CHAPTER

3

Hormones and Social Behaviour of Teleost Fish

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INTRODUCTION: A SHORT BRIEFING ON THE NEUROENDOCRINE SYSTEM OF TELEOSTS

Hormones and neuroendocrine regulatory mechanisms have been highly conserved across vertebrates, making fish good model species for studies on behavioural neuroendocrinology (e.g., Oliveira *et al.*, 2005b). In teleosts, as in other vertebrates, the neuroendocrine system is organized in a hierarchical fashion with the hypothalamus controlling the activity of the anterior pituitary gland that, in turn, controls the functioning of the numerous peripheral endocrine glands (gonads, anterior kidney, etc.; see Fig. 3.1). As in other vertebrates, the fish pituitary gland consists of two types of tissue, the adenohypophysis and neurohypophysis, and the secretion of the adenohypophysial hormones is under the control of releasing factors produced by hypothalamic neurons (Schreibman, 1986;

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Fig. 3.1). However, unlike other vertebrates, teleosts lack the hypothalamic-hypophysial portal vascular system, which is used in other vertebrates to pass the releasing factors from the hypothalamus to the pituitary, and the adenohypophysis receives direct innervation from the hypothalamus (Peter, 1990). Therefore, in teleosts, the relevant releasing factors controlling hypophysial function are still produced in neurosecretory hypothalamic neurons that project to the pars distalis of the adenohypophysis, thus making the hypothalamo-hypophysial axis. Within this axis, specific hypothalamic releasing hormones [e.g., gonadotropin-releasing hormone (GnRH), corticotropin-releasing hormone (CRH), growth-hormone-releasing hormone (GHRH)] control the release of specific trophic hormones [e.g., the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH), adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), growth hormone (GH)] produced by different populations of trophic hormone producing cells in the adenohypophysis (e.g., gonadotropes, corticotropes, thyrotropes, somatotropes). On the other hand, the neurohypophysis receives neural projections from the magnocellular neurons of the preoptic area, which end in a capillary network, where the neurohormones produced by these neurons are released into the bloodstream (Fig. 3.1). All of these vertebrate neurohormones known to date belong to one of two major neurohypophysial hormone families: vasopressin-like peptides and oxytocin-like peptides. In teleosts they are, respectively, arginine-vasotocin and isotocin (Urano *et al.*, 1994).

Of all the hormones mentioned above, few have received detailed attention in respect to their role in the expression of social behaviour. Most studies on teleost behavioural neuroendocrinology have focused on steroids (i.e., androgens, estrogens, progestins and glucocorticoids) and on peptides (mainly on the neuropeptides GnRH and AVT and on the prolactin family peptides) and, as a consequence, they will play a major role in this review. In the next section, we present a brief summary of the main behaviourally relevant hormones in teleost fish.

Steroids

Steroid hormones are mainly produced in the gonads and in the interrenal tissue (homologous to the adrenal cortex in tetrapods) and are classified into four major groups: progestins, corticoids, androgens and estrogens. In teleosts, progestins—named after their progestational role

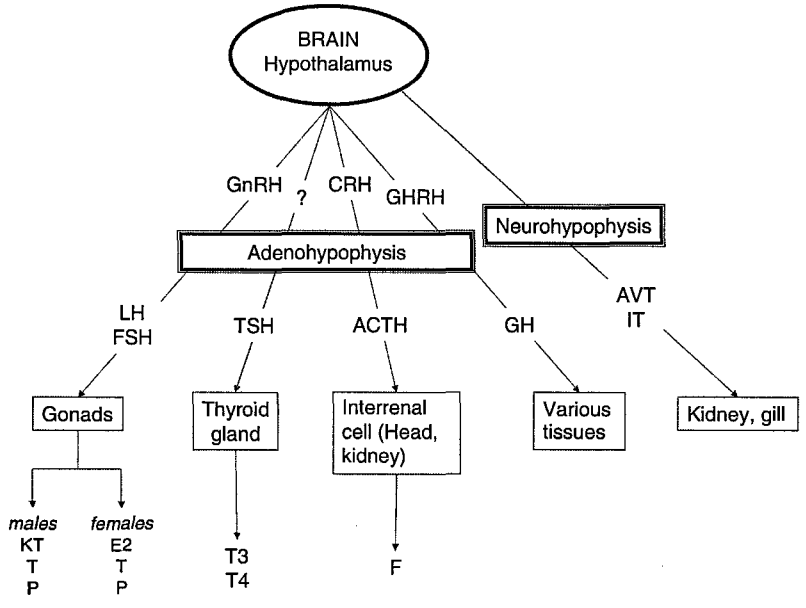

Fig. 3.1 Hierarchical organization of the neuroendocrine system with major hypothalamic-hypophysial axis for the control of peripheral glands and tissues represented. Abbreviations: GnRH = gonadotropin-releasing hormone; ? = unknown; CRH = corticotropin-releasing hormone; GHRH = growth hormone-releasing hormone; LH = luteinizing hormone; FSH = follicle stimulating hormone; TSH = thyroid stimulating hormone; ACTH = adrenocorticotropic hormone; GH = growth hormone; AVT = arginine-vasotocin; IT = isotocin; KT = 11-ketotestosterone; T = testosterone; P = progestogen; E2 = estradiol; T3 = triiodothyronine; T4 = thyroxine.

in mammals—play a major role in the final maturation of oocytes in females (Nagahama *et al.*, 1994) and in spermiation in males (Miura *et al.*, 1991, 1992; Miura and Miura, 2003). Two main maturation-inducing progestins have been identified among fishes: 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP; e.g., salmonids, catfishes, Nagahama *et al.*, 1994) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-P; e.g., Atlantic croacker, *Micropogonias undulates*, Trant and Thomas, 1989; spotted seatrout, *Cynoscion nebulosus*, Thomas and Trant, 1989; Lusitanean toadfish, *Halobatrachus dydactylus*, Modesto and Canário, 2002). In fish, cortisol represents over 80% of circulating corticoids (corticosterone and 11-deoxycorticosterone are also present in lower concentrations, Wendelaar Bonga, 1997), and mediates both glucocorticoid and mineralocorticoid responses (Mommsen *et al.*, 1999;

Baker, 2003). Contrary to mammals and birds, in which the typical androgens are testosterone (T) and dihydrotestosterone (DHT), in teleosts T and DHT are also present but the androgen with the highest biological activity is 11-ketotestosterone (KT), a non-aromatizable androgen (Borg, 1994). In male teleosts, the androgens most commonly found in circulation are T, 11-alfa-hydroxy-testosterone (11-OHT) and KT (Kime, 1993). Usually, there is a marked sex difference in circulating sex steroids, with very low or undetectable plasma concentrations of estradiol (E2) and high levels of KT in males and the reverse pattern in females (Borg, 1994). Interestingly, T circulating levels do not exhibit this sex difference, and in a number of species—especially during gonadal recrudescence—females display higher plasma concentrations of T than males (Borg, 1994). Similarly, in species with male alternative reproductive tactics, territorial males have higher levels of KT than sneaker males but no pattern was found for T (Oliveira, 2006). The function of androgens in males is related to different aspects of reproduction, namely gonadal differentiation (Strüssman and Nakamura, 2002), the regulation of spermatogenesis and spermiation (Schulz and Miura, 2002), the expression of secondary sex characters (Liley and Stacey, 1983; Borg, 1994) and the expression of reproductive behaviour (Liley and Stacey, 1983; Borg, 1994). In females, estrogens—in particular E2—stimulate vitellogenesis (Patiño and Sullivan, 2002) and regulate LH secretion (Trudeau, 1997).

The observation that both male and female reproductive behaviour can still take place in gonadectomized animals, often without significant qualitative or quantitative differences for intact fish, suggests that gonadal androgens are not essential for the expression of reproductive behaviours. More likely, sex steroids act as modulators of the neural pathways controlling reproductive behaviour, increasing or decreasing the motivation so as to exhibit the behaviour. Two neuropeptides—gonadotropin-releasing hormone (GnRH) and arginine-vasotocin (AVT)—have been shown to be involved in the expression of sexual behaviour across vertebrates and sex steroids are good candidates to interact with these systems (Foran and Bass, 1999; Goodson and Bass, 2001).

Gonadotropin-releasing Hormone (GnRH)

Primitive teleosts (salmonids, cyprinids, catfishes) have two GnRH forms: a species-specific form in the forebrain (GnRH-1) and an evolutionary

conserved variant (GnRH-2 = chicken GnRH II) in the midbrain tegmentum (King and Millar, 1997; Parhar, 2002). In more recently derived teleosts (e.g., cichlids, seabream), a third GnRH form (GnRH-3) is also present (White *et al.*, 1995; Parhar, 1997). There are differences in the localization and distribution of the different GnRHs within the brain leading to multiple GnRH neuronal systems: the terminal nerve system (GnHR-3), the preoptic-area/anterior hypothalamus system (GnHR-1) and the midbrain system (GnHR-2), where the different GnRH variants play diverse functions including neuroendocrine, neurotransmitter, neuromodulator, autocrine and paracrine regulation (Muske, 1993; King and Millar, 1997; Yamamoto, 2003). It has been suggested that these different GnRH systems may influence social behaviour through different routes: GnRH-1 influences reproductive behaviours, mainly via its regulation of the pituitary gonadotropins and ultimately of peripheral production of sex steroids (see Hofmann, 2006, for a recent review); GnRH-2, that has a neuromodulatory function, affects spawning behaviour in females (Volkoff and Peter, 1999); and GnRH-3, that plays a neuromodulatory role, affects both sexual and aggressive behaviour in males (Yamamoto and Kawashima, 1997; Ogawa *et al.*, 2006).

Arginine-Vasotocin (AVT) and Isotocin (IT)

Apart from the peripheral actions of AVT and IT on osmoregulation (Kulczykowska, 1998; Balment *et al.*, 2006), these peptides also act centrally on the neural mechanisms underlying the expression of behaviour. In fish, both AVT and IT neurons are located in the preoptic area and the anterior hypothalamus (POA-AH) and project widely to other brain regions, namely to the anterior and lateral hypothalamic areas, the midbrain tegmentum and peri-aqueductal gray, and the caudal medulla, in addition to the neurohypophysial projections (Goodson and Bass, 2001; Saito *et al.*, 2004). Therefore, these neuropeptidergic pathways may modulate the expression of social behaviour acting at different levels in the central nervous system (CNS), from the perception of relevant stimuli to motor output (Rose and Moore, 2002). Interestingly, GnRH neurons directly modulate the activity of POA AVT neurons, suggesting that GnRH may modulate AVT pathways involved in the regulation of reproductive behaviour (Saito *et al.*, 2003). A review of the effects of AVT and IT on fish social behaviour will be provided in later sections in this chapter.

The Prolactin Family

Although prolactin (PRL) has a wide range of effects in teleosts, the main and most primitive role of PRL in freshwater fish is supposed to be osmoregulation with PRL acting as a freshwater-adapting hormone in most euryhaline teleosts (Manzon, 2002). In some species, two PRL forms have been described. For example, in the Mozambique tilapia, *O. mossambicus*, the two PRL forms are classified as long (188 amino-acids; PRL I or tPRL188) and short (177 amino-acids; PRL II or tPRL177), and tPRL188 has a higher similarity to the PRLs of other fish (Yamaguchi *et al.*, 1988). The biological activities of these two PRL forms can be different in many aspects of the actions of PRLs, as for example in ion retention efficiency (Manzon, 2002). At the behavioural level, PRL has been implicated in parental care in teleosts (further details are given in the section on 'Hormones and reproductive behaviour').

MECHANISMS OF HORMONE ACTION ON BEHAVIOUR

The conceptual paradigm of behavioural endocrinology has been continuously shifting over the previous decades from a perspective of hormones as deterministic agents of behaviour towards a more probabilistic view. Classically, hormones were seen as causal agents of behaviour, acting directly on the expression of a given action pattern. This view was mainly supported by early studies of castration and hormone-replacement therapy that showed that some behaviour patterns were abolished by castration and restored by exogenous administration of androgens (Nelson, 2005). Currently, hormones are seen as modulators of behaviour. That is, the presence of the hormone would not be necessary for the expression of the behaviour but would increase or decrease the probability of its expression. This is achieved by acting as modulators of the neural mechanisms underlying the expression of that specific behavioural pattern. For example, the effects of androgens on the expression of aggressive behaviours in mammals are mediated by modulatory effects on central serotonergic and vasopressine pathways (Simon, 2002).

As described above, hormones may affect the expression of social behaviour by acting on neural circuits underlying behaviour at one of the three major functional compartments of the nervous system: sensory (or input) systems, central processing systems and effector (or output) systems (Nelson, 2005). If we consider social behaviours, hormones may

affect a social interaction by modulating the production of the signal in the sender, or the perception of the signal by the receiver or the central processing of the message in both senders and receivers (Oliveira, 2005). Hormones can further affect behaviour by acting peripherally on somatic releasers, i.e., somatic structures that elicit a behavioural response in conspecifics (sign stimuli *sensu* Tinbergen, 1951), thus influencing the behavioural response of conspecifics towards the subject (e.g., nuptial colouration). Below some examples of the action of hormones on behaviour at these four levels will be presented.

Hormonal Action on Output Systems

Hormones may affect the motor pathways underlying the expression of a social signal, namely visual displays, vocalizations, electric signals or pheromone production and/or release, thus potentially affecting social behaviours in different communication (sensory) channels. Below, a number of examples of the action of hormones on different effector systems will be illustrated.

Many fish species use complex visual displays in intraspecific communication, and the occurrence of stereotypic species-specific movements suggests that specific neuromuscular systems (i.e., motoneurons and their target muscles) may have evolved specifically for the production of these behaviours (e.g., gill cover erection in the Siamese fighting fish, *Betta splendens*, Simpson, 1968; Ma, 1995). It has not been investigated but it is quite plausible that the development of these muscles specifically involved in social displays is androgen-dependent as is the case in other vertebrates (e.g., muscles controlling the wingsnap display in the wild golden-collared manakin, *Manacus vitellinus*, Schultz and Schlinger, 1999; Schultz *et al.*, 2001). In fish, androgens also induce the development of somatic structures used in visual signalling such as the elongation of the dorsal and anal fins used in lateral displays and the thickening of the jaw used in mouth fighting (e.g., Siamese fighting fish, Leitz, 1987; Mozambique tilapia, Oliveira and Almada, 1998a).

Many teleost species use sounds to communicate (Fish and Mowbray, 1970; Ladich, 1997; Myrberg Jr., 1997), and toadfishes are one of the most vocal fish. Males of the batrachoidid *Porichthys notatus* produce loud humming calls to attract females to their nest site during the breeding season (e.g., Brantley and Bass, 1994). The mechanism of sound production involves a hindbrain vocal pacemaker circuit that innervates the paired sonic muscles attached to the lateral walls of the swimbladder

(Goodson and Bass, 2002; Bass and McKibben, 2003). The activity of the sonic pacemaker neurons leads to the bilateral firing of sonic motor neurons which, in turn, results in the synchronous contraction of the paired sonic muscles. Therefore, the periodicity of the firing of the sonic pacemaker neurons determines the frequency of contraction of the sonic muscles, which in turn establishes the fundamental frequency of the vocalization (Bass and Baker, 1990). Hormones have been shown to modulate the activity of this sonic swimbladder mechanism at different levels. Administration of androgens (KT and T), estrogens (E2) and glucocorticoids (cortisol) rapidly increases the duration of fictive vocalizations elicited by electric stimulation of the vocal motor circuit, which suggests a modulation by steroids of the hindbrain vocal circuits in this species (Remage-Healey and Bass, 2004). The exogenous administration of androgens also promotes the development of the sonic muscles in juvenile males, juvenile females, and sneaker males (that do not use the vocalizations to attract females), accompanied by an increase in the area of mitochondria-filled sarcoplasm in the myofibers (Brantley *et al.*, 1993). In juvenile males, an increase in the total number of fibers in sonic muscle was also observed. Taken together, these results indicate that not only the development but also the structure of the sonic muscle is androgen sensitive in *P. notatus*.

Two orders of teleosts produce weak electric signals that are used in communication: the Gymnotiformes from South America and the Mormyriformes from Africa (Zakon and Smith, 2002). These signals are produced in electric organs located in the tails and perceive by the receivers in specialized electroreceptors mainly located in the midline of the fish (Zakon and Smith, 2002). There are two types of electric organ discharges (EOD): pulse type and wave type, and each species only produces one or the other (Zakon and Smith, 2002). In wave-type Gymnotiform species, males produce signals of lower frequency than females and sex steroids—in particular androgens—which seem to be important in the determination of the EOD frequency. In *Sternopygus macrurus* circulating levels of androgens are negatively correlated with EOD frequency in males (Zakon *et al.*, 1991) and when their reproductive axis was challenged with human chorionic gonadotropin (hCG), they responded with an increase in circulating KT levels and a decrease in the frequency of their EODs (Zakon *et al.*, 1990). Moreover, treatment of wave gymnotiformes with androgens induces a masculinization of the waveform, with an increased in wave frequency duration (Meyer, 1983; Mills and Zakon, 1987; Dunlap and Zakon, 1998). In both mormyriformes

and gymnotiformes, with pulse type EODs, the androgen treatment of juveniles, females, castrated males or non-reproductive males masculinizes the pulse form (Bass and Hopkins, 1983, 1985; Hagedorn and Carr, 1985; Bass and Volman, 1987; Landsman and Moller, 1988; Freedman *et al.*, 1989; Landsman *et al.*, 1990; Herfeld and Moller, 1998). The effects of androgens on EOD parameters are mediated either by their effects on the morphology of the electric organ (e.g., shape or size of electrocytes) or by influencing the ionic currents of the electromotor system (e.g., Bass *et al.*, 1986; Bass and Volman, 1987; Mills and Zakon, 1991).

Also, a large number of teleosts use chemical signals in social interactions (Stacey and Sorensen, 2002) and, since reproductive events are associated with changes in circulating levels of sex hormones (e.g., gonadal steroids and prostaglandins), these evolved as sex pheromones conveying information on gender and reproductive status to conspecifics (Stacey and Sorensen, 2002). Unlike the above-mentioned examples, in chemical communication, hormones directly affect the signalling system without acting through the nervous system. This fact derives from the fact that hormonal pheromones represent an evolutionary stage in which 'true' signalling systems have not yet evolved, in that receivers are spying on the products of gonadal physiology released into the water, but senders have not evolved the release of these chemical cues for signalling purposes (Stacey and Sorensen, 1991). Henceforth, in systems where senders have evolved signalling mechanisms, it is expected that the production and release of the signal will be under neuroendocrine control independent of the chemical nature of the signal. Unfortunately, studies on such systems are still at an early phase among teleosts. One of the few examples is the Mozambique tilapia where males are able to control the release of urine into the water through the action of a sphincter in the urogenital papillae. Resident males release pulses of urine towards intruder males, which abstain from releasing urine when acting as intruders. Territorial males also emit pulses of urine towards females during courtship (Almeida *et al.*, 2005; E.N. Barata, pers. comm.). Moreover, holding water from sexually active males elicits large olfactory responses (measured by electro-olfactografy) and among the body fluids tested (bile, urine and faeces), urine is the strongest stimuli for females (Frade *et al.*, 2002). Thus, urine acts as a social signal in this species and its production and release must have an underlying physiological mechanism that might be regulated by neuroendocrine factors.

Hormonal Action on Input Systems

Hormones may influence sensory perception by acting on sensory systems, thus modulating the salience of socially relevant stimuli which, in turn, will influence the animal's responsiveness to those stimuli. Some examples of hormonal effects on sensory systems are presented below.

There are suggestions that sex hormones may be involved in the modulation of visual perception in teleost fish. In the three-spined stickleback, *Gasterosteus aculeatus*, males develop a red colouration in the throat and belly region during the breeding season which influences females in mate choice and by other males in detecting and assessing rivals. Interestingly, the sensitivity of the visual system to red wavelengths increases during the breeding season in both sexes (Cronley-Dillon and Sharma, 1968; Boulcott and Braithwaite, 2006), suggesting a potential role for sex hormones in modulating the spectral sensitivity. It could be argued that this effect could be a result of the hormonal modulation of visual perception either at the level of the sensory organ or at higher levels of visual information processing by the central nervous system (i.e., optic tectum). However, aromatase activity and estrogen receptors α or β have been described in the retina of the African cichlid *Astatotilapia burtoni* and of goldfish, respectively, indicating that cells in the retina are actively producing estrogens that can act locally (Callard *et al.*, 1993; Tchoudakova *et al.*, 1999; Hoke and Fernald, 2002). Together, these data support the hypothesis of a peripheral action of sex steroids on the modulation of visual sensitivity to biologically relevant stimuli. Recently, two GnRH receptor subtypes (R1 and R2) have also been described in the retina of the African cichlid *A. burtoni*, suggesting the hypothesis that the terminal nerve, which also sends projections to the plexiform layer of the retina and whose neurons produce GnRH-3, may be delivering GnRH-3 to the retina where it could act as a neuromodulator (Grens *et al.*, 2005). The two receptor subtypes have differential expression patterns within the retina: the GnRh-R1 has been identified in the amacrine layer, whereas the GnRh-R2 was located in ganglion cells; this pattern suggests that GnRH-3 released from the terminal nerve could modulate both lateral processing circuits, through the type 1 receptor, and vertical pathways, through the type 2 receptor, thus influencing the animal's perception of visual stimuli (Grens *et al.*, 2005). These results confirm earlier data on the innervation of the goldfish (*Carassius auratus*) retina by GnRH-fibers from the terminal nerve (Stell *et al.*, 1987),

suggesting that this can be a widespread neuroendocrine modulatory mechanism of visual processing in fish.

Auditory sensitivity also seems to be modulated by sex steroids. In the plainfin midshipman, *Porichthys notatus*, females locate and choose males based on their acoustic signals and use some call parameters of the male's humming call in mate choice (McKibben and Bass, 1998). Recently, the expression of the estrogen receptor α has been identified in auditory hair cells (Forlano *et al.*, 2005) and it has been demonstrated that during the summer, when females need to exert their mate choice preferences based on the male call, their auditory saccular units increase their temporal encoding capacity up to 340 Hz, compared to only 100 Hz during the winter females (Sisneros and Bass, 2003). This seasonal plasticity of the peripheral auditory system follows the seasonal variation in sex steroid profiles (Sisneros *et al.*, 2004b), suggesting that an increase in sex steroids at the beginning of the breeding season may induce changes in the frequency sensitivity of these hair cells. In fact, the exogenous administration of T or E2 to non-reproductive female midshipman induces an increase in the degree of temporal encoding of the frequency characteristics of the male vocalization (Sisneros *et al.*, 2004a).

Electroreception in weakly electric fish also seems to be modulated by androgens (Keller *et al.*, 1986; Sisneros and Tricas, 2000). Testosterone not only affects EOD frequency, as described above, but it also shifts the maximum receptivity of the electroreceptors to the new EOD frequency produced keeping the electroreceptors of a given individual fine-tuned to its own EOD (Meyer and Zakon, 1982; Bass and Hopkins, 1984).

Sex hormones are also involved in the modulation of olfactory sensitivity in fish. In many cyprinid fishes male courtship behaviour is elicited by a female pheromone (e.g., 15-keto-prostaglandin-15K-PGF-2-alfa in the tinfoil barb, *Puntius schwanenfeldi*, Cardwell *et al.*, 1995). The male's response to 15K-PGF-2-alfa is greatest during the breeding season. Moreover, juveniles implanted with androgens show an increased response to 15K-PGF-2-alfa measured by electro-olfactograms, and increased sexual behaviours directed towards stimuli fish (i.e., juveniles injected with 15K-PGF-2-alfa, Cardwell *et al.*, 1995). These results clearly demonstrate a peripheral effect of androgens on olfactory sensitivity.

Taken together, the data described here strongly suggests that the hormonal modulation of sensory systems is a common phenomenon affecting different sensory modalities that will influence how animals perceive social stimuli.

Hormonal Action on Central Processing Systems

The occurrence of hormone receptors in brain areas known to be involved in motivational systems underlying decision-making mechanisms or on learning and memory systems is a potential indicator of the modulatory action of hormones on central mechanisms fundamental to social behaviour. The presence of steroid receptors and/or aromatase activity in the hippocampus of mammals and birds (Kerr *et al.*, 1995; Saldanha *et al.*, 1999), a brain area known to be involved in relational memory processes, namely in spatial memory (Eichenbaum *et al.*, 1992; Squire, 1992), supports the potential role of sex steroids as modulators of cognitive mechanisms in birds and mammals. Androgen receptors as well as estrogen receptors, together with aromatase (an enzyme that metabolises androgens into estrogens), have also been found in the teleost lateral telencephalic pallium (Gelinas and Callard, 1997), which is the piscine homologous area to the mammalian/avian hippocampus, and that are selectively involved in spatial cognition in fish (Salas *et al.*, 2003). These results suggest a putative role of aromatizable androgens on cognitive functioning in fish.

There is a lack of studies on the effects of hormones on cognitive performance in fish. Recently, the effect of androgens on selective attention to social interactions has been investigated in Siamese fighting fish. Males of this species spent more time observing social interactions between pairs of conspecifics rather than observing pairs of conspecifics that were prevented from interacting (Oliveira *et al.*, 1998). Moreover, territorial males eavesdrop on agonistic interactions among conspecific neighbours, gathering information on relative fighting ability that they use in subsequent interactions with the previously observed individuals (Oliveira *et al.*, 1998). Androgen-treated males significantly increase the time spent observing conspecific interactions when compared to control males (Oliveira and Carneiro, unpubl. data). This result suggests that androgens may promote selective attention to relevant social stimuli in the environment.

Further evidence of hormonal effects on teleost cognitive function came from a recent study on the extinction of a conditioned response in artificially selected lines of rainbow trout, *Oncorhynchus mykiss*, (now in its third generation) of high- (HR) and low-response (LR) to confinement stress (Pottinger and Carrick, 1999). HR fish showed a more rapid extinction than individuals of the LR line (Moreira *et al.*, 2004), suggesting that the differences in cortisol levels between the two lines

may be acting on memory mechanisms in this species, as already shown for other vertebrates. To summarize, both sex steroids and glucocorticoids are potential modulators of cognitive processes in teleost fish.

Hormonal Action on Somatic Releasers

Sign stimuli (*sensu* Tinbergen, 1951) are somatic structures that evoke a behavioural response in conspecifics. The classic example of these releasers is the red belly of male three-spined sticklebacks that elicits aggressive responses in other male sticklebacks (Tinbergen, 1951). Social releasers have been described in many other species and they include nuptial colouration patterns, ornaments present in the fins (e.g., swordtail in swordtail fish), and dermal appendages in the genital papillae (e.g., tasselled genital papillae in male Haplochromine cichlids that elicits egg retrieval by spawning females, Fryer and Iles, 1972). The development of at least some of these somatic releasers is hormone dependent. For example, male nuptial colouration in African cichlids is suppressed in castrated males and restored in castrates and in females by exogenous administration of testosterone (Levy and Aronson, 1955; Wapler-Leong and Reinboth, 1974; Fernald, 1976). Also, in male sticklebacks, the nuptial colouration can be suppressed by castration (Ikeda, 1933) or by the exogenous administration of an anti-androgen (cyproterone acetate) (Rouse *et al.*, 1977). The development of the sword as an extension of the caudal fin in male swordtail fish (*Xiphophorus helleri*), which is used by females in mate choice (Rosenthal and Evans, 1998), is also induced by testosterone (Baldwin and Goldin, 1939). Thus, social behaviour can also be modulated by hormones that affect the expression of somatic releasers.

Organizational vs. Activational Effects

A classic dichotomy in behavioural endocrinology is the division of hormonal effects upon behaviour into activational vs organizational. An endocrine manipulation in an early stage of the life of the individual—usually during a sensitive or critical period—that has a permanent effect on its adult behaviour, is called an organizational effect (Arnold and Breedlove, 1985). On the other hand, in adulthood, hormones may affect behaviour in a transient way, by activating proximate mechanisms underlying behaviour, i.e., by having an activational effect upon behaviour (Arnold and Breedlove, 1985). This dichotomy was proposed by Phoenix

and associates while studying the sexual behaviour of female guinea pigs (Phoenix *et al.*, 1959).

In teleosts, most studies have concentrated on activational effects, and organizational effects have mainly been investigated in regard to the effects of sex steroids on sex determination and differentiation mechanisms (Devlin and Nagahama, 2002). In a number of teleost species, the early exposure of larvae or fry to sex steroids promotes a complete sex reversal in gonochoric species (e.g., Mozambique tilapia, Clemens and Inslee, 1968). For example, in the Mozambique tilapia XX sexually reversed individuals developed as males (i.e., differentiated functional testis) and when they reached sexual maturity, they adopted the black nuptial coloration typical of territorial males, and expressed the full male reproductive behavioural repertoire of this species, including the building of spawning pits in the substrate (Clemens and Inslee, 1968; Billy and Liley, 1985). It was also found that adult females exposed to androgens for a long period (40 days) acquired some male behavioural traits: they adopt a darker coloration, direct their male-courtship displays towards other females and exhibit lateral displays (an agonistic display used by males in initial phases of fighting). Thus, there is a critical period during which the exposure to androgens have a profound influence in gonadal differentiation, and after which the early exposure to androgens, although no longer effective in promoting sex reversal, still has an organizational action on adult reproductive behaviour.

These reports on the organizational effects of steroids on fish behaviour suggest the possibility of an endocrine mediation of parental effects on progeny. Although maternal and paternal effects in fish have been widely investigated (e.g., Chambers and Leggett, 1996; Kamler, 2005), the mechanisms underlying these effects have been poorly studied. Described parental influences on clutch, egg and larval size and viability and on sex ratios, can be mediated by hormones. Since there is a continuum between the maternal endocrine system and that of the offspring, maternal effects can be explained by the transference of specific hormones from the mother to their eggs (Schreck *et al.*, 1991). Therefore, socially induced variations in circulating steroid levels in breeding females can be reflected in their eggs and subsequently affect the development of the offspring. It has been shown that the physiological condition of the mothers during gametogenesis determines the provisioning of the eggs not only with nutrients but also with hormones, so that there is a close association between the circulating steroid levels during oogenesis and the steroid

egg contents (Schreck *et al.*, 1991; Hwang *et al.*, 1992; Mylonas *et al.*, 1994). As an example, in the ambon damselfish, *Pomacentrus amboinensis*, social interactions experienced by females prior to spawning (e.g., interactions with predators; density of females interacting with breeding females) influence the concentration of cortisol in their ovaries, leading to increased cortisol contents of the eggs and reduced size of the larvae (McCormick, 1998, 2006). Thus, maternally derived steroids may have a major impact on offspring development and survival and potentially, influence future behavioural displays via hormone organizational effects.

On the other hand, paternal hormones may affect the development of offspring in species with male parental care in which the eggs and/or the fry are exposed to the male's hormones either directly, when males physically incubate the eggs, or indirectly when males incubate the eggs in enclosed nests (e.g., tight crevices in rocky shores in blennies and gobies) and, thus, the offspring may be permanently exposed to the steroids released through the gills and/or excreted via the urine and faeces (Vermeirssen and Scott, 1996; Turner *et al.*, 2003; Ellis *et al.*, 2004). Two groups are of particular interest in this respect: seahorses and other syngnathids with well-developed brood pouches and biparental or paternal mouthbrooding cichlids. In seahorses, the males have a brooding pouch inside which the eggs are deposited by females during spawning and where embryos develop in close association with the pouch epithelium, resembling the embryo-placental relationship in mammals (Laksanawimol, 2006). The extensive vascularization of the brood pouch during gestation and the synthesis of estrogens and progestins in the testis, brood pouch and inter-renal tissues (Oconer *et al.*, 2003) raise the possibility of paternal-embryo transfer of circulating products in the blood, including hormones (Laksanawimol, 2006). In mouthbrooding cichlids, the bucal cavity is also widely vascularized and, therefore, the eggs and fry are potentially exposed to paternal hormones during mouthbrooding. Considering the increasing interest on comparative studies in maternal effects among vertebrates (Groothuis *et al.*, 2005), it is surprising that despite the opportunities offered by these species, no published studies are available so far on parental effects in teleosts. In particular, biparental mouthbrooding species offer the unique opportunity to study the relative effects of hormonally mediated maternal and paternal effects, within the same species. This area will certainly receive increasing attention in the coming years.

HORMONES AND REPRODUCTIVE BEHAVIOUR

Early studies on fish applying castration and hormone-replacement therapies have demonstrated an endocrine modulation of reproductive behaviours (Hoar, 1962). Since then, the influence of hormones on fish reproductive behaviour has been the subject of numerous investigations (for reviews: see Liley and Stacey, 1981, 1983; Borg, 1994). However, an overview of the available data shows significant variation on the effects of hormonal manipulations across species and the establishment of a general model for the neuroendocrine control of fish reproductive behaviour has been difficult to accomplish as the mechanisms of action underlying neural and hormonal influences on female and male sexual behaviours are still poorly understood. Most likely, the enormous variation in fish mating systems and reproductive displays will be paralleled by variation at the neuroendocrine level and, thus, the interaction between gonadal hormones, neural systems and reproductive behaviour may present a strong species-specificity.

Here, the existing data on the neuroendocrine regulation of fish sexual behaviour is reviewed briefly. Not surprisingly, most studies on this topic have focused on male behaviour as the males are usually the most actively courting sex and are more ornamented than females. The sections below on the neuroendocrine control of male and female reproductive behaviour thus reflect this asymmetry. In the search for the neuroendocrine mechanisms of male and female sexual behaviours, it is of interest to identify and discriminate between appetitive and consummatory behavioural patterns that may be regulated by different neuroendocrine mechanisms (e.g., Dermon *et al.*, 1999 and Taziaux *et al.*, 2006, present evidence for different neuroendocrine mechanisms underlying appetitive and consummatory sexual behaviours in birds). In teleosts, appetitive sexual behaviours include behavioural patterns displayed during the establishment and defence of a breeding territory, the preparation of a spawning site (e.g., nest) and the expression of courtship displays, whereas consummatory behaviours include copulation in internal fertilizers or the spawning reflex in external fertilizers (for an example of these two types of behaviour in the Mozambique tilapia, see Figure 3.2).

Male Sexual Behaviour

The effects of sex steroids on the different components of male behaviour are reviewed below. Although an attempt is made to establish a causal

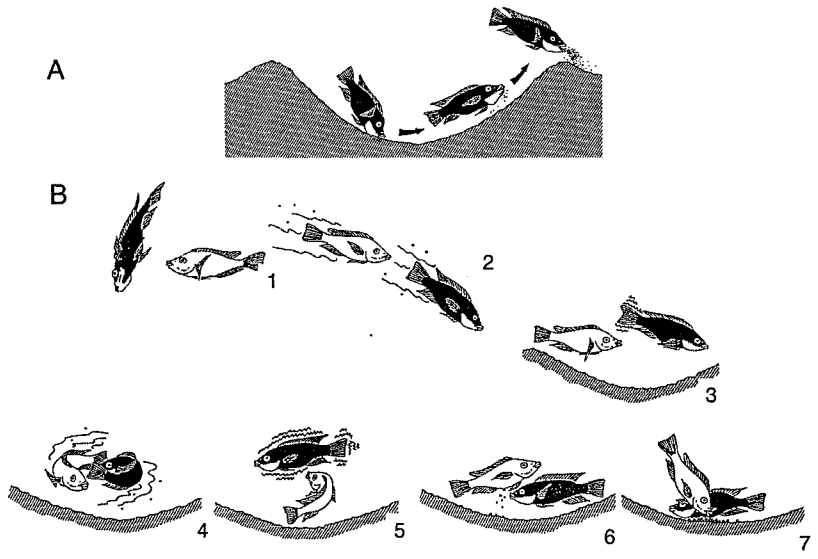

Fig. 3.2 Appetitive and consumatory sexual behaviours in the Mozambique tilapia, *Oreochromis mossambicus*. Male appetitive sexual behaviour includes digging a spawning pit (A) and courtship behaviour directed towards the female (B1-B6); female appetitive sexual behaviour includes receptivity of the male courtship, expressed by immobilization when approached by male and following the male to the spawning pit (B1-B5); female consummatory behaviour = egg release (B6); male consummatory behaviour = sperm release (B7).

relation between sex steroids and specific behaviours, in many studies it is difficult to disentangle the effects of hormonal manipulations in the several components of reproductive behaviour. For example, a reduction in spawning or copulation frequency due to castration may result from a direct effect on the behaviour of the decrease in circulating sex steroids or to a side effect from inhibition of courtship displays or nuptial colouration. Thus, although most experiments assessing the effects of hormones on fish reproductive behaviour have been conducted on males, carefully controlled experiments testing the effects of hormonal manipulations on specific behavioural patterns are scarce.

Reproductive Territory Acquisition and Nest/Spawning Site Building in Males

From a behavioural perspective, fish reproduction starts—in many species—by the acquisition and preparation of a breeding territory. This

includes male-male aggressive interactions during territory disputes and, in some species, nest building or spawning site preparation. Aggressive behaviour associated with reproductive territory acquisition has been proposed to be facilitated both by androgens (e.g., Cardwell and Liley, 1991) and, at least in some species, by AVT (Semsar *et al.*, 2001). However, because agonistic behaviours are also displayed in other contexts, their neuroendocrine regulation is treated elsewhere in this chapter.

The effect of castration in nest building behaviour has been described for several teleosts (Table 3.1). The results span from no effect to a complete suppression of nest-building behaviour (Table 3.1). A significant number of studies have investigated the effects of castration in nest building behaviour in the three-spined stickleback (Table 3.1). With one exception (Ikeda, 1933), all report a suppression of this behaviour following castration, suggesting gonadal androgens facilitate this behaviour (Table 3.1). Nevertheless, in other species, castration failed to suppress nest building (e.g., Aronson, 1951; Villars and Davis, 1977, Table 3.1). The role of androgens in mediating nest-building behaviour is also supported by androgen administration to castrates which, in general, restores the behaviour. Both T and 11-KT have been reported to restore the behaviour (Table 3.1) but, at least in sticklebacks 11-KA (11-ketoandrostenedione) seems to be more effective than T (Borg, 1987). This is in agreement with the fact that 11-KT, but not T, increases in male sticklebacks during the nest-building period (Mayer *et al.*, 1990; Páll *et al.*, 2002a). In the sunfish *Lepomis gibbosus*, cyproterone acetate, an androgen blocker, abolishes nest building and a mammalian gonadotropin (LH) restores the behaviour (Kramer, 1971). In species where nest building is a typical male activity, females and immature males administered with androgens may also exhibit the behaviour, corroborating the mediation of nest building behaviour by androgens (Table 3.1). As an example, female blue gourami, *Trichogaster trichopterus*, treated with MT (methyltestosterone) were reported to increase nest building (Kramer, 1972b). Thus, the present data suggests nest building is generally promoted by androgens but strong within-species variation occurs.

The neuropeptide GnRH has also been implicated in the control of nest-building behaviour. Besides controlling gonadal development via the HPG axis and thus, ultimately, the peripheral action of gonadal steroids, GnRH can directly regulate neurocircuits underlying the expression of reproductive behaviour (Propper and Dixon, 1997; Volkoff and Peter 1999). Displaying males usually have more or larger POA-GnRH cells

Table 3.1 Effects of hormonal manipulations in male reproductive behaviour. +, increase; –, decrease; 0, no effect; ND, not described, not applicable to the species.

Hormonal manipulation	*Sex*	*Hormone*	*Nest building*	*Nuptial colouration*	*Courtship displays*	*Spawning/ copulations*	*Reference*
Castration							
Centrarchidae							
Lepomis gibbosus and *L. megalotis*	Males		–	–	ND	ND	Smith (1969)
Cichlidae							
Sarotherodon melanotheron melanotheron	Males		0	–	NT	NT	Aronson (1951)
Sarotherodon melanotheron heudelotii	Males		0	ND	ND	ND	Heinrich (1967)
Oreochromis upembae	Males		0	ND	ND	ND	Heinrich (1967)
Hemichromis bimaculatus	Males		ND	0	0	ND	Noble and Kumpf (1936)
Pseudocrenilabrus multicolor multicolor	Males		–	–	–	ND	Reinboth and Rixner (1970)
Gasterosteidae							
Gasterosteus aculeatus	Males		–	ND	ND	ND	Borg (1987)
	Males		–	–	ND	ND	Wai and Hoar (1963)
	Males		–	–	–	ND	Hoar (1962)
	Males		–	–	–	ND	Baggerman (1957); Baggerman (1966)
	Males		0	–	ND	ND	Ikeda (1933)
	Males		ND	ND	0	ND	Páll *et al.* (2002b)
	Males		ND	ND	0	ND	Baggerman (1968)

(Table 3.1 Contd.)

(*Table 3.1 Contd.*)

Hormonal manipulation	*Sex*	*Hormone*	*Nest building*	*Nuptial colouration*	*Courtship displays*	*Spawning/ copulations*	*Reference*
Gobiidae							
Bathygobius soporator	Males		ND	ND	0	ND	Tavolga (1955)
Labridae							
Thalassoma bifasciatum	Males		ND	ND	0	0	Semsar and Godwin (2003)
Moronidae							
Morone americana	Males		ND	ND	–	ND	Salek *et al.* (2001)
Osphronemidae							
Macropodus opercularis	Males		0	ND	ND	–	Villars and Davis (1977)
Trichogaster trichopterus	Males		–	–	–	–	Johns and Liley (1970)
Salmonidae							
Oncorhynchus mykiss	Males		ND	ND	–	ND	Mayer *et al.* (1994)
Androgens							
Blenniidae							
Parablennius sanguinolentus parvircornis	Males	11-KT	ND	ND	0	ND	Ros *et al.* (2004)
Batrachoididae							
Opsanus beta	Males	11-KT	ND	ND	+	ND	Remage-Healey and Bass (2006)
Centrarchidae							
Lepomis gibbosus and *L. megalotis*	Castrated males	MT	+	+	ND	ND	Smith (1969)

(*Table 3.1 Contd.*)

(*Table 3.1 Contd.*)

Hormonal manipulation	*Sex*	*Hormone*	*Nest building*	*Nuptial colouration*	*Courtship displays*	*Spawning/ copulations*	*Reference*
Lepomis gibbosus	Females	MT	+	ND	ND	ND	Kramer (1972a)
	Males	Cyproterone acetate (androgen blocker)	–	ND	ND	ND	Kramer (1971)
Lepomis macrochirus	Males	T	0	ND	0	ND	Kindler *et al.* (1991b)
	Males	11-KT	0	ND	+	ND	Kindler *et al.* (1991b)
	Males	Cyproterone acetate (androgen blocker)	–	ND	–	–	Kindler *et al.* (1991b)
Cichlidae							
Haplochromis burtoni	Males	T	0	0	0	ND	Fernald (1976)
	Females	T	+	+	+	0	Wapler-Leong and Reinboth (1974)
Pseudocrenilabrus multicolor multicolor	Castrated males	T	+	+	+	ND	Reinboth and Rixner (1970)
	Females	T	+	+	+	ND	Reinboth and Rixner (1970)
Sarotherodon melanotheron melanotheron	Castrated males	MT	ND	+	+	ND	Levy and Aronson (1955)
Oreochromis mossambicus	Males	Cyproterone acetate (androgen blocker)	0	ND	0	ND	Kramer *et al.* (1969)
Cyprinidae							
Carassius auratus auratus	Females	T	ND	ND	0	0	Stacey and Kobayashi (1996)
	Females	11-KT	ND	ND	+	+	Stacey and Kobayashi (1996)

(*Table 3.1 Contd.*)

(*Table 3.1 Contd.*)

Hormonal manipulation	*Sex*	*Hormone*	*Nest building*	*Nuptial colouration*	*Courtship displays*	*Spawning/ copulations*	*Reference*
Carassius auratus langsdorfii	Gynogenetic females	11-KT	ND	ND	ND	+	Kobayashi and Nakanishi (1999)
Gasterosteidae							
Gasterosteus aculeatus	Castrated males	11-KA	+	+	+	+	Borg (1987)
	Castrated males	MT	ND	+	ND	ND	Wai and Hoar (1963)
	Castrated males	MT	+	+	ND	ND	Hoar (1962)
	Castrated males	11-KA	+	ND	ND	ND	Borg and Mayer (1995)
	Females	11-KA	0	0	0	ND	Borg and Mayer (1995)
	Males	Cyproterone acetate (androgen blocker)	0	-	-	ND	
	Males	T	0	0	0	ND	Rouse *et al.* (1977)
	Immature males	MT	0	+	0	ND	Hoar (1962)
	Castrated males	11-KA	ND	ND	0	ND	Páll *et al.* (2002b)
	Females	MT	ND	+	0	0	Wai and Hoar (1963)
Labridae							
Thalassoma bifasciatum	Ovariectomized females	11-KT	ND	+	+	ND	Semsar and Godwin (2003) Semsar and Godwin (2004)
Moronidae							
Morone americana	Castrated males	T	ND	ND	+	ND	Salek *et al.* (2001)
	Castrated males	11-KT	ND	ND	+	ND	Salek *et al.* (2001)

(*Table 3.1 Contd.*)

(*Table 3.1 Contd.*)

Hormonal manipulation	*Sex*	*Hormone*	*Nest building*	*Nuptial colouration*	*Courtship displays*	*Spawning/ copulations*	*Reference*
Osphronemidae							
Macropodus opercularis	Castrated males	T	0	ND	ND	+	Villars and Davis (1977)
	Females	MT + prolactin	+	ND	ND	ND	Machemer (1971)
Trichogaster trichopterus	Castrated males	MT	+	+	+	+	Johns and Liley (1970)
Colisa lalia	Females	T	+	ND	ND	ND	Forselius (1957)
Colisa labiosus	Females	T propionate	ND	+	ND	ND	Forselius (1957)
Poecilidae							
Poecilia reticulata	Females	MT	ND	ND	+	ND	Landsman *et al.* (1987)
	Males	Flutamide and Vinclozolin (androgen blockers)	ND	ND	–	ND	Bayley *et al.* (2002)
	Males	Flutamide and Vinclozolin (androgen blockers)	ND	ND	–	ND	Baatrup and Junge (2001)
Pomacentridae							
Chromis dispilus	Males	T	ND	ND	0	0	Pankhurst and Carragher (1995)
	Males	11-KT	ND	ND	0	0	Pankhurst and Carragher (1995)
Salmonidae							
Oncorhynchus mykiss	Castrated males	11-KA	ND	ND	0	ND	Mayer *et al.* (1994)
Estrogens							
Adrianichthyidae							
Oryzias latipes	Males	E_2	ND	ND	–	ND	Oshima *et al.* (2003)

(*Table 3.1 Contd.*)

(*Table 3.1 Contd.*)

Hormonal manipulation	*Sex*	*Hormone*	*Nest building*	*Nuptial colouration*	*Courtship displays*	*Spawning/ copulations*	*Reference*
Cyprinidae							
Carassius auratus	Males	E_2	ND	ND	–	ND	Bjerselius *et al.* (2001)
Gasterosteidae							
Gasterosteus aculeatus	Males	E_2	0	ND	0	ND	Wibe *et al.* (2002)
Poecilidae							
Poecilia reticulata	Males	E_2	ND	ND	–	ND	Bayley *et al.* (1999)
Gambusia holbrooki	Males	E_2	ND	ND	–	–	Doyle and Lim (2005)
Progestogens							
Salmonidae							
Oncorhynchus mykiss	Castrated males	17, 20βP	ND	ND	+	ND	Mayer *et al.* (1994)
Pomacentridae							
Chromis dispilus	Males	17, 20βP	ND	ND	0	ND	Pankhurst (1995)
Glucocorticoids							
Batrachoididae							
Opsanus beta	Males	Cortisol	ND	ND	0	ND	Remage-Healey and Bass (2006)
Prolactin							
Gasterosteidae							
Gasterosteus aculeatus	Males	PRL	ND	ND	–	ND	Páll *et al.* (2004)

(*Table 3.1 Contd.*)

(*Table 3.1 Contd.*)

Hormonal manipulation	*Sex*	*Hormone*	*Nest building*	*Nuptial colouration*	*Courtship displays*	*Spawning/ copulations*	*Reference*
Neuropeptides							
Cichlidae							
Oreochromis niloticus	Males	GnRH immunoneutralization[1]	–	ND	ND	ND	Ogawa *et al.* (2006)
Labridae							
Thalassoma bifasciatum	Females	AVT	ND	ND	0	ND	Semsar and Godwin (2003)
	Initial phase males	AVT	ND	ND	0	ND	Semsar and Godwin (2003)
	Terminal phase males	AVT	ND	ND	+	ND	Semsar *et al.* (2001)
	Terminal phase males	Manning (AVT antagonist)	ND	ND	–	ND	Semsar *et al.* (2001)
Moronidae							
Morone americana	Males	AVT	ND	ND	+	ND	Salek *et al.* (2002)

[1] Nest building was inhibited only with GnRH3 immunoneutralization

than both females and non-displaying males (Foran and Bass, 1999 and references therein), suggesting that this neuropeptide promotes male sexual behaviour. In the Nile tilapia *Oreochromis niloticus*, intracerebroventricular injections of antisera against all three forms of fish GnRH were accomplished in males and both nest-building activity and nest size decreased following GnRH-3 neutralization, suggesting a direct control by GnRH-3 of this behaviour (Ogawa *et al.*, 2006). It seems likely that gonadal androgens may promote nest building and other aspects of male reproductive behaviour by facilitating the action of GnRH neurons, and an up-regulation of GnRH by androgens has been demonstrated in several species (e.g., Amano *et al.*, 1994; Parhar *et al.*, 2001 but see Vetillard *et al.*, 2006).

Male Courtship Displays

Data from castration and hormone-replacement studies only partially support the causal relationship between androgens and male courtship displays. Some studies report the maintenance of male sexual displays after castration (e.g., Noble and Kumpf, 1936; Tavolga, 1955; Aronson, 1960; Heinrich, 1967; Semsar and Godwin, 2003) while others report a decrease (e.g., Johns and Liley, 1970; Villars and Davis, 1977; Table 3.1). Moreover, androgen administration to castrates restores male sexual displays in some species (e.g., Reinboth and Rixner, 1970; Borg, 1987) but not in others (e.g., Mayer *et al.*, 1994, Table 3.1).

Hormonal manipulations in females and immature males have also produced conflicting results. As an example, female goldfish treated with androgens will display male-like sexual behaviours and will not differ from males in their reply to a stimulus female (Stacey and Kobayashi, 1996), while in sticklebacks, androgen administration to females does not induce male-like behaviours (Borg and Mayer, 1995).

In mammals and birds, some of the behaviourally masculinizing effects of gonadal androgens rely on the local conversion of T to E2 by aromatase (reviewed in Baum, 2003; Ball and Balthazart, 2004). In fish, however, KT is the most abundant androgen in the plasma of most species and it was suggested to be more effective than T in eliciting male sexual displays (reviewed in Borg, 1994). Because KT is not aromatizable, it has been assumed that in fish male behaviour is less dependent on T aromatization. However, although KT has been shown to effectively promote the differentiation of male secondary sexual characters, its role in behavioural displays is less clear (Oliveira *et al.*, 2005b). Moreover, in a recent study

investigating the behavioural effects of aromatase in guppies, *Poecilia reticulata*, two of three male sexual displays were reduced by aromatase inhibition (Hallgren *et al.*, 2006), suggesting T aromatization into E2 facilitates these behaviours. Nevertheless, in all studies conducted so far where E2 was administered to males, an increase in male-like behaviours have never been reported and most studies described an inhibition of male sexual displays following E2 administration (Table 3.1). Thus, although it remains a possibility that as in mammals and birds, in some fish species local aromatization of T into E2 positively regulates the expression of some male sexual displays, the role of androgen aromatization on male behaviour needs to be further investigated.

Strong evidence that male sexual displays do not depend exclusively on sex steroids comes from research on the sex-changing bluehead wrasse, *Thalassoma bifasciatum*. In this species, sex change is under social control and females may undergo sex change following the removal of territorial terminal phase males (Warner and Swearer, 1991). In a field experiment, ovariectomized females responded to the removal of terminal phase males by occupying their territory and displaying the full-suite of male courtship and spawning behaviours, demonstrating that gonadal androgens are not necessary for the expression of male sexual behaviour (Godwin *et al.*, 1996). In this species, changes in the social environment are thought to directly trigger the neural mechanisms underlying the change from female to male behaviour. During sex change, AVT mRNA abundance increases in the brain of sex-changing females (Godwin *et al.*, 2000) and is higher even in ovariectomized dominant sex-changing females when compared with subordinate females (Semsar and Godwin, 2003). On the other hand, male displays in *T. bifasciatum* also seem to be promoted by KT. Subordinate ovariectomized females implanted with KT exhibited male-like colouration and increased the frequency of male-like courtship displays. KT implants had no effect on AVT mRNA abundance or AVT-immunoreactive soma size (Semsar and Godwin, 2003, 2004) and castrating dominant males reduces 11-KT circulating levels but has no effect on AVT mRNA expression (Semsar and Godwin, 2003). Together, these results suggest that in this species AVT plays a critical role in the modulation of male sexual behaviour but is not affected by gonadal hormones. The fact that 11-KT promotes male-like displays but it does not interact with the AVT neural system suggests that more than one neural mechanism may modulate male courtship behaviour in this species (Semsar and Godwin, 2004).

The facilitation of male reproductive displays by AVT has also been shown in other fish species. For example, in male electric fish, *Apteronotus*

leptorhynchus, AVT increased the production of electrical signals used in female attraction (Bastian *et al.*, 2001), and in male white perch, *Morone americana*, intracerebroventricular injections of AVT increased male sexual displays (Salek *et al.*, 2002). These findings are in agreement with what has been described for other vertebrates where AVT or its mammalian homologue AVP generally promotes male sexual displays (reviewed in Moore, 1992). The GnRH system has been shown to interact with AVT neurons. In rainbow trout *in vitro* GnRH administration to POA-AVT neurons stimulated their electrical activity (Saito *et al.*, 2003) and thus a modulation of reproductive behaviour by GnRH via AVT neurons is also possible.

Copulation/Spawning

Unlike pre-spawning behaviours that seem to be modulated in the long term by the action of gonadal steroids and presumably by more permanent changes in the underlying neurocircuitry, the neuroendocrine control of spawning or copulation behaviour *sensu stricto* seems to be more dependent on short-term actions of neurohypophysial hormones. The induction of the spawning reflex by pituitary extracts was first demonstrated in the killifish, *Fundulus heteroclitus* (Pickford, 1952). These results were further confirmed in males and females of the same species (Wilhelmi *et al.*, 1955), female medaka, *Oryzias latipes* (Egami, 1959), female bitterling, *Rhodeus sericeus* (Egami and Ishii, 1962) and male and female flagfish, *Jordanella floridae* (Crawford, 1975). In other species, however, neurohypophysial hormone administration failed to induce spawning behaviour (e.g., three-spined stickleback, T.J. Lam and Y. Nagahama, pers. comm. in Liley and Stacey, 1983; goldfish, G.E. Pickford in Macey *et al.*, 1974; weatherfish, *Misgurnus fossilis*, and Atlantic salmon, *Salmo salar* Egami and Ishii, 1962).

In the killifish, AVT was more effective than IT in eliciting the spawning reflex (Pickford and Strecker, 1977) and both oxytocin and IT were ineffective in inducing spawning behaviour in male seahorses, *Hippocampus hippocampus*, although they did induce parturition reflexes (Fiedler, 1970).

The neural substrate where neurohypophysial hormones act to induce the male spawning reflex remains unknown, and the mechanism associated with sperm release has been poorly documented. In the goldfish, central neural signals conducted by the genital nerve controls sperm duct contractions and, thus, sperm release (Demski *et al.*, 1975; Dulka

and Demski, 1986). It is possible that neurohypophysial hormones will act peripherally, facilitating the gamete release. In the killifish, telencephalon removal does not abolish the response to AVT, showing that AVT either acts on neural circuits in other brain or spinal cord regions or peripherally to induce the spawning reflex (Knight and Knight, 1996). However, Macey *et al.* (1974) reported that the destruction of the POA in the killifish eliminates the spawning reflex in response to neurohypophysial hormones and an indirect pathway from peripheral AVT-neurons to hypothalamic neurons has been proposed (Pickford *et al.*, 1980).

Female Sexual Behaviour

Neuroendocrine control of female reproductive behaviour has been less investigated than in males, as in most species female pre-spawning (appetitive) behaviour is less elaborate and temporally more restricted. In most cases, females do not play any role in reproductive territory acquisition or nest building and assume a passive role during courtship. For this reason, studies on the neuroendocrine regulation of female reproductive behaviour have been mainly focused on female receptivity and oviposition behaviour. However, the variability in fish mating systems and modes of reproduction offers an enormous potential for studying the neuroendocrine control of female reproductive behaviour which remains to be explored. As an example, sex-role reversed species where females play an active role in mating and perform complex courtship displays (e.g., syngnathids) have been poorly investigated from a neuroendocrine point of view. Nevertheless, data exist on the neural and hormonal regulation of female reproductive behaviour for some species, with goldfish being the most detailed studied species.

Female Receptivity

Stacey (1981) has proposed that while in external fertilizing species prostaglandins produced by mature oocytes may signal a ready-to-spawn state and thus promote sexual behaviours and egg release, in internal fertilizers sexual behaviour and fertilization are temporally dissociated and estrogens produced during follicular development may promote sexual receptivity and behaviours in anticipation to ovulation. As an example, in guppies, E2 administration to ovariectomized and hypophysectomized females restores sexual receptivity (Liley, 1972), while in the goldfish administering a PGF2α synthesis inhibitor to female goldfish blocks female

receptivity to males and injecting PGF2α reverses this effect (Stacey, 1976, reviewed in Kobayashi *et al.*, 2002; see Table 3.2).

Female Courtship

In most species, females do not exhibit courtship displays and as a consequence the neuroendocrine control of these behaviours have been poorly described (Table 3.2). Species with reversed sex-roles are good models to study these mechanisms but detailed investigations are still lacking. In one of these species, the peacock blenny, *Salaria pavo*, females take the initiative in courtship and exhibit a typical nuptial colouration (Almada *et al.*, 1995; Fig. 3.3). In this species, parasitic (sneaker) males reproduce by mimicking the female behaviour in order to approach and parasitize the nests of larger nest-holder males (Gonçalves *et al.*, 1996). Sneaker males implanted either with KT or with T decreased the expression of female-like displays and the nuptial colouration (Oliveira *et al.*, 2001c; Gonçalves *et al.*, in press), whereas AVT promoted these behaviours both in females and in sneakers (Carneiro *et al.*, 2003). It is possible that androgens suppress female-courtship in males via an inhibition of AVT neurons but this hypothesis remains to be tested.

Female Spawning/Oviposition

Female goldfish sexual behaviour closely follows the ovulation cycle and injecting non-ripe females with mature oocytes or with PGF2α stimulates female behaviour associated with oviposition (Stacey and Liley, 1974; Stacey, 1981; Liley and Stacey 1983; Sorensen and Goetz, 1993; Kobayashi *et al.*, 2002; Table 3.2). The response of ovariectomized female goldfish to PGF2α does not differ from intact or sham-operated fish, suggesting the PGF2α-mediated female reproductive behaviours are relatively independent from gonadal steroids production and, more likely, induced by PGF2α produced in the reproductive tract during ovulation (Kobayashi and Stacey, 1993). Male goldfish injected with PGF2α will exhibit female-like sexual behaviour not different from PGF2α -injected females (Stacey, 1976, 1977). Since this seminal work in the goldfish, the positive regulation of female sexual behaviour by PGF2α has been documented in other species with external fertilization. For example, in the cichlid, *Cichlasoma bimaculatum*, PGF2α injections induced females to clean the oviposition substrate and promoted oviposition behaviour (Cole and Stacey, 1984) and female paradise fish, *Macropodus opercularis*, increased the frequency

Table 3.2 Effects of hormonal manipulations in female reproductive behaviour. +, increase; –, decrease; 0, no effect; ND, not described, not applicable to the species.

Hormonal manipulation	*Sex*	*Hormone*	*Sexual receptivity*	*Nuptial colouration*	*Courtship displays*	*Spawning/ oviposition*	*Reference*
Ovariectomy							
Cichlidae							
Hemichromis bimaculatus	Female		ND	ND	–	ND	Noble and Kumpf (1936)
Sarotherodon melanotheron melanotheron	Female		ND	ND	–	ND	Aronson (1951)
Cyprinidae							
Carassius auratus	Female		ND	ND	0	0	Kobayashi and Stacey (1993)
Poecilidae							
Poecilia reticulata	Female		–	ND	ND	ND	Liley (1972)
	Female		ND	ND	0	ND	Liley (1968)
Androgens							
Poecilidae							
Poecilia reticulata	Ovariectomized females	MT	0	ND	ND	ND	Liley (1972)
Estrogens							
Poecilidae							
Poecilia reticulata	Ovariectomized females	E_2	+	ND	ND	ND	Liley (1972)

(*Table 3.2 Contd.*)

(*Table 3.2 Contd.*)

Hormonal manipulation	*Sex*	*Hormone*	*Sexual receptivity*	*Nuptial colouration*	*Courtship displays*	*Spawning/ oviposition*	*Reference*
Progestogens							
Cichlidae							
Cichlasoma bimaculatum	Females	PGF2α	ND	ND	ND	+	Cole and Stacey (1984)
Cyprinidae							
Carassius auratus	Females	Indomethacin (PGF2α synthesis inhibitor)	-	ND	ND	ND	Stacey (1976)
	Females	PGF2α	+	ND	ND	ND	Stacey (1976)
	Males	PGF2α	+	ND	ND	ND	Stacey (1976); Stacey (1977)
Barbonymus gonionotus	Females	PGF2α	+	ND	+	+	Liley and Tan (1985)
Poecilidae							
Poecilia reticulata	Ovariectomized females	Pro-gesterone	0	ND	ND	ND	Liley (1972)
Osphronemidae							
Macropodus opercularis	Females	PGF2α	ND	ND	ND	+	Villars and Burdick (1986)
Glucocorticoids							
Poecilidae							
Poecilia reticulata	Ovariectomized females	Cortisol and corticosterone	0	ND	ND	ND	Liley (1972)
Neuropeptides							
Blenniidae							
Salaria pavo	Females	AVT	ND	+	+	ND	Carneiro *et al.* (2003)

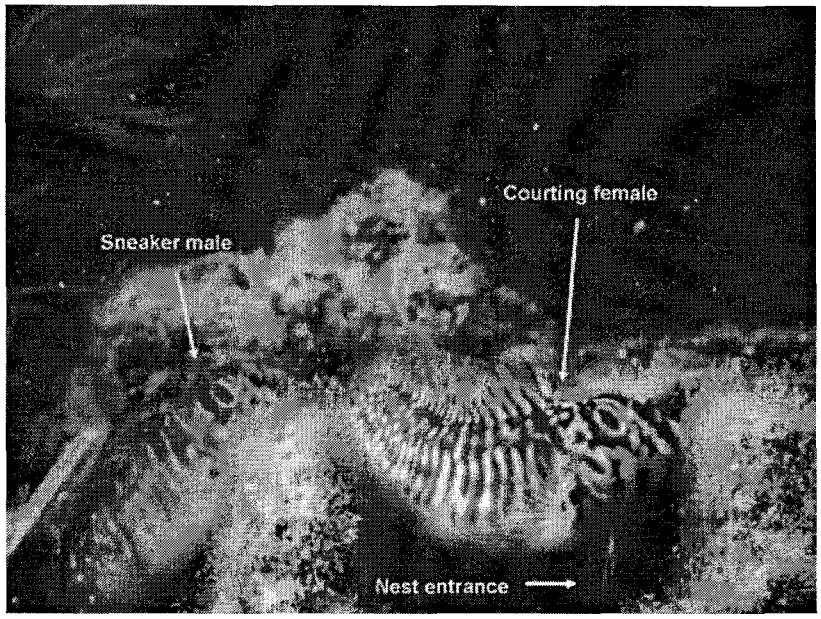

Fig. 3.3 Female courtship behaviour in a species with sex-role reversal, the peacock blenny (*Salaria pavo*) (underwater photograph by Rui Oliveira).

of spawning acts following PGF2α administration (Liley and Tan, 1985; Villars and Burdick, 1986; for other examples see Kitamura *et al.*, 1994; and Yamamoto *et al.*, 1997).

PGF2α has been proposed to enter the circulation and act on brain circuits in order to promote spawning behaviour (Stacey and Peter 1979; Stacey, 1981). However, the neural targets of PGF2α action in the brain presumably controlling female spawning behaviour are as yet unknown.

GnRH has also been implicated in female reproductive behaviour, particularly in spawning behaviour. In *A. burtoni*, the size of POA-GnRH neurons is smaller in females carrying broods in comparison with females that have never spawned, are in the act of spawning or are in a post-reproductive state (White and Francis, 1993). The involvement of GnRH in female reproductive behaviour was further demonstrated in a study on the effects of salmon GnRH (GnRH-3), chicken GnRH II (GnRH-2), and a mammalian GnRH antagonist on the spawning behaviour of female goldfish (*C. auratus*; Volkoff and Peter, 1999). Intracerebroventricular injections of low doses of GnRH-3 and GnRH-2 promoted spawning behaviour in female goldfish, while high doses of

these peptides or the GnRH antagonist inhibited spawning (Volkoff and Peter, 1999).

Parental Behaviour

In fish that provide parental care to the eggs or juveniles, male care is more common than that in females (Breder and Rosen, 1966). In males, androgens have been proposed to have suppressive effects on parental behaviour. In the generality of species studied to date, male androgen levels decrease during the parenting phase when compared with the mating phase, even in species where males continue to reproduce after the initiation of parental care. An explanation for the functional significance of this variation stems from the challenge hypothesis (Wingfield, 1984a, b), which postulates an androgen-mediated trade-off between territorial and parental behaviour. During the mating phase, animals often have to compete for sexual partners and androgens are thought to be causally linked to aggression during these periods. Engaging in intrasexual competition for mates leaves less time for parental duties and, thus, during periods of social instability, an increase in androgens would mediate the necessary trade-off between parental care and aggressive interactions.

In most fish species tested so far, androgen levels drop during the parenting phase thus supporting this hypothesis (Oliveira *et al.*, 2002; Table 3.3). However, there are exceptions to this general pattern (e.g., Ros *et al.*, 2003a; Bender, 2006; Desjardins, 2006; Rodgers *et al.*, 2006, Table 3.3) and studies manipulating androgens levels have generally failed to find the predicted suppressive effect of androgens on parental care (Table 3.4). The inconsistency of these results suggests the endocrine mediation of parental care in fish may be more dependent on non-gonadal hormones.

Prolactin (PRL) has been suggested to be a good candidate for the regulation of parental care in both male and female vertebrates (Bridges *et al.*, 1985; Schradin and Anzenberger, 1999). The first demonstration of the role of PRL in fish parental behaviour was described in the wrasse, *Symphodus ocellatus*, where mammalian PRL administration induced egg-fanning behaviour in males (Fiedler, 1962). Following this study, others have described a stimulating effect of this hormone on fish parental behaviour (see Table 3.4). However, other studies failed to associate PRL with parental behaviour. In *Neolamprologus pulcher*, PRL administration failed to induce parental fanning behaviour (Bender, 2006) and in the Nile tilapia, pituitary and plasma circulating levels of the two

Table 3.3 Plasma hormone levels variation in parenting fish when compared with non-parenting fish. Symbols: +, increase; –, decrease; 0, no variation; ND, not described.

	Sex	*Hormone levels variation in parenting phase when compared with non-parenting phase*						*Reference*
		T	11KT	E2	PRL	cortisol	GH	
Batrachoididae								
Porichthys notatus	Males	–	–	ND	ND	0	ND	Knapp *et al.* (1999)
Blenniidae								
Lipophrys pholis	Males	–	–	ND	ND	ND	ND	Oliveira and Canário (unpubl.)
Parablennius parvircornis	Males	–	–	ND	ND	ND	ND	Oliveira *et al.* (2001a)
Centrarchidae								
Lepomis macrochirus	Males	–	–	ND	ND	ND	ND	Kindler *et al.* (1989)
	Males	–	–	ND	ND	+	ND	Magee *et al.* (2006)
Cichlidae								
Sarotherodon melanotheron	Males and females	–	–	–	ND	ND	ND	Specker and Kishida (2000)
	Males	–	–	–	ND	ND	ND	Kishida and Specker (2000)
Sarotherodon galileus	Males	0	0	ND	ND	ND	ND	Ros *et al.* (2003)
Neolamprologus pulcher	Males	0	0	ND	ND	ND	ND	Desjardins (2006)
	Males and females	0[1]	0[1]	0[2]	ND	0	ND	Bender (2006)
Oreochromis mossambicus	Females	ND	ND	ND	+	ND	+	Weber and Grau (1999)
	Females	+	ND	0	ND	ND	ND	Smith and Haley (1988)
Oreochromis niloticus	Females	–	ND	–	0	ND	+	Tacon *et al.* (2000)
Gasterosteidae								
Gasterosteus aculeatus	Males	ND	–	ND	ND	ND	ND	Páll *et al.* (2002a)

(*Table 3.3 Contd.*)

(*Table 3.3 Contd.*)

	Sex	*Hormone levels variation in parenting phase when compared with non-parenting phase*						*Reference*
		T	11KT	E2	PRL	cortisol	GH	
Gobiidae								
Lythrypnus dalli	Males	ND	+	ND	ND	ND	ND	Rodgers *et al.* (2006)
Pomacentridae								
Chromis dispilus	Males	-	-	ND	ND	ND	ND	Pankhurst (1990)
Hypsypops rubicundus	Males	-	-	ND	ND	ND	ND	Sikkel (1993)
Syngnathidae								
Syngnathus acus	Males	-	-	ND	ND	ND	ND	Mayer *et al.* (1993)
Syngnathus typhae	Males	-	-	ND	ND	ND	ND	Mayer *et al.* (1993)

[1]Only measured in males; [2]only measured in females.

PRL isoforms did not differ significantly between incubating or non-incubating females (Tacon *et al.*, 2000). In the Nile tilapia, strong individual variation in the tilapia PRL II was observed during the period of maternal behaviour and, thus, a role for this isoform in the control of maternal behaviour could still be possible (Tacon *et al.*, 2000). In support of this hypothesis, in the closely related Mozambique tilapia, serum concentrations of PRL II were higher in females mouthbrooding post-yolk sac larvae when compared with non-brooding females, and the pituitary levels of this isoform were also significantly higher for females mouthbrooding eggs or yolk sac larvae in comparison with non-incubating females (Weber and Grau, 1999 but see also Wendelaar Bonga *et al.*, 1984). No difference was recorded for PRL I pituitary or serum levels between incubating or non-incubating females, suggesting that this isoform is unrelated with maternal care (Weber and Grau, 1999). In some studies, the positive effects of PRL on parental care were dependent or intensified by co-administration of testosterone, progesterone or gonadotropins (*Pseudocrenilabrus multicolor multicolor* and *Oreochromis mossambicus*, Bartmann, 1968; *Macropodus opercularis*, Machemer, 1971; *Gasterosteus aculeatus*, Molenda and Fiedler, 1971; *Lepomis gibbosus*, Kramer, 1973). Sex steroids have been shown to interact with PRL secretion. With the exception of the platyfish *Xiphophorus maculatus* (Kim *et al.*, 1979), where no effects were detected, E2 has been found to have a stimulatory effect on PRL secretion or mRNA expression during reproductive periods (Wigham *et al.*, 1977; Barry and Grau, 1986; Borski *et al.*, 1991; Williams and Wigham, 1994; Poh *et al.*, 1997; Brinca *et al.*, 2003; Onuma *et al.*, 2005). Both T and 11-KT have also been found to stimulate PRL release and mRNA production and also to potentiate the stimulatory effects of GnRH on PRL release (Wigham *et al.*, 1977; Barry and Grau, 1986; Borski *et al.*, 1991; Onuma *et al.*, 2005). Prolactin was also shown to stimulate steroidogenesis (e.g., Singh *et al.*, 1988; Rubin and Specker, 1992) and, thus, a positive feedback between sex steroids and PRL is likely to occur. These findings are difficult to interpret since according to life-history theory, a trade-off between androgens and parental care should be expected (Wingfield *et al.*, 1990), and thus PRL and androgens are expected to have opposite effects in the regulation of these behaviours. Experimental studies on species with different mating systems and modes of parental care are necessary to confirm the hypotheses that androgen suppresses and PRL promotes parental care behaviour. Also, the identification of the neural substrates upon which PRL and sex steroids are possibly acting to regulate parental care behaviour remains unknown,

Table 3.4 Effects of hormonal manipulations in parental care behaviour.

	Treatment	*Effect on parental care*	*Reference*
Androgens			
Blenniidae			
Parablennius sanguinolentus parvircornis	11KT implants to nesting males	No effect	Ros *et al.* (2004)
Gasterosteidae			
Gasterosteus aculeatus	Castration and 11-KA implants to castrated spawned males	No effect of castration or of 11-KA implants on fanning rate	Páll *et al.* (2002b)
	Castration and MT administration to castrated males	Castration reduces and MT restores fanning behaviour	Smith and Hoar (1967)
Osphronemidae			
Trichogaster trichopterus	MT administration to females	Increase in parental behaviour in response to egg batches	Kramer (1972b)
PRL			
Centrachidae			
Lepomis macrochirus	Administration of the PRL-secretion antagonist bromocriptine to males with eggs	Decrease in fanning rate	Kindler *et al.* (1991a)
Cichlidae			
Neolamprologus pulcher	PRL injections to breeding males and helpers	No effect	Bender (2006)
Symphysodon aequifasciatus	PRL administration to males	Increase in fanning rate only at low dosages	Blüm and Fiedler (1964, 1965)
Pterophyllum scalare	PRL administration to males	Increase in fanning rate only at low dosages	Blüm and Fiedler (1965)

(*Table 3.4 Contd.*)

(*Table 3.4 Contd.*)

	Treatment	*Effect on parental care*	*Reference*
Archocentrus nigrofasciatus	Administration of L-DOPA, a putative inhibitor of PRL synthesis, to females	Decrease in fanning rate	Fiedler *et al.* (1979)
Gasterosteidae			
Gasterosteus aculeatus	PRL administration to males not guarding eggs	Increase in fanning rate	Páll *et al.* (2004)
	PRL injections to males not guarding eggs	Increase in fanning rate	Molenda and Fiedler (1971)
	Implantation of homologous pituitaty PRL-lobes to males not guarding eggs	Increase in fanning rate	de Ruiter *et al.* (1986)
	PRL injections to males	No effect	Smith and Hoar (1967)
Labridae			
Symphodus ocellatus	PRL administration to males	Egg-fanning behaviour induction	Fiedler (1962)

and this is a fundamental step in understanding the mechanisms of endocrine regulation of parental care.

Finally, the growth hormone (GH) may play a direct role on the regulation of fish parental behaviour, as suggested for mammals (Bridges and Millard, 1988). In both *O. niloticus* and *O. mossambicus*, GH plasma levels increase during mouthbrooding (Weber and Grau, 1999; Tacon *et al.*, 2000). In *O. mossambicus*, the pattern of change in GH serum levels between mouthbrooding females or fasted females differed, suggesting that the GH plasma surge observed during mouthbrooding is not fully explained as a response to fasting (Weber and Grau, 1999). However, the hypothesis of GH regulating parental behaviour remains to be tested as no experimental studies have been conducted so far where GH levels were manipulated so as to assess its effects on paternal behaviour.

HORMONES AND AGGRESSIVE BEHAVIOUR

Sex Steroids and Aggressive Behaviour

Gonadal steroids have been viewed as major modulators of aggressive behaviour. This view was mainly based on two types of evidence: (a) dominant males having higher androgen levels than subordinates and (b) experiments on the effects of castration followed by the exogenous administration of androgens (Liley and Stacey, 1983; Villars, 1983; Borg, 1994). For example, among species with alternative reproductive tactics, KT levels are higher in the breeding (bourgeois *sensu* Taborsky, 1997) male than in the alternative (subordinate) male type for 13 out of 16 species (from 9 different families) for which data is available (Oliveira, 2006), and androgen treatment enhances aggressive behaviour in bourgeois males but not in alternative male types (Table 3.5). Although there is some variation in the results of the castration-androgen replacement studies (Table 3.5), a recent meta-analysis has confirmed the reinforcing effect of exogenous androgens on male aggressiveness in teleost fish (Hirschenhauser and Oliveira, 2006).

Data on endocrine correlates of female aggression in teleosts are even more rare (Table 3.5). Basically, three contexts offer the possibility of studying different types of female aggression: (a) maternal aggression displayed by brooding females defending their broods, (b) territorial aggression in species in which females also participate in the defence of a breeding territory (e.g., substrate-brooding biparental cichlids) and (c) direct female-female competition in sex-role reversed species

Table 3.5 Effects of sex hormones on aggressive behaviour in teleost fish.

Family/species	*Treatment (subjects)*	*Effect*	*Reference*
Belontiidae			
Betta splendens	Castration (breeding males)	0*	Weiss and Coughlin (1979)
Macropodus opercularis	Castration (breeding males)	0*	Villars and Davis (1977); Villars (1983)
	Methallibure (castrated males)	-	
	T enanthate (castrated males)	+	
Trichogaster trichopterus	Castration (breeding males)	0	Johns and Liley (1970)
Gasterosteidae			
Gasterosteus aculeatus	Castration (pre-nesting males; SD)	0	Hoar (1962)
	Castration (pre-nesting males; LD)	+	
	Castration (pre-nesting males)	0	Baggerman (1966)
	Castration (nesting males)	-	Baggerman (1966)
	Castration (pre-nesting males)	0	Wootton (1970)
	Castration (nesting males)	-	
	T propionate (nesting males)	0	Rouse *et al.* (1977)
	Cyproterone acetate (nesting males)	-	
Centrarchidae			
Lepomis megalotis	Castration (territorial males)	0	Smith (1969)
Lepomis gibbosus	Castration (territorial males)	0	Smith (1969)
	Cyproterone acetate (males)	-	Kramer *et al.* (1969); Kramer (1971)
	Methyl-T or T propionate (males)	-	Kramer *et al.* (1969)
	Methyl-T (males pre-treated w/GtH inhibitor)	+	Kramer (1971, 1972a, 1973)

(*Table 3.5 Contd.*)

(*Table 3.5 Contd.*)

Family/species	*Treatment (subjects)*	*Effect*	*Reference*
	Methallibure (males)	0	Kramer (1973)
	Ovine LH or perch GtH (males)	+	Kramer *et al.* (1969)
	mammalian LH (males pre-treated w/methallibure)	+	Kramer (1971, 1973)
Lepomis macrochirus	Cyproterone acetate (males and females)	+	Avila (1976) in Villars (1983)
	Methyl-T or T propionate (males)	0	Avila (1976) in Villars (1983)
Cichlidae			
Aequidens pulcher	Cyproterone acetate (males)	-	Molenda, unpub. in Fiedler (1974)
	T (males)	+	Molenda, unpub. in Fiedler (1974)
	T propionate (females)	+	Munro and Pitcher (1985)
	Estradiol-17β benzoate (females)	-	Munro and Pitcher (1985)
Astatotilapia burtoni	Castration (males)	-	Francis *et al.* (1992)
	T (males)	+	Fernald (1976)
Oreochromis mossambicus	Cyproterone acetate (males)	-	Molenda, unpub. in Fiedler (1974); Kramer *et al.* (1969)
	T (males)	-	Molenda, unpub. in Fiedler (1974)
	T (male and female fry)	+ (as adults)	Billy and Liley (1985)
Pseudocrenilabrus multicolor	Castration (males)	-	Reinboth and Rixner (1970)
Pterophyllum scalare	LH (males)	+	Blüm and Fiedler (1965)
Symphysodon aequifasciata axelrodi	LH (males)	+	Blüm and Fiedler (1965)
Cyprinodontidae			
Cyprinodon variegatus	T (juveniles)	+	Higby *et al.* (1991)

(*Table 3.5 Contd.*)

(*Table 3.5 Contd.*)

Family/species	*Treatment (subjects)*	*Effect*	*Reference*
Poecilidae			
Xiphophorus maculatus	Castration (males)	-	Chizinsky (1968)
Xiphophorus helleri	T propionate (females)	+	Noble and Borne (1940)
Gobiidae			
Bathygobius soporator	Castration (breeding males)	-	Tavolga (1955)
Blenniidae			
Parablennius parvicornis	KT implants (nest-holder males)	+	Ros *et al.* (2004)
	KT implants (sneaker males)	-	Oliveira *et al.* (2001b)
	17α-methyl-T implants (sneaker males)	-	Oliveira *et al.* (2001b)
Salaria pavo	KT implants (sneaker males)	0	Oliveira *et al.* (2001c)
Batrachoididae			
Porichthys notatus	KT implants (sneaker males)	0	Lee and Bass (2005)
Apteronotidae			
Apteronotus leptorhynchus	T (females)	+**	Dulka and Maler (1994)
Apteronotus albifrons	Castration + T	0**	
	Castration + KT	0**	
	Castration + DHT (males)	0**	Dunlap *et al.* (1998)

(*) incomplete gonadectomy or evidence for rapid testicular regeneration; (**) chirping rate was used as a measure of aggressiveness.

(e.g., syngnathids; Eens and Pinxten, 2000). These different types of female aggression not only serve different functions but may also have different causal mechanisms. Unfortunately, despite the wide diversity of modes of reproduction and social systems among teleosts that offer the possibility to study female aggression in natural contexts and with a comparative perspective, there are almost no studies on this topic.

In the Mozambique tilapia, maternal aggression was studied during the mouthbrooding cycle. Brooding females become more aggressive as the brooding cycle progresses, reaching a peak in advanced phases of the cycle when they begin to defend fry that are starting to forage outside

their mouth (Oliveira and Almada, 1998b). This suggests that maternal aggression in mouthbrooding cichlids may play an important role as a means of defending a mobile feeding territory for the fry around the female. Interestingly, female circulating T levels have a bimodal distribution during the brooding cycle, with one of its peaks occurring at the final phase of oral incubation (Smith and Haley, 1988) when the peak in maternal aggression is also observed. It should be stressed upon that the agonistic repertoire of brooding females lacks the stereotyped displays and fighting behaviours exhibited by males in territorial disputes, consisting mainly of more direct agonistic patterns, like charging, chasing, and butting (Oliveira and Almada, 1998b).

In the substrate-brooder cichlid *N. pulcher*, breeding pairs jointly defend a breeding territory where they guard and care for their brood; females are more aggressive than males towards conspecific intruders and also have higher androgen levels than their mates (Desjardins *et al.*, 2005). Therefore, their higher androgen levels may reflect their higher investment in brood defence when compared with males.

Finally, the only investigation on the hormonal control of aggression in female groups has been performed in the Blue Acara (*Aequidens pulcher*). In this species, T increased and estradiol reduced female aggressive behaviours (Munro and Pitcher, 1985).

The positive effects of androgens on the expression of aggressive behaviour among teleosts are most probably mediated by their action on central motivational systems underlying aggression. Usually, the effects of steroids, including androgens, on motivational mechanisms involve the regulation of neuropeptide gene expression in the limbic system (e.g., AVT and IT), and/or the direct modulation of central neurotransmitter systems (e.g., catecholamine, serotonin and GABA), that subsequently influence the central states that control the behavioural output.

Cortisol and Aggressive Behaviour

The potential role of the hypothalamic-pituitary-adrenal axis in aggressive behaviour in mammals was investigated only in the mid-1960. Soon after, it was found that both glucocorticoids and ACTH affect aggressiveness (Leshner, 1983). In teleost fish, the relationship between the HPI axis and aggressive behaviour only started to be explored in the mid-1980s using water-borne exposure of female Blue Acara (*Aequidens pulcher*) to cortisol and to its synthesis blocker metyrapone (Munro and Pitcher, 1985). In this study, cortisol affected agonistic behaviour in a selective

manner. In social groups, cortisol increased the charging behaviour of dominant males, which was interpreted by the authors as a consequence of increased submissiveness in lower ranks. It also promoted foraging behaviour in the absence of food, which was interpreted as a displacement activity (Munro and Pitcher, 1985). In isolated fish, cortisol increased aggressive encounters towards models but not towards mirror images. Again, this was interpreted as a sign of an increase in submissiveness since in the mirror situation, in which there is the perception of an aggressive response from the stimulus (not present in the dummies), there was no increase in aggressiveness. Metyrapone reduced aggression in all cases and promoted schooling in social groups. Since this drug inhibits the enzyme 11-β-hydroxylase that is present not only in the synthesis of cortisol but also in the biosynthesis of KT from T, the observed effects could be due to an androgenic effect via KT. To rule out this hypothesis, a combined treatment of metyrapone and cortisol was used. However, this combined treatment led to results comparable to those obtained with metyrapone alone which suggests a toxic effect of the dosage used (Munro and Pitcher, 1985).

In the rainbow trout lines selected for high- (HR) and low-responsiveness (LR) to stress (Øverli *et al.*, 2005), LR fish tend to be socially dominant over HR fish in paired encounters (Pottinger and Carrick, 2001), but there is no difference between HR and LR individuals in their response towards an intruder in their home tank, which suggests that the cortisol effect on aggressive behaviour is context specific (i.e., HR and LR fish differ in unfamiliar environments but not when challenged in their home environment, Schjolden *et al.*, 2005). In a non-selected population of rainbow trout, cortisol levels before contests were also found to be higher in individuals that become subordinates in comparison with individuals that became dominants (Sloman *et al.*, 2001a). In another experimental study using staged encounters, the usually dominant larger animals lost their competitive advantage when treated with an intraperitoneal cortisol implant prior to the test, suggesting a direct negative effect of cortisol in the fish competitive ability (Gilmour *et al.*, 2005). In a subsequent study, juvenile rainbow trout were given an intraperitoneal implant of either cortisol or cortisol plus the GC receptor antagonist RU486 (mifepristone) and were paired either with smaller (< 5% body size) opponents or with size-matched opponents. Cortisol treatment increased the probability of fish becoming subordinates in both experimental situations, and this effect was not present when the GC receptor antagonist was given together with cortisol, which confirms the

specificity of the effect (DiBattista *et al.*, 2005). Moreover, it was also found that cortisol-treated fish had higher serotonergic activity and lower dopaminergic activity in the telencephalon, but not in the hypothalamus, an effect that was abolished by the combined treatment with RU486 (DiBattista *et al.*, 2005). These results suggest that cortisol may be acting on behaviour through the modulation of central executive mechanisms rather than on motivational systems.

Finally, in a recent study with the cichlid *A. burtoni*, males were challenged by a video presentation of a dominant male displaying aggressive behaviour. The response was moderated by the cortisol level of the subjects: non-territorial males with intermediate cortisol levels reacted directly to the video image, while males with either high or low cortisol levels showed more displaced aggression towards their tank mate. These results suggest an optimal cortisol value that promotes direct reply towards challenging individuals, which may further lead to success in social groups (Clement *et al.*, 2005).

It is also interesting to note that cortisol can affect the expression of specific behavioural patterns. The weakly electric fish, *Apteronotus leptorhynchus*, produces two electrocommunication signals: a continuous EOD and rapid EOD modulations named 'chirps'. Chirping has been interpreted as an aggressive signal since chirp rate increases during agonistic interactions, and individuals respond to playbacks of EOD of approximately the same frequency as their own EOD (EOD frequency is an indicator of sex and social status, Hagedorn and Heiligenberg, 1985; Dunlap *et al.*, 1998) with chirping often accompanied by attacks to the electrodes used to present the EOD signals (Dunlap *et al.*, 1998, 2002). In this species cortisol-treatment induced higher chirp rates, but had no effect in EOD frequency (Dunlap *et al.*, 2002).

The association between cortisol and social status has been documented for other species of teleosts and will be discussed below.

The Neuropeptides AVT and IT and Aggressive Behaviour

Divergent effects of AVT on aggression have been documented among teleosts, with examples of AVT promoting, decreasing or having no effect on aggressive behaviour. In a natural population of the bluehead wrasse (*Thalassoma bifasciatum*), territorial males injected with AVT tended to decrease chases towards initial phase individuals, while in non-territorial terminal phase males, AVT increased both aggression towards initial

phase males and territorial behaviour (Semsar *et al.*, 2001). The specificity of the AVT effect was not conclusive since the administration of the AVP-V_{1a} receptor antagonist (Manning compound) to terminal-phase males decreased the chases towards initial phase individuals but not towards other terminal phase males (Semsar *et al.*, 2001). These apparently contradictory results may be explained by dose-related effects of AVT, as suggested from another field study with the beaugregory damselfish (*Stegastes leucostictus*). In this work, AVT injections increased and Manning compound administration decreased aggression by territorial males towards intruders in simulated territorial intrusion tests (Santangelo and Bass, 2006). However, this effect was only recorded for medium dosages, with both low and high AVT doses eliciting a response similar to saline controls (Santangelo and Bass, 2006). These results suggest that AVT effects are dependent on the dynamics of AVT binding to its receptor in target tissues. In contrast, in the other three species studied so far, exogenous administration of AVT decreased aggressive behaviours in territorial males, both in natural and laboratory settings (plainfin midshipman, Goodson and Bass, 2000; weakly electric fish, *A. leptorhynchus*, Bastian *et al.*, 2001, and Amargosa pupfish, *Cyprinodon nevadensis amargosae*, Lema and Nevitt, 2004).

Regarding the effects of isotocin (IT) on aggressive behaviour, only two studies are available to date. In the plainfin midshipman, where males use grunt vocalizations as an aggressive acoustic display (Brantley and Bass, 1994), IT elicited fictive grunt vocalizations in a neurophysiological preparation of parasitic (sneaker) males but not of territorial males (Goodson and Bass, 2000). In the beaugregory damselfish, IT had no effect on aggressive behaviour (Santangelo and Bass, 2006). Therefore, the effects of these two neuropeptides on the regulation of aggressive behaviour in teleosts seem to be species dependent and also vary with sex type and context, as suggested by Goodson and Bass (2001) in a wider phylogenetic review of AVP/AVT systems and behaviour.

Growth Hormone, Somatostatin and Aggressive Behaviour

Hormones involved in the regulation of somatic growth have also been implicated in control of aggressive behaviour in teleosts, in particular the growth-hormone (GH) and somatostatin (SS). GH release is stimulated by GHRH and inhibited by SS, and both are synthesized by hypothalamic neurons that project to somatotropes in the pituitary (Björnsson, 1997).

Apart from its effects on GH secretion, SS also plays an important role as a regulatory peptide in a variety of physiological contexts, also acting as a neuromodulator in the CNS. In the cichlid *A. burtoni*, the treatment of territorial males with the SS agonist octreotide decreased chasing behaviour, whereas treatment with the SS antagonist cyclosomatostatin increased the frequency of chases in a dose-related fashion (Trainor and Hofmann, 2006). Since GH promotes aggressive behaviour in fish (see below), the behavioural effects of SS analogs may be explained by an action of SS on GH release at the pituitary level. In fact octreotide suppresses GH secretion in rainbow trout (Very *et al.*, 2001), and the use of a GHRH antagonist also inhibits chasing; however, no direct effects of GH on aggressive behaviour were detected (Trainor and Hofmann, 2006). It must be stressed here that the SS analogs and the GHRH antagonist only affected chasing behaviour but had no effect on threatening behaviour, suggesting a selective action on overt aggression but not on display behaviours. Since SS also modulates androgen production in the mammalian testes (Gerendai *et al.*, 1996), it could be hypothesized that the behavioural effects of SS were mediated via a reduction of androgen release by the testes. However, octreotide injections increased circulating T levels in dominant males (Trainor and Hofmann, 2006). Unfortunately, no results on KT levels were reported, which makes the interpretation of the data difficult. If KT had been measured and no effects of octreotide had been detected, than the elevated T levels could reflect an inhibitory effect of SS on the conversion of T into KT which is the behaviourally active androgen in teleosts (as mentioned above KT and not T has been associated with dominance status in teleost fish), and the data would be compatible with an androgen mediation of the behavioural effects of SS. In addition, the expression of SS receptors subtype 2 and 3 in the testes were negatively correlated, both with androgen levels and with threatening behaviour (Trainor and Hofmann, 2006). Taking into consideration the diversity of SS receptor subtypes in fish (Lin and Peter, 2003; Nelson and Sheridan, 2005), and their differential expression in different tissues, it is possible that the SS effects on different behavioural sub-systems (e.g., chasing vs displaying) are being mediated through different physiological mechanisms (central vs peripheral). Finally, the data on SS effects on aggression described above are in apparent contradiction with the previously reported association between social dominance and SS activity in *A. burtoni* males. Since territorial males have larger SS-ir neurons than non-territorials in the POA (Hofmann and Fernald, 2000), it could be expected that SS

would increase aggression. However, as the original study already stressed, the soma size of SS-ir neurons was responding to manipulations in social status, and thus the causal link should be interpreted as a social modulation of the SS system and not the reverse.

GH has been found to correlate with aggression. For example, in dyadic encounters of rainbow trout, aggression was higher between two GH-treated fish than between two control fish (Jönsson *et al.*, 1998). Since in GH-control pairs there was no difference in the number of encounters won by GH-treated when compared with control fish (Jönsson *et al.*, 1998), GH is probably not affecting the fighting ability per se but increasing aggression in an indirect manner, for example, by increasing swimming activity and, therefore, the encounter rate between the opponents. This result is in accordance with similar plasma levels of GH previously found in paired dominant and subordinate fish (Johnsson *et al.*, 1996). More recently, intracerebroventricular injections of GH into the third ventricle of the brain of juvenile rainbow trout increased their swimming activity and dopaminergic activity in the hypothalamus when compared to sham-injected fish (Jönsson *et al.*, 2003), which further supports the hypothesis of an indirect route of action of GH on the expression of aggressive behaviour in rainbow trout. In contrast, in Atlantic salmon (*S. salar*) parr, GH-treatment of subordinate individuals stimulates an increase in the dominance rank in a semi-natural environment, without having an impact on space use (Martin-Smith *et al.*, 2004). No explanation has been found for these contrasting results.

NEUROTRANSMITTER PATHWAYS AND AGGRESSION

As mentioned above, steroids may act on central neurotransmitter pathways underlying the expression of social behaviour, namely on catecholamine, serotonin and γ-amino butyric acid (GABA) systems. Evidence for the involvement of these three systems on the modulation of aggressive behaviour in vertebrates is quite robust (for recent reviews: see Miczek *et al.*, 2002; de Almeida *et al.*, 2005). In fish, the involvement of these systems in aggression and their modulation by sex steroids has received less attention.

Dopamine

In mammals, dopaminergic neurons are found in the forebrain and midbrain. The diencephalic dopaminergic neurons are involved in the

regulation of the endocrine system, whereas the midbrain dopaminergic neurons are classically divided into two sub-systems: (a) the nigrostriatum, that projects from the substantia nigra to the dorsal striatum and that is involved in the organization of motor programs and (b) the mesocorticolimbic system, that projects from the ventral tegmentum to the ventromedial striatum, the forebrain limbic system and parts of the cortex, and acts on motivational and executive functions (Björklund and Lindvall, 1984). In teleosts, the diencephalic component is well described and has been studied in relation to endocrine control (e.g., dopaminergic innervation of the pituitary from the preoptic recess in the hypothalamus inhibits LH release in some fish species, in particular cyprinids; Yaron and Sivan, 2006). The telencephalon also receives dopaminergic innervation but its origin has not been clearly established as yet (Meek, 1994; Kaslin and Panula, 2001). The traditional view is that the homologs of the mesencephalic dopaminergic nuclei are missing in teleosts (Meek, 1994). Nevertheless, both dopamine agonist (apomorphine) and antagonist drugs (e.g., d-amphetamine) have behavioural effects in different teleost species, both on locomotory behaviour, and on appetitive behaviours suggesting that a motivational circuit is being targeted (Munro, 1986; Kunze and Wezstein, 1988; Lett and Grant, 1989; Mok and Munro, 1998). Of particular interest are the effects of apomorphine on aggressive behaviour in the cichlid fish *Aequidens pulcher* (Munro, 1986) and the reward-like effect of amphetamine in goldfish (Lett and Grant, 1989). Recently, an immuno-cytochemical study has identified glial cells with the enzyme aromatase (that produces estrogens from androgens) in the same brain regions as tyrosine hydroxilase immuno-reactive neurons, suggesting a potential interaction between neurosteroidogenesis and the dopaminergic system. Since the areas of co-regionalization of both systems (i.e., POA-hypothalamus) are important brain areas for the regulation of social behaviour, these data suggests that sex steroid-dopamine interactions may be implicated in the control of male sexual and aggressive behaviour (Marsh *et al.*, 2006).

Serotonin

Like in other vertebrates (Nelson and Chiavegatto, 2001), in teleosts serotonergic activity are inversely associated with social status (e.g., arctic charr, *Salvelinus alpinus*, Winberg *et al.*, 1991, 1992; rainbow trout, Winberg *et al.*, 1993 and the cichlid fish, *A. burtoni*, Winberg *et al.*, 1997b).

Moreover, in the bluehead wrasse, the administration of fluoxetine—a selective serotonin reuptake inhibitor (SSRI) that enhances serotonergic activity—reduced the aggressive behaviour of territorial males towards intruders both in the laboratory and in nature (Perrault *et al.*, 2003). Consistently, the treatment of male firemouth cichlids (*Cichlasoma meeki*) with p-chlorophenylalanine, a serotonin synthesis inhibitor, resulted in an increase of mirror-elicited aggression (Adams *et al.*, 1996). Finally, in rainbow trout fish fed on a dietary supplement of L-tryptophan, the precursor of serotonin, for 7 days significantly reduced their aggressive behaviour in a resident-intruder test and showed elevated levels of serotonergic activity in the brain (Winberg *et al.*, 2001). These results are in agreement with similar data on rodents that shows that different pharmacological strategies to increase serotonin levels result in a decrease in aggressive behaviour (Nelson and Chiavegatto, 2001). Interestingly, and in parallel with findings from rodents (e.g., Ferris *et al.*, 1997), the fluoxetine-treated male wrasses exhibited lower AVT mRNA expression in the POA, suggesting that the serotonergic effect on aggressive behaviour was being mediated by an interaction with the AVT system (Semsar *et al.*, 2004). Apart from their action as a SSRI, fluoxetine is also known to increase the production of neurosteroids, in particular allopregnanolone, which is a modulator of the $GABA_A$ receptor and has inhibitory effects on AVP expression in mammals (Majewska *et al.*, 1986; Hansen *et al.*, 2003). Thus, the anti-aggressive effect of fluoxetine can be in part mediated by allopregnanolone actions on GABA and on AVT (e.g., Pinna *et al.*, 2003).

GABA

The neurotransmitter GABA is present in most inhibitory synapses of the mammalian central nervous system and has an inhibitory role on aggressive behaviour in mammals (Miczek *et al.*, 2002; de Almeida *et al.*, 2005). GABAergic neurons are widely distributed throughout the forebrain of salmonids and are particularly abundant in the POA, an area rich in sex steroid receptors (mainly estrogen receptors) (Anglade *et al.*, 1999). Therefore, the localization of GABA neurons is ideal for them to interact with the HPG axis and, consequently, with reproductive behaviour (including territorial aggression). In fact, the involvement of GABA in the control of gonadotropin release has been documented both for male and female teleosts, and the inhibitory effect of estrogens on GABA has been viewed as part of the negative feedback of estrogens on the HPG

axis (Kah *et al.*, 1992; Mañanos *et al.*, 1999). Recently, sex differences in the reactivity of the GABAergic system to sex steroids have been described in the goldfish during the breeding season: five-day intra-peritoneal implants either of T or of progesterone decreased the levels of the GABA-synthesizing enzyme glutamic acid descarboxilase (GAD) in the telencephalon of males and had no effect on the females. On the other hand, progesterone treatment reduced the GAD mRNA levels in the hypothalamus of females but not of males (Larivière *et al.*, 2005). This steroidal modulation of the GAD expression in the male telencephalon may modulate GABA-mediated behavioural outputs. In fact, experimental lesions in the ventral area of the telencephalon, an area that expresses high GAD mRNA levels in trout (Anglade *et al.*, 1999), disrupt male sexual behaviour in goldfish (Kyle and Peter, 1982). However, very few studies have been conducted on the direct role of GABA on aggressive behaviour in fish. In gymnotiform weakly electric fish, the GABAergic system has been implicated in the control of the EOD and on the skeletomotor activity underlying defensive behavioural patterns (e.g., *Hypopomus brevirostris*, Kawasaki and Heiligenberg, 1990 and *Gymnotus carapo*, Duarte *et al.*, 2006a, b).

HORMONES AND AFFILIATIVE BEHAVIOUR

Although teleost fish display the widest variation in mating systems, there have been few investigations on the neuroendocrine basis of affiliative behaviours. From the several monogamous teleost species with pair-bond formation, in only one case have the physiological mechanisms been investigated (Ros *et al.*, 2003a, b). In the St. Peter's fish (*S. galilaeus*; Cichlidae), breeding pairs establish pair bonds that last for long periods of time (Ros *et al.*, 2003a). However, there is great flexibility in male mating behaviour with males that may or may not stay with their partners after spawning and thus contribute or not to parental care of the offspring (Balshine-Earn, 1996; Fishelson and Hilzerman, 2002). To test whether males showing more intense pair bond would have lower androgen levels, paired males were offered the access to a novel female out of sight from their female partner and the time spent with each female was measured. At the end of the behavioural test, a significant negative correlation between KT levels and partner preference was detected, suggesting that the males with a weaker pair bond had higher androgen levels (Oliveira *et al.*, 2001e). However, the exogenous administration of androgens to paired males subsequently tested in the same set-up revealed that T-treated males had a similar partner preference to control males. This

suggests that the association between pair-bond strength and androgen levels in St. Peter's fish males is not due to a causal effect of androgens on partner preference, but most probably reflects the variation in partner preference behaviour observed among males (Oliveira *et al.*, 2001e). This conclusion is supported by results from a study in semi-natural conditions in Lake Kinneret (Israel) where it was found that polygynous males did not differ from monogamous males on their androgen levels (Ros *et al.*, 2003a).

In mammals, the neuropeptides AVP and oxytocin (OT) have been implicated in the regulation of pair bonding in monogamous mating systems. The most thoroughly investigated system has been the prairie vole, *Microtus ochrogaster*, in which partner preference is promoted by OT in females and by AVT in males (Young *et al.*, 1998). The only study that has addressed the relationship between AVT and IT (the piscine homologues of AVP and OT), and affiliative behaviours has used the goldfish (*C. auratus*) and was done in a non-reproductive context. In this species, either AVT or IT was directly infused in the brain and approach behaviour towards conspecifics was recorded. AVT inhibited and both a V1 receptor antagonist (Manning compound) and IT promoted approach behaviour (Thompson and Walton, 2004).

SOCIAL FEEDBACK ON ENDOCRINE MECHANISMS

Hormones not only act as facilitators of the expression of social behaviours but their signalling pathways are also affected by the social environment in which the animal is living. Nowadays, there is ample evidence for the social modulation of different neuroendocrine systems, including the hypothalamic-pituitary-gonadal (HPG) axis and the hypothalamic-pituitary-inter-renal (HPI) axis. For example, in teleosts, the exposure to social and sexual stimuli elicits endocrine responses. In male salmonids, a rise in sex steroid and gonadotrophin levels and an increase in milt production is observed in the presence of ovulated females (Liley *et al.*, 1986, 1993; Rouger and Liley, 1993). Moreover, anosmic males in the presence of sexually active females have lower levels of sex steroids and a lower sperm production than males with intact olfactory epithelium, which suggests that chemical signals may play an important role in this social modulation of hormone levels. In the Mozambique tilapia, males are sensitive to the maturation stage of females courting more intensively ovulated females (Silverman, 1978). This effect also seems to be mediated by chemical signals emitted by receptive (i.e., pre-ovulatory) females (Miranda *et al.*, 2005). In Mozambique tilapia males, 11-ketotestosterone

circulating levels also increase in response to courtship interactions (Borges *et al.*, 1998). Male-male competitive interactions may also induce an endocrine response in the participating individuals, a response especially sensitive in the case of the androgens and glucocorticoids (Oliveira *et al.*, 2002).

THE SOCIAL MODULATION OF THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS

The modulation of androgen levels by the social challenges faced by the individual has been described for a wide range of vertebrate species, ranging from fish to primates, including humans (Oliveira, 2004; Hirschenhauser and Oliveira, 2006). Since androgens also have an effect on the expression of aggressive behaviour, the social modulation of androgen levels has been interpreted as an adaptation for the individuals to adjust their agonistic motivation to the social environment in which they live. This reciprocal relationship between androgens and behaviour was first formally conceptualized by Mazur (1976) and later led to the proposal of the 'challenge hypothesis' by John Wingfield and associates (Wingfield *et al.*, 1987, 1990), according to which the social interactions in which the subject is involved would determine its androgen levels. The challenge hypothesis proposes that at the beginning of the breeding season, androgen levels rise from a non-breeding baseline to a higher breeding baseline sufficient for the animal to enter a reproductive stage (i.e., gametogenesis, the expression of secondary sexual characters, and the performance of reproductive behaviour). Subsequently, in response to social stimuli such as male-male interactions and the presence of receptive females, androgen levels can further increase until they reach a maximum physiological level (Wingfield *et al.*, 1990). However, the increase of androgen levels above the breeding baseline should have no additional effect on reproduction and may, therefore, be regarded as a direct short-term response to the social environment (Wingfield *et al.*, 1990; Oliveira, 2004). A number of predictions can be generated from the challenge hypothesis. Below, some of these predictions will be analyzed with special consideration to their validity in teleost fish.

Testing the Challenge Hypothesis in Teleosts

A first prediction of the challenge hypothesis is that the androgen fluctuations in relation to territorial intrusions should be moderated by the mating system. In fish, few studies have analyzed the effects of

simulated territorial intrusions on androgen levels. In a natural population of the stoplight parrotfish (*Sparisoma viride*), Cardwell and Liley (1991) found that peaks of androgens could be induced in established territorial males by experimental intrusions of other males. In a study specifically designed to test the challenge hypothesis in cichlid fishes, a standardized laboratory simulated territorial intruder protocol was applied to males from 5 different species of cichlids with different mating systems (*N. pulcher*: monogamous biparental, territorial pair with helpers of all sizes and both sexes; *Lamprologus callipterus*: polygynous biparental with parasitic sneaker males; *Tropheus moorii*: polygynous maternal mouthbrooders with temporary pair formation; *Pseudosimochromis curvifrons*: polygynous maternal mouthbrooders, lek breeders and *O. mossambicus*: polygynous maternal mouthbrooders, breeding in leks, with facultative parasitic males; Hirschenhauser *et al.*, 2004). In all these species, resident males KT levels—but not T levels—responded to the territorial intrusions and the magnitude of the response was associated to the type of mating system (monogamous > polygynous > lek breeding) (Hirschenhauser *et al.*, 2004). The KT responses to interactions with ovulating females were also observed in maternal mouthbrooders but not in biparental species (e.g., Lamprologini). In another laboratory study, Neat and Mayer (1999) failed to detect any differences in plasma concentrations of T or KT between winners and losers of staged fights. It should be noted that in contrast with the two previous studies, in this case a resident-intruder paradigm was not used and, therefore, an asymmetry was not present at the start of the interaction and this might explain the lack of androgen response in the social interaction.

Although the challenge hypothesis was initially proposed to explain the social modulation of androgens among males, its validity has recently been tested in females from species where the females also defend territories (e.g., California mice, *Peromyscus californicus*; Davis and Marler, 2003). In teleosts, the response to territorial intrusions in females has been documented for two species (Desjardins *et al.*, 2005; Hay and Pankhurst, 2005). In the cooperatively breeding *N. pulcher*, an increase in both T and KT levels in paired females defending a breeding territory occurs following a staged intrusion by a conspecific (Desjardins *et al.*, 2005). The magnitude of the androgen response was inclusively higher in females than in males (Desjardins *et al.*, 2005). In contrast, in the spiny damselfish, *Acanthochromis polyacanthus*, where both parents contribute to territory and brood defence, it has been shown for a laboratory population that resident females that are more aggressive towards

intruders than their mates show no endocrine (T or cortisol) response to paired encounters with intruder females or males (Hay and Pankhurst, 2005). On the contrary, resident males showed increased T, but not KT, levels when challenged by intruder males (Hay and Pankhurst, 2005). These results suggest inter-specific variations in the response of the female endocrine system to the social environment that need to be analyzed in greater detail in the future.

A second prediction of the challenge hypothesis is that since territorial/dominant males have to defend their territories and status against competitors, it would be expected that they would have higher androgen levels than non-territorial/subordinate males. In a recent meta-analysis of the challenge hypothesis in vertebrates, it was found that among the 12 teleost species included in the study, there was a strong effect of the social status on androgen levels (Hirschenhauser and Oliveira, 2006). However, the causal link between androgen levels and dominance can be explained in two ways: (a) androgen levels are the predictors of social status; or (b) the social status itself is the cause and not the consequence of higher androgen levels. In an attempt to disentangle these two hypotheses, one can compute temporal cross-correlations between androgen levels and social rank in groups where the social hierarchy is being established. The rationale behind this approach is that if androgen levels are the causal factors of social status acquisition, it is expected that androgen levels before group formation would be good predictors of the social status achieved after group formation. In contrast, if androgen levels are a response to the acquired social status, it is predicted that only after group formation the correlation between androgen levels and social status should be present. Oliveira *et al.* (1996) computed such correlations between androgen levels and a social dominance index during group formation in males of the cichlid fish *O. mossambicus* and found a lack of correlation between the androgen levels prior to group formation (both T and KT) and the social status achieved, but strong correlations between androgen levels measured after group formation (both T and KT) and the acquired social status. Therefore, the associations found between androgen levels and social status may be potentially explained by the challenge hypothesis in different species, reflecting a more challenging social environment for territorial/dominant males than for non-territorial/subordinate ones.

This association between social status and androgen levels may only be present at periods of social instability, when social challenges are frequent. An illustration of this hypothesis comes from studies with the

swordtail fish *Xiphophorus helleri*. Despite the fact that in dyadic interactions between males of this species some aspects of aggressive behaviour are associated with high levels of androgens (Hannes, 1986), no relationship between social dominance and androgens is found in a socially stable community tank (Hannes, 1984). It is possible that the association between androgen levels and social status is only present at periods of social instability and that in stable social groups the androgen levels become dissociated from the social status.

Additionally, it should be noted that the social environment might modulate the androgen responsiveness not only through fluctuations in androgen levels but also by influencing the expression of androgen receptors in target tissues. This alternative (or complementary) mechanism has been demonstrated in the cichlid fish *A. burtoni*, in which dominant males have higher levels of mRNA expression of the steroid receptors AR-α, AR-β, ER-βa, and ER-βb, but not ER-α, in the anterior brain than subordinate males (Burmeister *et al.*, 2007).

A third prediction of the challenge hypothesis is that since the probability of a territorial male suffering a territorial intrusion is higher in more dense populations, it is expected that males breeding in more dense populations have higher androgen levels.

This prediction should be treated cautiously because there may be mechanisms to avoid aggression in situations of increased population density. In any case, a positive association between the density of breeding territories and higher levels of androgens have also been found in the meta-analysis mentioned above (available data from 6 teleost species was used in this analysis; Hirschenhauser and Oliveira, 2006), thus confirming this prediction among fish.

Androgens as Mediators of the Adjustment of Social Behaviour to Social Context

As mentioned above, social modulation of androgens allows individuals to adjust their aggressive behaviour to the current social context according to their relative competitive ability. This mechanism would allow subordinate individuals to down-regulate the expression of their aggressive behaviour, thus avoiding the costs associated with agonistic interactions that they have low probabilities of winning and conversely promoting the persistence of aggressive behaviours in dominant individuals, thus reinforcing their social status (Oliveira, 2004). As an example, in the Gulf toadfish, *Opsanus beta*, territorial males exposed to acoustic playbacks

of a putative male intruder responded with an increase in androgen levels and vocal activity (Remage-Healey and Bass, 2005). The rapid increase (within 10 min) in the advertisement call can be induced by an exogenous administration of androgens that induces a raise in circulating levels that mimics the androgen response to the territorial challenge, thus suggesting a rapid (non-genomic) effect of androgens on the neural circuits underlying the vocal behaviour (Remage-Healey and Bass, 2006).

In social species, fish interact with each other frequently and these interactions modulate subsequent interactions among them and with other group members (e.g., in dominance hierarchies, on territories) forming social networks, which raises the possibility that dyadic interactions can be both observed and influenced by the presence of conspecifics (McGregor, 1993). This scenario potentially increases the complexity of the interplay between hormones and behavior in the interacting dyad and any other conspecifics exposed to the interaction. Thus, a number of group phenomena that have been described in social ethology (e.g., bystander effects, audience effects, winner-loser effects, dear enemy effects) may be mediated at the physiological level by transient changes in androgen levels. For example, in the Mozambique tilapia, bystander males that were able to observe—through a one-way glass—two conspecific neighbours fighting, presented higher androgen levels (both T and KT) than control bystanders that observed two neighbours resting or performing maintenance activities (Oliveira *et al.*, 2001d). The increase in androgen levels in bystanders potentially mediates the priming effect of aggressive motivation in spectators of agonistic interactions (Clotfelter and Paolino, 2003). On the other hand, the presence of an audience also affects both the behaviour and the androgen levels of the contestants. In the Siamese fighting fish, dyads of fighting males increase the intensity of conspicuous displays and decreased highly aggressive acts in front of a female audience, whereas the frequency of more aggressive acts increased in the presence of a male audience (Doutreland *et al.*, 2001; Matos and McGregor, 2002). In parallel with these results, it has been shown recently that the androgen response to the presence of an audience was dependent on the type of audience: males fighting in the presence of a female audience had lower KT levels than when fighting with no audience present, and KT levels were significantly higher when fighting in front of a male audience (Dzieweczynski *et al.*, 2006). In summary, the androgen response induced by the social network where an animal lives can help the individuals to adjust their internal state (motivation) according to the multiple facets of the social interactions

they were exposed to. In fact, a recent study has demonstrated that the androgen responsiveness to social challenges in fish is dependent on the availability of information on the outcome of the fight (Oliveira *et al.*, 2005a), suggesting that in fish a cognitive activation of the androgen response to social competition is present, similar to the cognitive activation theory of stress described for other vertebrates (Eriksena *et al.*, 2005).

Mechanisms Underlying the Social Modulation of Androgen Levels and Behaviour: Social Control of the GnRH Systems

Since androgen production is under the control of the HPG axis, it is also expected that other hormones of this axis be open to the influences of the social environment. In fact, in the African cichlid fish *A. burtoni*, territorial males that have higher androgen levels (Parikh *et al.*, 2006) also have larger GnRH-1 immuno-reactive neurons in the POA than non-territorials, and the size of the soma of these neurons is sensitive to changes in social status (Francis *et al.*, 1993). There is shrinkage of the cell body of the GnRH-1-ir neurons in the POA of dominant males that become subordinates and, conversely, there is an enlargement of the soma of these neurons in subordinates that acquired the dominant status (Francis *et al.*, 1993). These changes in the size of GnRH-1 neurons are accompanied by changes in GnRH-1 gene expression in the POA (White *et al.*, 2002) and by GnRH receptor 1 expression in the pituitary (Au *et al.*, 2006), suggesting that the social modulation of the reproductive signalling pathways can occur at multiple levels. It must be stressed that these changes can occur in subordinates within minutes of an opportunity to increase their social status, as shown by the activation of the immediate early gene erg-1 specifically in the anterior POA, a region with high density of GnRh-1 cells (Burmeister *et al.*, 2005).

This set of studies supports the hypothesis that the rapid modulation of androgens can be mediated by the HPG axis through the regulation of the activity of GnRH neurons.

THE SOCIAL MODULATION OF THE HYPOTHALAMIC-PITUITARY-INTER-RENAL AXIS

Social interactions are a potential stressor in fish (Sloman and Armstrong, 2002; Gilmour *et al.*, 2005). Typically, agonistic encounters stimulate an increase in cortisol levels in both contestants at its onset, and after the assessment of dominance status cortisol plasma concentrations rapidly

return to baseline levels in dominants, whereas they remain elevated in subordinates for a long period after the interaction (Øverli *et al.*, 1999a; Sloman *et al.*, 2001a). Therefore, in paired-encounters, most studies have found elevated levels of cortisol levels or higher activity of the cortisol producing cells in the inter-renal tissue in subordinate fish (Table 3.6), and the increase is cortisol levels is directly linked to the intensity of the agonistic encounters (e.g., Winberg and Lepage, 1998; Sloman *et al.*, 2000a). However, it must be stressed that in these studies the encounters were very prolonged in time (between 1 day and 6 weeks of physical contact between the pair within a relatively small tank) and, thus, the subordinate's high cortisol levels may reflect chronic social stress rather than the natural physiological consequence of a single social interaction. These chronically subordinate individuals are unable to activate their HPI axis in response to subsequent challenges, as indicated by their blunted cortisol response to an ACTH challenge (Sloman *et al.*, 2002a) or to handling stress (Øverli *et al.*, 1999b), which suggests that they have achieved their maximum physiological levels. This chronic activation of the HPI axis in subordinates is confirmed by elevated expression of CRH mRNA in the POA (Doyon *et al.*, 2003), of the ACTH precursor proopiomelanocortin (POMC) mRNA in the pituitary (Winberg and Lepage, 1998) and of circulating ACTH levels (Höglund *et al.*, 2000). Brain serotonergic activity is also increased by social subordination in fish (Winberg and Nilsson, 1993) and may also be involved in the up-regulation of the HPI axis in subordinate individuals (Winberg *et al.*, 1997a; Höglund *et al.*, 2002). Chronic elevated levels of cortisol in subordinates are associated with an inhibition of aggressive behaviour and a promotion of submissive behaviour (Øverli *et al.*, 2002), which contrasts with acute elevation in cortisol levels which seem to facilitate the expression of aggressive behaviour (see above).

In contrast to the above-mentioned long-term dyadic social interaction studies, in the few cases in which cortisol levels have been measured after a single fighting episode (Øverli *et al.*, 1999a; Earley *et al.*, 2006), or that have used a short exposure time of the subordinate individuals to the dominants (Corrêa *et al.*, 2003), no differences in cortisol levels have been found between winners and losers (Table 3.6).

The emerging cortisol patterns in studies of social hierarchy formation in small captive groups is less clear, with increased levels of cortisol in subordinates being found in some species but not in others (Table 3.6). Interestingly, few studies have documented cortisol levels in relation to more naturalistic group settings. In the cooperative breeding cichlid

Table 3.6 Relationship between cortisol levels and social status in teleost fish.

Family/species	*Social context (group composition/size)*	*Duration of social interaction*	*Relative cortisol levels (D = dominants; S = subordinates; I = intermediate rank)*	*Reference*
Cichlidae				
Archocentrus nigrofasciatum	Dyadic interaction	< 1 h	D = S	Earley *et al.* (2006)
Astatotilapia burtoni	Dyadic interaction	3-7 wk	D < S	Fox *et al.* (1997)
	Communal tanks (12-14 males + 24 females)	7 wk	D < S	Fox *et al.* (1997)
Neolamprologus pulcher	Dyadic interaction (2 size-matched helpers)	2 d	D = S	Buchner *et al.* (2004)
	Dominance hierarchy (4 immatures)	57 d	D = S	Buchner *et al.* (2004)
	Family groups (breeding pair + helpers)	21 d	D > S	Buchner *et al.* (2004)
	Family groups (breeding pair + helpers)	30 d	D = S	Bender *et al.* (2006)*
Oreochromis niloticus	Dyadic interaction	6 h	D = S	Corrêa *et al.* (2003)
Poecilidae				
Xiphophorus helleri	Dyadic interaction	< 1 d	D < S	Hannes *et al.* (1984)**
	Dominance hierarchy (4 individuals)	10 d	D < S (=I)	Scott and Currie (1980)***
Salmonidae				
Oncorhynchus kisutch	Dominance hierarchy (6 individuals)	2 wk	D < S	Ejike and Schreck (1980)
Oncorhynchus mykiss	Dyadic interaction	5 min 3 h	D = S D < S	Øverli *et al.* (1999a)
	Dyadic interaction	< 1 d	D < S	Pottinger and Carrick (2001)
	Dyadic interaction	1 d 7 d	D < S D < S	Winberg and Lepage (1998)

(Table 3.6 Contd.)

(*Table 3.6 Contd.*)

Family/species	*Social context (group composition/size)*	*Duration of social interaction*	*Relative cortisol levels (D = dominants; S = subordinates; I = intermediate rank)*	*Reference*
	Dyadic interaction	1,2,7 d	D < S	Sloman *et al.* (2001a)
	Dyadic interaction	6 d	D < S	Sloman *et al.* (2002a)
	Dyadic interaction	2 wk	D < S	Laidley and Leatherland (1988)
	Dyadic interaction	2 wk	D < S	Sloman *et al.* (2000a)
	Dyadic interaction	6 wk	D < S	Pottinger and Pickering (1992)
	Dominance hierarchy (6 individuals)	2 wk	D < S	Noakes and Leatherland (1977)***
	Dominance hierarchy (5 individuals)	6 wk	D = S	Pottinger and Pickering (1992)
	Dominance hierarchy (4 individuals)	2 wk	D = S	Sloman *et al.* (2001b)
Salmo trutta	Dyadic interaction	1,2,7 d	D < S	Sloman *et al.* (2001a)
	Dominance hierarchy (4 individuals)	2 wk	D = S	Sloman *et al.* (2000b)
	Dominance hierarchy (4 individuals)	2 wk	D = S	Sloman *et al.* (2002b)
Salvelinus alpinus	Dyadic interaction	4 d	D < S	Elofsson *et al.* (2000)
	Dyadic interaction	5 d	D < S	Höglund *et al.* (2000)
	Dominance hierarchy (3 individuals)	5 d	D < S	Höglund *et al.* (2002a)
	Communal tanks (200 individuals)	undetermined	D = S < I	Øverli *et al.* (1999b)

(*) cortisol levels measured from fish-holding water; (**) blood and whole-body corticosteroid levels; (***) cortisol levels inferred from inter-renal cell activity.

N. pulcher, there are no differences in cortisol levels between the breeding male and helper males and the dominant helpers have higher cortisol plasma concentrations than subordinates in natural family groups (breeding pair plus helpers) that mimics the social structure found in wild populations (Buchner *et al.*, 2004; Bender *et al.*, 2006). Conversely, in communal tanks housing groups of *A. burtoni* of similar sizes to those found in nature, non-territorial (subordinate), individuals have higher cortisol levels than territorials, and spontaneous switches in social status that occurred during the study were followed by changes in cortisol levels (i.e., the same individual had higher cortisol levels as a non-territorial than as a territorial; Fox *et al.*, 1997). Finally, in the longear sunfish (*Lepomis megalotis*)—a species with alternative reproductive tactics—parasitic males have higher levels of cortisol than territorial (dominant) males (Knapp, 2004).

PROSPECTS FOR THE FUTURE OF TELEOST SOCIAL NEUROENDOCRINOLOGY: THE BRAIN 'SOCIAL BEHAVIOUR NETWORK'

The idea of different motivational systems underlying varying social behaviours have implicitly led a large number of researchers to adopt a view of the underlying mechanisms of social behaviour as being composed of a number of independent modules, each performing a specific function. According to this classic view, we would expect to be able to map different neural networks sensitive to the action of hormones for each type of social behaviour and, thus, we will have a multitude of segregated neural systems, each allocated to each kind of social behaviour. Therefore, there would be assigned neural circuits for sexual behaviours (usually expected to be structurally different between the sexes), for aggressive behaviour (usually multiple ones are expected for different forms of aggression), for parental behaviours, for affiliation, and so on, until we would have covered all possible forms of social behaviour. Although people have for long recognized that some of these circuits shared components and were overlapping to some extent, the concept of a direct correspondence between one neural circuit and one form of social behaviour has persisted. These are the circuits that hormones are expected to modulate in order to influence the expression of behaviour.

In a recent paper, Jim Goodson (2005) has challenged this classic view by elaborating the concept of an 'extended amygdala'. Initially proposed by Newman (1999), this suggests that the regulation of social

behaviour in mammals arises through a 'social behaviour network' present in all vertebrates, and that this is composed of a set of brain nodes, all of which are differentially activated by a variety of social stimuli. Therefore, each node is not specifically allocated to any particular type of social behaviour, but rather each social context activates a specific pattern of response across the nodes (Goodson, 2005; Fig. 3.4).

The nodes have been identified based on three major criteria: (a) that they have been implicated in the control of multiple forms of social behaviour, (b) that bi-directional connections link exist between pairs of nodes and (c) that each node has hormone receptors, which opens the

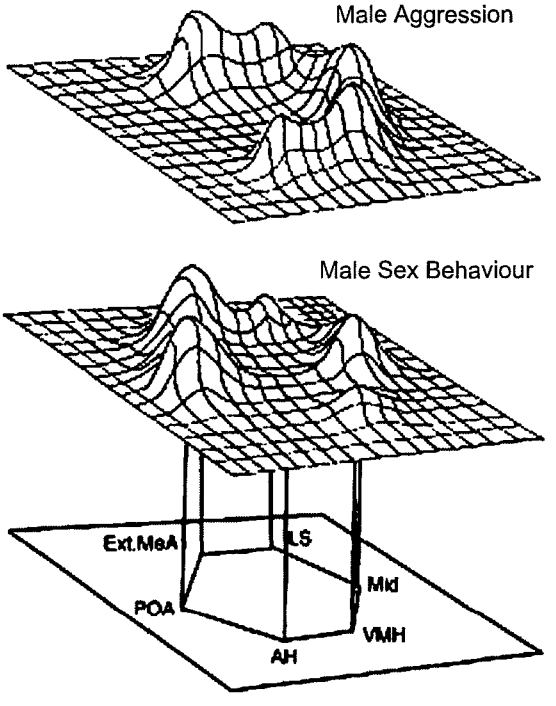

Fig. 3.4 Aggressive and sexual behaviour elicit differential patterns of activation in the 6 nodes of the social behaviour network in the vertebrate brain, corresponding to data of immediate early gene expression in each region (see text for details). Abbreviations of the network nodes: LS = lateral septum; POA = preoptic area; AH = anterior hypothalamus; VMH = ventromedial hypothalamus; Mid =midbrain, including periaqueductal grey (reprinted from *Hormones and Behavior, J.L. Goodson, The Vertebrate Social Behavior Network: Evolutionary Themes and Variations,* Vol. 4811-22, copyright 2005, with permission from Elsevier).

functioning of the network to hormonal modulation. Based on these criteria, six nodes have been identified that are likely to be present in all vertebrate taxa, from fish to mammals:

(1) The medial amygdala and the medial bed nucleus of stria terminalis in mammals, and the homologue supracomissural nucleus of the ventral telencephalon in teleosts;
(2) The lateral septum in mammals, to which corresponds the ventral nucleus of the ventral telencephalon in bony fish;
(3) The preoptic area;
(4) The anterior hypothalamus;
(5) The ventromedial hypothalamus in mammals, corresponding in part to the piscine anterior tuberal nucleus; and
(6) The periaqueductal gray and motor areas of the tegmentum in the midbrain (Goodson, 2005).

This new approach offers a new conceptual framework for more solid comparative investigations of hormone-behaviour mechanisms across different vertebrate classes, and for the understanding of the evolution of the causal mechanisms underlying the wide diversity of social behaviours found in vertebrates, both at the inter-specific and at the intra-specific levels. Surprisingly, one teleost species has already been studied using this pioneering approach (Goodson, 2005). We look forward for more to follow.

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