due to dopaminergic action through different receptor classes in the hypothalamus compared to the telencephalon.

The other neurotransmitter-active drugs studied in this meta-analysis also had effects on IST. A 2-week fluoxetine treatment resulted in a fivefold decrease of IST mRNA in the hypothalamus (Mennigen et al., unpublished). The $GABA_B$ receptor agonist, baclofen, had an inhibitory effect on IST (down ∼1.5-fold) whereas the $GABA_A$ receptor agonist, muscimol, had a stimulatory effect on IST (up ∼1.3-fold) (Fig. 4). It is unclear at this time whether, or which, neurotransmitter(s) are having direct effects on IST release. However, electrophysiological studies in the rat support the hypothesis that DA neurons directly innervate OXT neurons as they express functional D_2 receptors (Yang et al., 1991), and TH-ir fibres are present in the anterior part of the parvocellular preoptic nucleus of the zebrafish brain (Kaslin and Panula, 2001). Furthermore, *in situ* hybridization studies in the rainbow trout revealed a strong signal for of the D_2 receptor mRNA in the parvocellular preoptic area, but no signal was found in the magnocellular preoptic area (Vacher et al., 2003). As no 5HT-ir fibres were found in close proximity of the magnocellular or parvocellular preoptic areas of the zebrafish (Kaslin and Panula, 2001), the effects of 5HT on IST mRNA expression are hypothesized to be indirect, potentially by modulation of DAergic neurons. There is currently no evidence of the co-localization of GABA receptors on IST neurons in fish; however there is strong evidence for direct GABAergic regulation of OXT neurons in the rat (Pittman et al., 1998). It is speculated that GABA in goldfish may be affecting IST mRNA levels both directly and indirectly by modulating DAergic neurons (Fig. 1).

9. Activins are growth factors implicated in neuroendocrine control and neuronal repair

The activins, members of the transforming growth factor β (TGF-β) superfamily (Massague, 1987), are homo- or heterodimeric proteins. They are composed of 2 βA subunits (activin A), 2 βB subunits (activin B) or 1 βA and 1 βB subunit (activin AB). Activins were originally isolated because of their abilities to stimulate FSH release from the mammalian pituitary. In goldfish, activin B stimulates FSHβ mRNA expression while inhibiting LHβ mRNA expression *in vitro* (Yam et al., 1999). Furthermore, the activin binding protein follistatin was found to have the opposite effect; it inhibits FSHβ while stimulating LHβ expression in the goldfish pituitary (Yuen and Ge, 2004). Activin βA, βB, as well as activin receptor subunits IB and IIB are also expressed in the goldfish brain (Lau and Ge, 2005). Activin, once bound to the Type II receptor, forms a complex along with the Type I receptor which activates the Smad family of transcription factors (Shi and Massague, 2003). Full-length cDNAs for Smads2 and 3 (regulatory, or R-Smads), Smad4 (co-Smad), as well as Smad7 (an inhibitory, or I-Smad) have recently been cloned in goldfish (Lau and Ge, 2005). Although the authors focused on these transcription factors in goldfish pituitary, it is interesting to note that they are also expressed in the brain. In rat brain, activins and their receptors are expressed in neurons (Tretter et al., 2000).

In the present analysis, activin βA was stimulated by the $GABA_B$ agonist baclofen but not by the $GABA_A$ agonist muscimol (Martyniuk et al., 2007a,b). What the effect of activins and the role GABA-mediated increases in activin may have on the fish brain are a matter of speculation but are likely to be involved in LH release by enhancing GnRH production in the brain (Martyniuk et al., 2007a,b; Ge et al., 1992; Gregory and Kaiser, 2004).

The activins are implicated in many processes, including development, reproduction, behaviour, neuronal stem cell differentiation and neuronal repair (Tretter et al., 2000; Hughes et al., 1999; Ma et al., 2005; Satoh et al., 2000). Given that GABA is a major neurotransmitter and is implicated in many of the same functions, it may be that $GABA_B$ receptor mediated stimulation of activin βA expression is also important for maintenance of neuronal function in addition to a role in neuroendocrine signaling in the adult brain.

10. Conclusions

Endocrine systems regulate reproduction, development and growth, and exist for the purpose of the organism to propagate their genomes. Model systems are essential to the advancement of biology and the goldfish has featured prominently in numerous fundamental discoveries concerning neuroendocrine regulation of reproduction. The central role of DA as the potent inhibitor of LH release, and GnRH and a multitude of other stimulatory neurohormones has helped to advance the concept of inhibitory–stimulatory duality of control mechanisms in the brain and pituitary. Part of the utility of the model resulted from the pioneering work of R.E. Peter and his brain atlas (Peter and Gill, 1975) and classic electrolytic lesioning studies delineating the brain regions important for neuroendocrine signalling (Peter et al., 1986). Many reagents are now available, including recombinant hormones and cytokines and there are numerous fields employing the goldfish model.

Recently, we initiated an expressed sequence tag (EST) sequencing project to address one of the main limitations of the goldfish model: a lack of gene sequence information. Increasing availability of ESTs for goldfish, together with the ∼40 K ESTs available for the common carp, *Cyprinus carpio* (Cossins, unpublished sequences and Gracey et al., 2004) and the recent development of a goldfish bacterial artificial chromosome library (Luo et al., 2006) is beginning to address this limitation. Similar efforts for genes in the goldfish immune system are underway (Barreda et al., 2004). We have also demonstrated the utility of microarray analysis and associated bioinformatics for research on neuroendocrine signaling. With these techniques, we have found that multiple genes involved in reproduction are differentially regulated by various neurotransmitter systems in the brain, and have begun to elucidate the complexity of the neural pathways involved in reproduction. It will soon be possible to study the concept of neuroendocrine–immune interactions in fish (Hanington et al., 2006; Barreda et al., 2004; Metz et al., 2006). Moreover, there are numerous other gut, pituitary and brain peptide genes being sequenced, and certainly the goldfish model will continue to contribute significantly to our understanding of neuroendocrine control of growth and feeding (Volkoff et al., 2005; Matsuda et al., 2008).

Highly complementary resources are available for the common carp (carpBASE; http://legr.liv.ac.uk), a closely related species. While zebrafish are without doubt excellent models for developmental biology and toxicology, they remain a challenging organism for basic physiological studies because of their small size and lack of pituitary hormone assays. Thus physiological genomic analysis of reproduction is somewhat limited in zebrafish. Importantly, however, the vast resources for zebrafish as well as *Fugu* and medaka models make it relatively easy to categorize goldfish ESTs using comparative genomic approaches. Meta-analysis of expression data from numerous neuropharmacological experiments in goldfish helps to focus attention on key neuroendocrine systems, for example IST (Goodson and Bass, 2000) and activins (Yam et al., 1999), whose functions in the teleost brain are only partially characterized. What we have discovered is that hundreds of goldfish ESTs derived from brain cannot be found in the other fish genomes. The 1415 unidentified ESTs derived from both goldfish and carp that respond to neuroendocrine manipulations in the goldfish brain may be derived from the highly divergent untranslated regions of mRNAs already isolated. Others may be unique sequences from coding regions that we cannot yet characterize, representing new genes. They may also represent non-protein coding transcriptional products that participate more widely in biological regulation through RNA/RNA, RNA/DNA and RNA/protein interactions. Together these uncharacterized products provide rich biological material for future analysis of neuroendocrine signaling in the vertebrate brain.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mce.2008.06.017.

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