The integration of sociality, monoamines, and stress neuroendocrinology in fish models: Applications in the neurosciences[1]

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Abstract

Animal-focused research has been crucial for scientific advancement however, in this matter, rodents are still taking a starring role. Coming out from merely being supportive of evidence found in rodents, the use of fish models has slowly taken a more central role and expanded its overall contributions in areas such as social sciences, evolution, physiology, and recently in translational medical research. In neurosciences, zebrafish has been widely adopted, contributing to our understanding of the genetic control of brain processes, and the effects of pharmacological manipulations. However, discussion continues regarding the paradox of function versus structure, when fish and mammals are compared, and on the potentially evolutionarily conserved nature of behaviour across fish species. From the behavioural stand point we explorted aversive/stress and social behaviour in selected fish models, and refer to the extensive contributions of stress and monoaminergic systems. We suggest that, in spite of marked neuroanatomical differences between fish and mammals, stress and sociality are conserved at the behavioural and molecular levels. We also suggest that stress and sociality are mediated by monoamines in predictable and non-trivial ways, and that monoamines could “bridge” the relationship between stress and social behaviour. To reconcile the level of divergence with the level of similarity, neuroanatomy, pharmacology, behavioural analysis, and ecology studies conducted in the laboratory and in nature need to add to each other and enhance our understanding of fish behaviour and ultimately how this all may translate to better model systems for translational studies.

Keywords: Neuroendocrinology, Social behaviour network, Mesolimbic reward system, Aversive behaviour network

1. Introduction

While the study of fish behaviour goes back to the origins of ethology, with von Frisch's (1938) studies on alarm pheromones and Tinbergen's (1951) on behavioural co-adaptations in sticklebacks, increased interest in that (very heterogeneous) group has soared in the last few years (Gerlai, 2014). The use of classical and modern tools for brain research is enabling the means to answer mechanistic questions in relation to fish behaviour. Moreover, the enormous diversity of behavioural and physiological adaptations in fish allows for these methods to be applied to questions on specific adaptations as well as general neurobehavioural trends. As a result, fish behaviour – especially stress-related and social behaviour – is increasingly being recognized as a tool in the neurosciences (Gerlai, 2014; Maximino et al., 2015; Stewart et al., 2015; Soares et al., 2017). A picture is emerging in which complex relationships between fish social behaviour and stress are becoming prominent, and special attention now is given to the applications of these relationships (Oliveira, 2013; Soares et al., 2017).

The present article discusses different neurobehavioural questions using fish as models, focusing on stress-related behaviour, monoamines, and sociality in model species. We set the stage by discussing the neuroanatomical underpinnings of social and stress-related behaviour and its equivalents in teleosts, as well as by briefly presenting the monoaminergic innervation of the "social behaviour network", the "aversive behaviour network", and the "mesolimbic reward system". The modulation of aversive behaviour by monoamines follows that, as well as a discussion on coping strategies in fish and its relations to stress and monoamines. Stress both modifies social behaviour (e.g., the defensive functions of shoaling) and is modified by it (e.g., the stressful nature of dominant-subordinate interactions), and different coping styles are associated with different reactivities to social interaction. An overview of the monoaminergic modulation of social behaviour is given, focusing on shoaling in zebrafish and cooperation in cleaning wrasses and gobies. Finally, applications in biological psychiatry and psychopharmacology are outlined. The article provides a larger picture on the frontiers between social behaviour and stress in different fish species, as well as their common modulation by neurotransmitter systems, as well as the open avenues in fish neurobehavioural research on these fields.

2. Stress and sociality networks in the vertebrate brain

Monoaminergic systems modulate the activity of specific behavioural circuits which have been implicated in a plethora of functions, from cognition to emotional behaviour (Cools et al., 2007; Rogers, 2010). For vertebrates, a "social behaviour network" (SBN; Newman, 1999) has been proposed which overlaps a "mesolimbic reward system" to form a "social decision making network" (O'Connell & Hofmann, 2011a, 2011b, 2012). The SBN involves, in mammals, the lateral septum, extended medial amygdala, preoptic area/paraventricular nucleus (POA/PVN), anterior hypothalamus, ventromedial hypothalamus, and periaqueductal gray area (Figure 1). The SBN is involved in multiple forms of social behaviour, including sexual behaviour and courtship, aggression, and parental care, and its nodes are reciprocally and massively connected (Goodson, 2005; O'Connell & Hofmann, 2011b). By definition, these nodes express sex hormone receptors (Forlano & Bass, 2011; O'Connell & Hofmann, 2011a, 2012). The mesolimbic reward system of mammals involves projections from the ventral tegmental area (VTA) to the nucleus accumbens, lateral septum, ventral pallidum, striatum, pallial amygdala, and hippocampus (Figure 1A). This latter circuit has been proposed to be involved in reward and/or reward seeking (Ikemoto & Panksepp, 1999b; Alcaro & Panksepp, 2011) by allowing the individual to evaluate the relative value and consequence of making choices among external stimuli, and by "mobilizing" goal-directed behaviour that directs the animal to important environmental resources.

In addition to its roles in reward and resource seeking, the mesolimbic system is also involved in coping and in the individual's ability to adapt to chronic social stress (Kvetnansky et al., 2009;

Trainor, 2011). Mice that mount active responses after social stress show increased excitability of VTA neurons and associated brain-derived neurotrophic factor (BDNF) release in the nucleus accumbens (Krishnan et al., 2007). Mesolimbic DA has also been implicated in encoding predictors of aversive stimuli and forming operant associations as to avoid them (Ilango et al., 2012). Stress-induced increases in tonic levels of DA in the mesolimbic system have been implicated in supporting active coping responses – that is, responses that aim to remove and avoid stressors (Cabib & Puglisi-Allegra, 2012).

The latter examples also underline the role of these circuits in regulating responses to stressful and aversive stimuli. Indeed, another layer that could be added to the overlap between SBN and mesolimbic reward system is that of an “aversive behaviour network” (Misslin, 2003; Cezario et al., 2008; Panksepp, 2011; LeDoux, 2012a; Sternson, 2013; Canteras & Graeff, 2014; Andersen et al., 2016). This system is equivalent to the stress/anxiety/fear circuit. In mammals, this network consists of the septum, frontotemporal and striatal amygdaloid systems, periaqueductal gray area (PAG), and extensive and mutual projections with the hypothalamus-pituitary-adrenal axis (Figure 1). These structures are highly responsive to stressful and aversive stimuli, including social and non-social stressors, in a variety of species. In addition, this circuit selects appropriate defensive and stress coping responses as a function of threat probability (Fanselow & Lester, 1988; McNaughton & Corr, 2004a; Perusini & Fanselow, 2015). Part of this circuit comprises the fight/flight/freeze “fear” system that has been widely studied in behavioural neuroscience (Misslin, 2003; Panksepp, 2006). Another part of this circuit is associated with responses to uncertain threat, with important behavioural correlates of anxiety-like states (Davis, 2002; Gray & McNaughton, 2003; McNaughton & Corr, 2004a; Maximino, 2012). In theory, anxiety-like behaviour is triggered by uncertain threat, while fear-like behaviour is triggered by distal or proximate threat, i.e. clearly detectable aversive stimuli (Fanselow & Lester, 1988; Perusini & Fanselow, 2015). Whether or not, in fish, those responses are also segregated at the behavioural and neuroanatomical levels is still debated (Kalueff et al., 2012).

2.1. A tale of homologies

It is difficult to establish direct homologies between structures found in the SBN, the mesolimbic system, and the aversive behaviour network of amniotes and anamniotes (O'Connell & Hofmann, 2011a), to the point that Goodson & Kingsbury (2013) argued that the SBN is "not yet supported as a pan-vertebrate model". One of the main reasons of that difficulty is that in teleosts, the developmental eversion of the telencephalon makes it very difficult to establish direct homologies on the basis of topology (Nieuwenhuys, 2011); moreover, the lack of cytoarchitectonic and hodological data on a variety of species also impairs judgments of homology. Numerous homologies that have been proposed for these regions in teleost fish are only partial (Goodson, 2005; O'Connell & Hofmann, 2011b; Goodson & Kingsbury, 2013); and important structures (such as mesolimbic DAergic pathways) are absent in teleosts (Rink & Wullimann, 2001; Wullimann & Mueller, 2004; Maximino et al., 2015a). Based on topology, hodology, and expression of receptors for sexual hormones, O'Connell & Hofmann (2011b) proposed direct one-to-one homologs of the periaqueductal gray area and POA in fish, and the anterior and ventral tuberal regions as homologues for anterior and ventromedial hypothalamus, respectively; these structures would comprise the social behaviour network in fish. In the mesolimbic reward system, homologues have been proposed for the basolateral (pallial) amygdala (= dorsomedial telencephalon, Dm; Maximino et al., 2013); and hippocampus (= dorsolateral telencephalon, Dl; Demski, 2013). Subpallial structures in the mesolimbic reward system probably represent partial homologies, with the ventral telencephalon (Vd, Vv, Vc) presenting a pallidal portion and a striatal portion that are equivalent to the pallidum and striatum (including a subpallial amygdala), respectively (Ganz et al., 2012, 2014; Maximino et al., 2013a). While no homologue of the VTA has been described in teleosts, a functional (analogical) equivalent is the ascending dopaminergic projection that originates in the posterior tuberculum (Rink & Wullimann, 2001, 2004; Tay et al., 2011). In relation to the aversive behaviour network, based on expression of ancillary markers, topology, and function, it has been proposed that the suppracommissural subpallium (Vs) is a partial homologue of the mammalian extended amygdala, including the striatal component and the bed nucleus of the stria terminalis, while the dorsomedial telencephalon (Dm) is homologous to the frontotemporal component of the amygdala (Maximino et al., 2013a). Finally, the shared structures (bed nucleus of the stria terminalis, lateral septum) are partially homologized to the Vs and ventral (Vv) and lateral (Vl) parts of the ventral telencephalon, respectively (Moreno et al., 2009; Ganz et al., 2012). These homologies and analogies are summarized in Table 1 and Figure 1B.

2.2 Monoaminergic innervation in teleosts

These three circuits are heavily innervated by monoamines in all vertebrates (O'Connell & Hofmann, 2012). However, the anatomical organization of monoaminergic systems presents significant differences between teleosts and mammals (Kaslin & Panula, 2001; Herculano & Maximino, 2014; Maximino et al., 2015a, 2016). For example, the serotonin (5-HT) neurons in amniotes are restricted to the raphe nuclei (cluster 5-8), while anamniotes present four extra clusters (Lillesaar, 2011; Gaspar & Lillesaar, 2012; Cornide-Petronio et al., 2013; Herculano & Maximino, 2014; López & González, 2014). Moreover, genomic events (the "fish-specific duplication event; Meyer & Van de Peer, 2005) resulted in duplication of most the synthesizing enyzme tryptophan hydroxylase, 5-HT receptors, the serotonin transporter, and the vesicular monoamine transporter in teleosts (Norton et al., 2008; Sourbron et al., 2016); similar changes were observed in the catecholaminergic systems, with duplication of the synthesizing enzyme tyrosine hydroxylase and most dopamine receptors (Yamamoto & Vernier, 2011). Teleosts also lack a mesolimbic DAergic system, as described above (Rink & Wullimann, 2001, 2004; Tay et al., 2011).

Teleosteans present pretectal and hypothalamic 5-HTergic clusters whose projections and function are poorly described (Lillesaar, 2011; Maximino et al., 2013c; Herculano & Maximino, 2014). Nevertheless, one of the most well characterized teleosts in this respect is the zebrafish (*Danio rerio*). The axonal projections from the superior and inferior raphe of zebrafish have been described, with moderate to dense innervation of regions in the mesolimbic reward network, such as the Dl, Dm, Vv/Vl, and posterior tubercular area; low to moderate innervation of nodes in the SBN, including POA, tuberal regions and GC; and moderate innervation of regions in the aversive behaviour network, including Dm, Vs, and caudal hypothalamus (Lillesaar et al., 2009). Other regions which receive serotonergic innervation (Kaslin & Panula, 2001) do not appear to be part of the SBN, aversive behaviour network, or mesolimbic reward systems.

Catecholaminergic projections have also been characterized in zebrafish (Rink & Wullimann, 2001; Ma, 2003; McLean & Fetcho, 2004; Tay et al., 2011). The POA receives only weak local DAergic projections, and does not appear to receive noradrenergic projections in zebrafish larvae (Tay et al., 2011). In adult animals, low to moderate catecholaminergic innervation of regions of the SBN are found, with moderate innervation of regions in the aversive behaviour network and moderate to high innervation of regions in the mesolimbic reward system (Kaslin & Panula, 2001). A summary of this innervation patterns, as well as the homologies proposed for the teleostean brain, can be found in Table 1.

3. The aversive behaviour network of fish: Modulation by 5-HT and DA

Stress is an important risk factor in many diseases, including psychiatric disorders. In the latter case, stress is especially relevant to anxiety and mood disorders, as well as to trauma- and stressor-related disorders (Belzung & Lemoine, 2011). The neurocircuitry associated with these disorders is partially elucidated (Coplan & Lydiard, 1998; Etkin & Wager, 2007; Hartley & Phelps, 2009; Hayes

& Northoff, 2011; Jovanovic & Norrholm, 2011), and overlaps extensively with the aversive behaviour network (LeDoux, 2012b).

Anxiety-like behaviours have been explored in different fish species, including *Xiphophorus nigrensis* (Ramsey et al., 2014), sticklebacks (Thompson et al., 2016), and guppies (Maximino et al., 2010b). Nonetheless, zebrafish is the most widely studied species when it comes to fear, anxiety, and stress (Jesuthasan & Mathuru, 2008; Maximino et al., 2010a; Kalueff et al., 2012; Gerlai, 2013). Two assays – the novel tank test (Egan et al., 2009) and the light/dark test (Maximino et al., 2010b) – are widely used in this species, with good pharmacological validation and construct validity for both tests (Maximino et al., 2012; Kysil et al., 2017). In the novel tank test, the novelty of the environment elicit bottom-dwelling (also termed a "diving" response) that is associated with erratic swimming and freezing, representing anxiety-like behavior in this assay (Egan et al., 2009). In the light/dark test, the preference for a black compartment over a white one, associated with risk assessment behaviors, erratic swimming, thigmotaxis, and freezing is interpreted as anxiety-like behavior (Maximino et al., 2010b). Moreover, a relationship between stress and behaviour in these tests is established, as both acute (Giacomini et al., 2016) and chronic (Chakravarty et al., 2013; Marcon et al., 2016) stress increases anxiety-like behaviour in these assays, and exposure to the apparatuses induce cortisol levels (Kysil et al., 2017). A causal link between neuroendocrine and behavioural responses to stress has not been ascertained; since these are modulated by monoamines, these upstream modulators could link both functions. However, as we will show, the behavioural roles of monoamines in unstressed animals are discordant from its functions in neuroendocrine responses, underlining the need for more thorough analyses at both the behavioural and the physiological levels.

3.1. Serotonin and the aversive behaviour network of zebrafish

Some of the behavioural functions of the serotonergic system have been described in zebrafish (Herculano & Maximino, 2014). It has been proposed that serotonergic projections to the telencephalon compute the aversive expectation value necessary for the zebrafish to mount an active response to aversive stimuli (Amo et al., 2014). Consistent with that hypothesis, drugs which increase 5-HT levels in the zebrafish brain increase anxiety-like behaviour in the light/dark test and decrease it in the novel tank test (Egan et al., 2009; Sackerman et al., 2010; Maximino et al., 2011, 2013b, 2014a; Iturriaga-Vásquez et al., 2012; Kyzar et al., 2013; Stewart et al., 2013; Herculano & Maximino, 2014; Cheng et al., 2016). Moreover, there is a correlation between 5-HT levels in the extracellular fluid *ex vivo* and behaviour in the light/dark test, with higher levels associated with more anxiety-like behaviour in the light/dark test and lower anxiety-like behaviour in the novel tank test (Maximino et al., 2013b).

A role for specific 5-HT receptors has also been proposed. In both the light/dark and novel tank tests, the 5-HT$_{1A}$ receptor antagonist WAY 100,635 decreases anxiety-like behaviour (Maximino et al., 2013b); however, the antagonist p-MPPF *increases* anxiety-like behaviour in the novel tank test (Nowicki et al., 2014). Antagonists at 5-HT$_2$ and 5-HT$_3$ receptors were shown to increase anxiety-like behaviour in the novel tank test (Nowicki et al., 2014), while 5-HT$_{1B}$ receptor antagonists decrease it (Maximino et al., 2013b; Nowicki et al., 2014).

These results suggested that serotonin has a dual role in controlling anxiety-like behaviour in zebrafish, with opposite effects on the light/dark test and the novel tank test. These discrepancies could be due to differences in stimulus control in both tests: while white avoidance/dark preference in the light/dark test is controlled by an approach/avoidance conflict, bottom-dwelling/top avoidance in the novel tank test is controlled by escape from the top (Maximino et al., 2012). This stimulus control is reminiscent of Gray’s theory on the difference between fear and anxiety (Gray & McNaughton, 2000; McNaughton & Corr, 2004b), suggesting that 5-HT could have a differential role in fear vs. anxiety in zebrafish (as suggested, for mammals, by the Deakin/Graeff hypothesis;

cf. Deakin & Graeff, 1991; Graeff et al., 1996; Paul et al., 2014). Alternative explanations have been proposed; for example, Stewart et al. (2013) suggested that the effects of 5-HT on the novel tank test are better explained as a “serotonin syndrome-like” phenotype. To test both hypotheses, a clearer threatening stimulus is needed, an example of which is the alarm substance, a “panicogenic” stimulus.

The alarm substance is produced by club cells of Ostariophysan fish; when these cells are damaged (by, for example, predator attack), the substance is released to the water, immediately producing a dramatic fear-like behaviour (von Frisch, 1938; Jesuthasan & Mathuru, 2008; Døving & Lastein, 2009). When zebrafish are exposed to this alarm substance and subsequently tested in the light/dark test, an increase in aversive behaviour is observed that is blocked by acute pre-treatment with fluoxetine (Maximino et al., 2014b). Interestingly, the same fluoxetine dose *increases* the same behaviour in this test when given to animals that were not exposed to alarm substance (Maximino et al., 2014b), suggesting that this drug is anxiogenic and panicolytic. alarm substance also increases plasma levels of norepinephrine, epinephrine, and glucose, effects which were blocked by fluoxetine (Maximino et al., 2014b). Both behavioural and autonomic effects, however, were not blocked by treatment with WAY 100,635 (Maximino et al., 2014b); interestingly, when animals are exposed to the alarm substance during the novel tank test, WAY 100,635 and methysergide (a 5-HT_2 receptor antagonist) potentiate the effect of alarm substance (Nathan et al., 2015).

These results are in line with clinical data (Mortimore & Anderson, 2000; Graeff et al., 2001; Garcia-leal et al., 2014), as well as preclinical data on mammals (Pinheiro et al., 2007; Guimarães et al., 2010; Paul et al., 2014; Graeff, 2016). The participation of this monoamine in behavioural and neuroendocrine responses to stressors appears to be different, however. While it has long been shown that social stressors increase brain 5-HTergic activity in fish that is accompanied by increased cortisol levels (Winberg & Nilsson, 1993), a causal role is more difficult to ascertain. 5-

HT_{1A} receptors expressed at all levels of the hypothalamus-pituitary-interrenal (HPI) axis of teleosts, but the mRNA levels in the preoptic region and the head kidney are 12- to 16-fold higher than in the pituitary (Norton et al., 2008; Lim et al., 2013). In goldfish and Arctic charr, 5-HT_{1A} and 5-HT_4 receptor activation increase cortisol responses by acting directly in steroidogenic cells in the interrenals (Lim et al., 2013). However, these effects appear to be different in stressed animals, as fluoxetine blocks cortisol responses after chasing stress in zebrafish (de Abreu et al., 2014), and the 5-HT_{1A} receptor agonist 8-OH-DPAT does the same in the Arctic charr (Höglund et al., 2002). Höglund et al. (2002) observed that fish receiving this drug with a permanent intraperitoneal catheter, thus decreasing the stress of injection, showed increased cortisol and adrenocorticotropic hormone (ACTH) levels, while animals which received (stressful) intraperitoneal injections responded with decreased plasma cortisol and ACTH levels. Similarly, 8-OH-DPAT increases cortisol levels in catheterized rainbow trout (Winberg et al., 1997). Thus, it looks as if 5-HT phasically increases basal cortisol levels but decreases cortisol responses to stressors, as well as behavioural and physiological responses to stress.

There are some methodological issues in mapping behavioural and neuroendocrine roles for 5-HT in stress, as well. One of them regards concerns the post-stress time intervals chosen for testing. Fluoxetine blocks the anxiogenic effect of chasing stress in the novel tank test 2 min after stress (Giacomini et al., 2016). However, peak levels of whole-body cortisol are observed 15 min after stress (de Abreu et al., 2014), an effect that is also blocked by fluoxetine. If stress-induced cortisol responses were causally related to the anxiogenic effect of stress, the temporal order of these effects should be inverted. As it stands, it appears that serotonin phasically inhibits both behavioural and neuroendocrine effects of stress independently.

3.2. Dopamine and the aversive behaviour network of zebrafish

Similarly to the serotonergic system, a plethora of differences can be found in the dopaminergic system of teleosts in relation to mammals. As described above, important neuroanatomical (absence of the A9/mesolimbic projection) and genomic (duplication of tyrosine hydroxilase and of some receptors) differences exist between teleosts and mammals, and many of these differences are plesiomorphic in Gnathostomata (Yamamoto & Vernier, 2011). Some roles for dopamine receptors have been described in zebrafish anxiety-like behaviour. Treatment with the dopamine transporter blocker D-amphetamine increases anxiety-like behaviour in the novel tank test without apparent locomotor effects (Kyzar et al., 2013). Similarly, the D_1 receptor antagonist SCH 23390 decreases anxiety-like behaviour (Kacprzak et al., 2017) and reduces social preference (Scerbina et al., 2012) in zebrafish. A developmental role for catecholamines is also suggested by experiments with morpholino knockdown of the tyrosine hydroxylase-coding gene *th1*, which decreases anxiety-like behaviour when the animal reaches adulthood (Formella et al., 2012). Moreover, dopamine transporter-null zebrafish are more sensitive to the D_2/D_3 receptor antagonist sulpiride (Kacprzak et al., 2017).

There is also some evidence for a role of dopamine receptors in behavioural and neuroendocrine stress responses, although they are much less developed than in the case of serotonin. Stress induces dopaminergic activity in the brain of tilapia *Oreochromis mossambicus* (Chabbi & Ganesh, 2015). Risperidone, which acts as an antagonist at dopamine D_2 and serotonin 5-HT_{2A} receptors, blocks stress-induced cortisol increases in zebrafish, without apparent behavioural effects (Idalencio et al., 2015). The tyrosine hydroxylase blocker α-methyltyrosine produces a similar neuroendocrine effect (Idalencio et al., 2017). Differently from serotonin, then, catecholamines appear to have concordant effects on basal anxiety-like behaviour and on neuroendocrine responses to stress in zebrafish, promoting stress-induced cortisol release.

In both the case of 5-HT and catecholamines, the assumption of anatomical and neurochemical conservation in zebrafish (in relation to mammals) is not fully supported by the data; in fact, the most parsimonious interpretation is that mammalian state is derivative (Parent, 1984; Yamamoto & Vernier, 2011; Herculano & Maximino, 2014; Maximino et al., 2015a). The paradox, however, is that, at least in the case of some roles of monoamines in the aversive behaviour network, function appears to be conserved instead of neuroanatomy and genetics (Herculano & Maximino, 2014; Maximino et al., 2016). Questions remain on whether this conservation of function can be explained at the molecular level – that is, if active sites from duplicated proteins are conserved, or if their function is duplicated.

3.3. Coping mechanisms and monoamines in fish

While these responses to stressful manipulations appear to differentiate between the neuroendocrine and behavioural roles of 5-HT (but not of catecholamines) in zebrafish, there is a potential confounding variable that has not been accounted for: coping. While many different definitions exist for the term, “coping” usually refers to coherent and stable sets of individual behavioural and physiological differences in responses to stressors. Coping styles are a result of individual differences in perceiving and reacting to the environment. From an eco-physiological point of view, coping styles fundamentally affect the robustness of individuals to challenges such as stress and diseases (Koolhaas et al., 1999; Réale et al., 2007; Coppens et al., 2010; Vindas et al., 2017). behavioural and physiological differences prompted classifying animals into “proactive” and “reactive” coping styles, with the first describing individuals which are “bolder” (which can mean lower anxiety and/or increased impulsivity), more aggressive, dominant, and less flexible to fluctuations in the environment, and the second describing individuals which are “shyer”, less aggressive, usually submissive, and more flexible to fluctuations (Øverli et al., 2007; Coppens et al., 2010). In outbred populations, however, there is consistent evidence (e.g., Frost et al., 2007, 2013; Thomson et al., 2012, 2016) that bold rainbow trout are more flexible than shy fish. These discrepancies suggest that the reduced flexibility in bold individuals results from inbreeding and/or artificial selection, and is rare in the wild.

Neuroendocrine responses usually vary between proactive and reactive individuals. For example, it has been suggested that proactive individuals show low basal stress axis activity and less prominent cortisol responses to stress, with higher sympathetic responses to challenges (Koolhaas et al., 1999). For example, Pottinger & Carrick (1999) selected rainbow trout (*Oncorhynchus mykiss*) selected for responsiveness of the HPI axis by assessing cortisol responses to repeated confinement stress, and selectively breeding animals at extremes of the endocrine response. These lines show divergent behavioural profiles, with high responding (HR) fish more frequently becoming subordinate (Øverli et al., 2005), habituating more slowly to transfer to a new tank (Øverli et al., 2005; Ruiz-Gomez et al., 2008), showing more behavioural arousal after the introduction of an intruder (Øverli et al., 2005) and more attacks to the intruder (Schjolden et al., 2005a) than low responding (LR) fish. Interestingly, differences were also observed in the intestinal mucosal barrier: using a similar selective breeding ("experimental evolution") technique as that of Pottinger, Rosengren et al. (2017) generated high responding trout that show lower intestinal permeability during basal conditions, but this was reversed after stress. Thus, it appears that HR trout show a reactive coping style, while LR trout show a proactive style.

The notion that coping styles are causally related to cortisol levels, however, has been disputed many times (Øverli et al., 2007; Koolhaas et al., 2010). In juvenile rainbow trout, a mild environmental stressor (exposure to an unfamiliar environment) produces consistent individual differences in cortisol responses, with some animals showing high reactivity and other showing low reactivity (Schjolden et al., 2005b). Moreover, individual differences in aggression in a resident-intruder context and speed of recovery after social isolation were also observed; however, these behavioural differences, while consistent over time and contexts, did not correlate with the individual differences in cortisol responsiveness (Schjolden et al., 2005b). In zebrafish larvae, proactive animals (selected on the basis of latency to emerge into a novel well-lit environment) show higher cortisol levels after netting stress, but faster recovery (Tudorache et al., 2015). These observations might be restricted to larvae, however, as adult proactive animals do not show more cortisol after stress, although their recovery is faster (Tudorache et al., 2013).

In zebrafish, proactive vs. reactive coping is usually assessed at the behavioural level, because the size of the animal impairs cortisol analysis without euthanasia. A “boldness-aggression syndrome” is sometimes identified in zebrafish that is reminiscent to the proactive-reactive dimension, with a correlation between aggression and boldness at the population level (Wright et al., 2003; Moretz et al., 2007; Dahlbom et al., 2011; Norton et al., 2011). Animals selected for 5 generations on the basis of position on the tank (bold animals were defined by higher position in the tank in relation to shy animals) did not differ in terms of cortisol levels; however, shy animals had higher expression of the 11β-hydroxysteroid dehydrogenase-coding gene *11b-hsd*, the glucocorticoid receptor-coding genes *gr-a* and *gr-b*, the corticotropin releasing factor-coding gene *crf*, and the brain-derived neurotrophic factor-coding gene *bdnf* in relation to bold animals (Oswald et al., 2013). It should be noted, however, that it is yet to be addressed whether these bold/shy animals correspond to proactive/reactive coping styles.

Zebrafish selected for low vs. high stationary behaviour (LSB vs. HSB), which have been argued to exhibit characteristics of proactive and reactive coping styles, respectively, show interesting behavioural and transcriptomic differences (Wong et al., 2012, 2015). LSB animals display less anxiety-like behaviour in the novel tank test and light/dark test, a lower fear-like behaviour during alarm substance exposure, lower latency to feed after a disturbance, and a higher probability of orienting towards a human observer than HSB animals (Wong et al., 2012). 62 genes were found to be upregulated in the brains of proactive (LSB) animals, including genes involved in the biosynthesis and metabolism of organic acids, carboxylic acids, and fatty acids (Wong et al., 2015). The authors suggested that these differences could be related to dealing with oxidative stress in the brain. Moreover, stationary behaviour was positively correlated, in LSB animals, with the expression of the methylsterol monoxygenase-coding gene *msmo1* and 11β-hydroxysteroid dehydrogenase-coding gene *hsd11b2*, and negatively correlated with *gabbr1a* expression, a gee which codes for the GABA$_B$ receptor.

As described above, there is some evidence for a role of the mesolimbic reward system on coping styles in vertebrates – especially in coping with chronic stress (Kvetnansky et al., 2009; Trainor, 2011). There are some suggestions that monoamines participate in these coping responses (Coppens et al., 2010; Koolhaas et al., 2010). Telencephalic levels of *mra* (mineralocorticoid receptor A), *slc6a4b* (serotonin transporter B), and *htr1ab* (5-HT$_{1AB}$ receptor) are higher in HR trout (Rosengren et al., 2017). Likewise, *spiegeldanio* zebrafish – which show increased aggression, exploration, and boldness (i.e., proactive coping) – show higher levels of *slc6a4a* (serotonin transporter A) in the raphe nucleus (Norton et al., 2011); this does not appear tot be causal, however, since acute treatment with a serotonin transporter inhibitor does not rescue the behavioural differences. In Atlantic salmon (*Salmo salar*) that escaped an imposed hypoxia by swimming into an adjacent normoxic tank (proactive coping) showed lower DA and DOPAC levels in the Dl than reactive animals (i.e., animals which did not escape hypoxia) (Vindas et al., 2017). While 5-HIAA levels were identical between reactive and proactive animals, it increased in the Dm of proactive animals after stress (Vindas et al., 2017). These later effects might be related to the 5-HT$_{1A}$ receptor, as proactive animals had higher levels of *htr1aa* (5-HT$_{1AA}$ receptor) and *htr1ab* (5-HT$_{1AB}$ receptor) mRNA levels in the Dm than reactive animals (Vindas et al., 2017); while differences in *crf* and *crfbp* (CRF binding protein) were not observed, proactive animals had higher levels of *crhr1* (CRF receptor 1) in the Dl (Vindas et al., 2017). Reactive individuals also showed higher levels of the neural proliferation marker *pcna* (proliferating cell nuclear antigen) in the Dl (Vindas et al., 2017). This suggests that proactive fish are characterized by a stress-induced increase in 5-HT signaling in the Dm and lower DAergic neurotransmission in the Dl.

In conclusion, coping styles appear to be a promising avenue of investigation that could solve the paradox of a dissociation between neuroendocrine and behavioural roles of monoamines in fish. A more thorough operational definition of coping is still needed, to underline discrepancies between results from experimental evolution experiments and tests assessing inter-individual differences, as well as between experiments that select of the basis of behaviour and cortisol responses. Nonetheless, the research program carries the potential to suggest solutions linking behavioural and neuroendocrine functions of these amines.

4. Shoaling and the dopaminergic system in zebrafish

The last common ancestor of modern teleosts (bony fishes) and mammals lived about 400 million years ago. Despite this long period of time, the zebrafish has turned out to possess numerous evolutionarily conserved features (Gerlai, 2014), and has been proposed for the modelling and analysis of more complex human neuropsychiatric and neurodevelopmental brain disorders (Gerlai, 2010, 2012; Kalueff et al., 2014). A number of such human disorders, albeit mechanistically and causally perhaps unrelated, are characterized by abnormal social behaviour. For example, symptoms and/or diagnostic criteria of Autism Spectrum Disorders (ASD), schizophrenia or fetal alcohol spectrum disorders (FASD) all contain some form or level of social behaviour abnormality (Gillberg et al., 1996; Greenbaum et al., 2009; Hoertnagl & Hofer, 2014). These disorders represent substantial unmet medical needs mostly because their mechanisms and/or causes are poorly understood, and thus development of proper treatments has been difficult.

The zebrafish has been proposed to model such disorders to facilitate discovery of their mechanisms and development of treatment (Kalueff et al., 2014; Stewart et al., 2015; Shams et al., 2018). However, this type of research suffers from an inherent paradox: in order for one to properly model the chosen human disorder, one has to first know its mechanism, the latter being the reason why the disease model would be created at the first place. Breaking through this conundrum, similar to what is described in the novel *Catch 22* by Joseph Heller, is not an easy task. One way to start the process is to focus on phenotypical features (face validity of the model), and subsequently test whether these features have similar mechanistic underpinnings (construct validity of the model). For example, social behaviour in humans, although a lot more sophisticated and complex than in zebrafish, may share common mechanisms with social behaviour in fish (Oliveira, 2013). For one to model a human disorder with abnormal social behaviour, one first needs to understand unaltered "normal" social behaviour in the chosen model organism. Thus, studying zebrafish social behaviour and its mechanisms, may allow to eventually identify underlying mechanisms common to human and fish, and to start the modeling and analysis of human disorders associated with abnormal social behavioural responses.

In this section, we describe the first steps of this research line. First, we will discuss how one can induce and measure social behaviour in zebrafish. Next, we will examine a potential mechanism that may underlie social behaviour in mammals and fish, a proof of concept analysis. Last, we will present an experimental example on how one can utilize the analysis of social behaviour of zebrafish and the knowledge we gained about its mechanism in an empirical model of a human disorder, fetal alcohol spectrum disorder.

4.1. Zebrafish social behaviour

Although a small and simple vertebrate, the zebrafish possesses a sophisticated social behavioural repertoire. Detailed description of this repertoire is beyond the scope of this review, but briefly, zebrafish have an elaborate courtship display/spawning behaviour, show transient territoriality, exhibit numerous agonistic behaviours, and most importantly shoal, the focus of the current review (Darrow & Harris, 2004; Miller & Gerlai, 2007, 2011; Spence et al., 2008; Suriyampola et al., 2016; Teles & Oliveira, 2016). Shoaling is a form of group forming, or aggregation behaviour, in which subjects stay in close proximity to each other. Fish in a shoal stay in a relatively well-defined distance from each other. Shoaling is a dynamic behaviour in which shoal members monitor each other and constantly adjust their position relative to each other. This dynamism translates to rapid oscillation of shoal cohesion (Miller & Gerlai, 2008), a short time scale (tenth of a second to a second) change. Shoal cohesion also changes on a longer time scale (hours to days). For example, when fish habituate to a novel environment shoal cohesion gradually decreases (Miller & Gerlai, 2007). Whereas when a predator approaches, shoal cohesion is rapidly broken (escape reaction), which is followed by a robust tightening of the shoal, increase of shoal cohesion (Miller & Gerlai, 2007; Speedie & Gerlai, 2008). The strength of shoaling also changes (increases) as zebrafish develop (Buske & Gerlai, 2011). Another dynamic feature of the shoal is its polarization, i.e. its synchronized movement direction. In case of synchronized movement, the shoal is called a school, and the behaviour is called schooling. A recent study confirmed that, as suspected based upon anecdotical accounts, zebrafish group forming is bimodal: fish either shoal (unpolarized aggregate of shoal members moving in an asynchronous manner), or school, polarized group in which members move in synchrony, i.e. in the same direction (Miller & Gerlai, 2012).

4.2. Induction and quantification of shoaling

Shoaling is an inherent feature of the zebrafish, a behaviour that can be observed in nature. But even more importantly, shoaling may be easily induced and quantified in the laboratory too. There are two distinct ways with which shoaling may be induced. One is to allow a group of zebrafish to swim freely in their test tank, the other is to present the single experimental fish with social stimuli. First, we discuss the former.

Three or more zebrafish will always form a group in the aquarium and move so that the individuals remain in relative close proximity to each other. This type of social behavior must be distinguished from other social interactions, including reproductive behaviors and aggression. In case of the latter two behaviors, the physical distance between or among interacting fish and the type of interaction, behavioural patterns, differ from what one observes in shoaling. Reproductive behaviors and aggressive responses are characterized by idiosyncratic motor patterns whose sequence is often fixed, these species-specific action patterns are missing in shoaling, where the fish just simply swim or stay close to one another. Another important distinguishing feature of shoaling from these other behaviors is that the distance among shoal members is relatively stable. Although it can change in the short and longer time scale (see below), the change does not follow the ritualized and often fast temporal pattern typical of aggressive or reproductive behaviors. Last, aggressive and reproductive behaviors are also narrowly defined in terms of the number of interacting members, whereas shoaling has been described in nature among as few as three and as many as few hundred zebrafish, although the question of what constitutes an ideal, or usual, or natural, shoal size has started to be empirically investigated only recently (Fernandes et al., 2015b).

Shoal cohesion of freely moving zebrafish group can be measured using video-tracking systems that can quantify distances among the fish. Two of the most frequently employed measures to quantify shoal cohesion have been the inter-individual distance, Delaunay distance, or the nearest neighbor distance (Miller & Gerlai, 2007; Saverino & Gerlai, 2008; Lima et al., 2016). The first two are the average of all distances between the focal fish and its shoal members (inter-individual distance) or between all shoal members (Delaunay distance). The latter is the distance between the focal fish and another shoal member closest to it. Each measure has some advantages and disadvantages. Inter-individual distance utilizes all distances one can measure, and thus it does not suffer from loss of information, unlike the nearest neighbor distance. But the value of inter-individual distance is dependent upon the size of the shoal, whereas the value of nearest neighbor is not. Thus, as long as the same number of shoal members are used in experimental analyses, interindividual distance is the recommended measure. Irrespective of which measure one uses, the problem with using live shoals to induce and measure shoaling is that behaviour of the shoal is dependent upon all shoal members: fish in the shoal interact, and this dynamism may complicate interpretation of results.

The second way to induce and evaluate shoaling responses in zebrafish is to present social stimuli (live, or computer animated images of, conspecifics) to a single experimental subject (Saverino & Gerlai, 2008; Fernandes & Gerlai, 2009; Qin et al., 2014). A single experimental subject in a novel test tank is highly motivated to join the shoal, thus upon presentation of the social stimuli, it usually approaches the stimuli and stays in close proximity of the stimuli. This response is simple to quantify using video-tracking, as one can just measure the distance between the test fish and the stimulus presentation tank or computer screen. Another advantage of using a single subject presented with social stimuli is that the social stimuli, especially in the case of computer animated images, is experimentally controlled and remain consistent across the test session and in between different sessions too. This reduces experimental error variation and contributes to enhanced replicability of results (Gerlai, 2018). Further advantage of inducing and measuring shoaling in this manner is that one can monitor the behaviour of a single subject, which allows identification of mutation or drug induced changes more precisely than what may be achieved when one has to monitor the complex interactions among multiple subjects, as is the case in live shoals.

But does presentation of animated images really induce shoaling responses? Is this artificial stimulus equivalent to live, moving and interactive, conspecifics? This question was addressed in a recent study in which the effect of live conspecifics inside the test tank (cues of all modalities available, stimulus fish interacting), live conspecifics outside the test tank (only visual cues available, stimulus fish interacting), live stimulus fish video-taped and the recording replayed (only visual cues available, stimulus fish not interacting, but move in 3D), computer animated images (only visual cues available, stimulus fish not interacting, and move in 2D) using two different software applications were presented (Qin et al., 2014). The results indicated that experimental zebrafish responded equally well to all these stimuli, i.e., interaction with conspecifics, 3D movement, and cues other than visual are not necessary to induce maximal shoaling responses, as defined by the distance between test fish and the stimulus, in zebrafish (Qin et al., 2014). In summary, presentation of animated conspecifics is a reliable, and consistently controllable stimulus that induces a robust shoaling response that lasts as long as the stimulus is presented.

Experiments exploring what may be the “Platonian” essence of “zebrafishness”, i.e. what specific aspects of the shoaling stimulus zebrafish are sensitive to, has also started to be explored (Saverino & Gerlai, 2008; Gerlai, 2017). This line of research is also useful, as it will allow investigators to test potentially subtle abnormalities in the way mutant or drug treated zebrafish may alter their responses to social stimuli (Seguin & Gerlai, 2018). Although at a preliminary stage of research, results already showed that zebrafish are sensitive to certain cues, while ignore others, when making decisions about whether the stimulus is a shoal-mate or not. For example, rectangles moving with the same speed as live zebrafish and occupying the same overall area on the screen as the image of an adult zebrafish and containing scrambled pixels (overall matching color to that of a zebrafish) do not induce a shoaling response (Saif et al., 2013a). Although such moving objects elicit an exploratory approach, the experimental subject quickly moves away from them. On the other hand, in response to a realistic moving image of a zebrafish, the experimental zebrafish not only approaches, but also stays in close proximity of the stimulus. Altering the stripe pattern (vertical vs horizontal stripes, or no stripes at all) makes no difference in the effectiveness of the stimulus. But elongating the stimulus, while keeping its overall surface area constant induces avoidance. Making the stimulus more yellow enhances the shoaling response compared to what a normal (wild type) image of conspecific would induce (Saverino & Gerlai, 2008). In summary, computerized presentation of animated conspecifics is a precisely controlled and reliable way with which shoaling may be induced and with which the importance of particular aspects of the social stimuli may be dissected. After achieving robust induction and precise quantification of behaviour, one may be able to explore the biological mechanism underlying the behaviour. What are the mechanisms of shoaling in zebrafish?

4.3. Mechanisms of shoaling

Likely, shoaling is under the control of, or influenced by, many neurobiological mechanisms, brain areas, neuronal circuits, biochemical pathways and molecular targets. Where should one begin unrevealing such complexity? Systematic analysis of this question would require labor intensive screening of drug targets, mutations, genome wide gene expression changes, and or mapping of gene expression or other neuronal activity related phenotypes in the brain. While ultimately such studies must be conducted, we decided to start instead with a relatively simple proof of concept analysis. That is, we picked a single mechanism, one target, and explored whether it is involved in shoaling. The target was the dopaminergic system.

We chose the dopaminergic system as our focus for two main reasons. One, we observed that zebrafish is highly motivated to join shoals. That is, we found social stimuli (sight of conspecifics) to be able to serve as an Unconditioned Stimulus (US), a reward, in learning tasks employed for zebrafish (Al-Imari & Gerlai, 2008). Two, the dopaminergic system has been shown to be involved in reward/reinforcement in mammals (Ikemoto & Panksepp, 1999a; Ikemoto, 2010). We assumed that observing, approaching and/or joining a shoal is a rewarding experience for the zebrafish, one which engages the dopaminergic system. This idea is supported by the notion of the intersection between “social behaviour network” (SBN) and the “mesolimbic reward system” (O’Connell & Hofmann, 2011b).

4.4. The dopaminergic system and shoaling

The above described assumption turned out to be correct. Using HPLC, neurochemical responses to the presentation of conspecific images were quantified, and the authors found that the images induced a robust increase of the level of dopamine and the dopamine metabolite DOPAC, indicating elevated dopamine production (dopamine levels) as well as increased dopamine release (elevated DOPAC levels) (Saif et al., 2013a). The results also indicated that the strength of the dopaminergic response was not linearly correlated with the length of stimulus presentation, a temporal trajectory that suggested that it was the appearance not the constant presence of the social stimuli that triggered the elevated dopaminergic response. It is also important to note that the increased dopaminergic response was found to be specific, i.e. other neurotransmitter systems, e.g. the serotoninergic system, were found not to respond to the social stimuli.

Another independent piece of evidence suggesting the involvement of the dopaminergic system in shoaling came from a psychopharmacology analysis. A D1 dopamine receptor antagonist was found to dose dependently disrupt shoaling (Scerbina et al., 2012) without altering motor function or perception (but see (Echevarria et al., 2008). 5-HTergic signalling has also been implicated, as a 5-HT$_{1A}$ receptor agonist promotes shoaling (Barba-Escobedo & Gould, 2012). Furthermore, strong correlation between developmental changes in dopamine levels (relative to total brain protein) and the strength of shoaling during ontogenesis of zebrafish was also demonstrated (Buske & Gerlai, 2011). Last, Mahabir et al. (2013) found alcohol administered during embryonic development to significantly impair both shoaling and the dopaminergic system, a result that brings up the last point to be discussed in this section: how one can utilize the knowledge one has gained about social behaviour of zebrafish in modelling and the analysis of a human CNS disorder.

4.5. Fetal Alcohol Spectrum Disorders: Zebrafish as a potential model system

Despite the clear and known adverse effects of alcohol on the developing fetus, pregnant women continue to consume this substance. Fetal Alcohol Spectrum Disorders (FASD) still occur in high frequency (about 1% of live births) and represent the largest number of patients suffering from preventable mental retardation (Lange et al., 2017). FASD means a lifelong suffering for the patient and his/her family and caregivers. The societal burden of FASD in terms of lost productivity and health care costs is also enormous (Popova et al., 2016). There is no cure, nor even appropriate treatment for the disease, mainly because the neurodevelopmental and functional changes resulting from exposure of the embryo to alcohol are poorly understood. The zebrafish has been suggested as a potentially useful tool or model organism for uncovering the effects and mechanisms of embryonic alcohol exposure (Seguin & Gerlai, 2018). Earlier studies utilized high concentrations of alcohol administered for prolonged period of time during embryonic development of the zebrafish and indeed found abnormalities resulting from this treatment that appeared similar to what has been reported for the most severe forms of FASD in humans (Arenzana et al., 2006). We attempted to model/mimic a less severe form of the disease, one which is most prevalent among FASD patients. We employed only low concentrations of alcohol and administered the substance solely for a short period of time during embryonic development of zebrafish (Fernandes & Gerlai, 2009). The low concentrations ranged between 0.25 and 1.00 % (vol/vol %) external bath concentration. We emphasize that these concentrations are of the external bath, into which the zebrafish eggs (with the embryo inside the egg developing) were immersed. It is notable that if these alcohol concentrations were blood alcohol levels, they would be certainly lethal. However, the chorion (the eggshell) of the zebrafish egg protects the embryo and, according to our own findings, allowed only 1/25th of the external concentration to of alcohol to enter the egg (Fernandes & Gerlai, 2009). In other words, the actual alcohol concentration that reached the brain of the developing zebrafish embryo was below of the legal limit of blood alcohol level for driving in North America, i.e. less than 0.08 %.

Phenotyping of drug and mutation effects requires a thorough and systematic analysis (Gerlai, 2002). We tested many aspects of the behaviour of the embryonic alcohol exposed fish, including their motor function, perception, fear and anxiety, and found no significant alterations. The treated fish appeared to develop normally, looked healthy, and suffered from no obvious defects. However, one test did reveal a robust change. Fish exposed to alcohol at their 24th hour post-fertilization developmental stage for 2 hours exhibited impaired shoaling responses when tested 6 months later, i.e. at their fully grown adult stage. Furthermore, the alcohol effect was found highly dose dependent (Fernandes & Gerlai, 2009). Fish exposed to 0.25% alcohol showed a modest but significant impairment in shoaling, 0.5% and 0.75% alcohol exposed fish showed a stronger impairment in shoaling, and 1% alcohol exposed fish showed such robust impairment that their response to social stimuli was statistically indistinguishable from chance, i.e. they exhibited no shoaling at all (Fernandes & Gerlai, 2009).

Interestingly, the impaired shoaling response was found to be accompanied by altered dopaminergic function. When isolated for 24 hours, fish of all groups, i.e. both control and embryonic alcohol treated fish showed similar baseline dopamine and DOPAC levels. However, upon presentation with social stimuli (animated images of zebrafish), control fish robustly increased their dopamine and DOPAC levels, but fish treated with 0.5% and 1.0% alcohol during their embryonic development did not respond to these stimuli at all. i.e. exhibited no change in their dopamine and DOPAC levels (Fernandes et al., 2015).

The strong correlation between the blunted or abolished behavioural and neurochemical responses to social stimuli is highly suggestive, but the question of how embryonic alcohol may have impaired the development of dopaminergic system remains unanswered. Alcohol is known to enhance apoptotic cell death. In zebrafish, using tunnel staining, we have found evidence that embryonic alcohol treatment results in elevated number of apoptotic neurons in the developing zebrafish brain. Furthermore, we have also found increased expression of Bax in the brain, a pro-apoptotic protein whose level we quantified using Western blot (Mahabir, Chatterjee, Gerlai, unpublished results). Whether these changes directly affected the development of dopaminergic neurons, or whether other neurons connecting to, or mediating the activity of, the dopaminergic neurotransmitter system have been affected is unknown at this point.

Although clearly at the early stages of model development, the zebrafish mild embryonic alcohol exposure model already showed good face and construct validity. Whether the model will be useful for unraveling the potentially complex and target rich mechanisms underlying fetal alcohol spectrum disorders remains to be seen. Similarly, whether the model will turn out to have predictive validity, i.e. treatments, e.g. pharmacotherapeutic applications, would work the same way in the zebrafish and humans, is also an open question.

Nevertheless, the past decade of behavioural neuroscience research conducted with the zebrafish suggests this small teleost will continue to make headway in biomedical research, and may enhance our ability to model and mechanistically analyze human central nervous system disorders.

5. Stress, monoamines and cooperation: Insights from the cleaner fishes

This section explores the proximate and behavioural links between stress, monoamines and cooperation in fish, aiming specifically at the cleanerfish system. Marine cleaning interactions between varied species have long been considered as textbook examples of mutualistic cooperation (Trivers, 1971; Cushman & Beattie, 1991; Vaughan et al., 2016). By definition, cleaners are usually small fish and shrimps that inspect the body surface, the gill chambers and mouth of other visiting larger fishes, known as clients, in search of ectoparasites, mucus and dead or diseased tissue (Côte, 2000). These cleaners are found at specific sites or territories known as cleaning stations which clients actively visit, several times a day and sometimes repeatedly to the same cleaner (Grutter, 1995; Bshary & Côté, 2008). For instance, the Indo-pacific cleaner wrasse *Labroides dimidiatus* is able engage in thousands of interactions a day and may feed on more than 1200 “clients” daily (Grutter, 1999). These cleaners’ behaviour has been described in detail during the last few decades (Bshary & Côté, 2008; Soares, 2017; Soares et al., 2017c), which deemed them to be appropriate for developing and testing new paradigms on the proximate mechanisms that render altruistic behaviour as psychologically rewarding (Soares, 2017).

5.1. Stress mediation of cleaning behaviour: fine-tuning cleaners' performance

One of the most notable behaviours by these animals, is the entering of predators' mouths (while cleaning), a behaviour that has been interpreted as altruistic on behalf of the clients that may simply eat the cleaner (Trivers, 1971). This seemingly fearless behaviour became a paradoxical feature of cleaners' proactivity and 'gutsy' cooperative behaviour but also of putative anxiety and stress control.

Indeed, cleaners change behaviour crucially when dealing with piscivorous clients, that is, they seem to have evolved behavioural strategies that enable them to remain safe despite putting themselves at risk. For instance: cleaning gobies dash to inspect predators' despite of not gaining more foraging benefits (i.e. ectoparasites; Soares et al., 2007) and, cleaner wrasses are known to start any interaction with predators with the exclusive provision of tactile stimulation (also known as 'massages' – cleaners touch clients with their pectoral and pelvic fins; a behaviour that is incompatible with foraging), particularly when inspecting hungry piscivorous (Bshary & Wurth, 2001; Grutter, 2004). This raised questions regarding cleaners' appraisal of fear, or if these fish could be suffering from a kind of "heterospecific boldness syndrome" which would enable them to happily deal with dangerous clients. This hypothesis was later refuted, as cleaners were found to respond with higher cortisol levels when exposed (visually) to piscivorous compared to other harmless clients (Soares et al., 2012b). Furthermore, authors interpreted cleaners' proactivity towards these clients, by rapidly approaching predators, both in captivity and in the wild, and by reducing the time elapsed between client approach and the start of the interaction process, as a way would interrupt the potentially harm of the physiological consequences elicited by predatory clients, securing a safe outcome to these interactions (Soares et al., 2012b). Moreover, it has been observed that interactions following those involving predators, are increasingly more cooperative (Bshary et al., 2008; Gingins et al., 2013), which pointed for a deeper role of stress mechanisms into the short-term modulation of cleaners' cooperative levels. These mechanisms were further investigated in natural conditions, when the exogenous effects of cortisol were found to propitiate cleaners behavioural switch from cooperation to cheating (Soares et al., 2014). Cleaners behavioural change occured in both directions, under influence of rising cortisol levels: providing more tactile stimulation to smaller clients to gain access to bigger ones that are then bitten or mediated by the antagonism of the glucocorticoid receptors, producing a positive improvement of cleaning service (more tactile stimulation to those clients that are truly valuable – i.e. have more ectoparasites and more mucus), that will be responsible to reinforce current and future relationships (Soares et al., 2014). However, knowing that the scope of stress-mediated effects is also in dependence to animals' learned repertoire (Binning et al., 2017), much research is yet to be done with this system.

To facilitate the mechanistic approach to this system, 6 main cognitive modules have been proposed (Soares, 2017), aiming to summarize and organize the main categories of behaviour used by these individuals but also to establish the grounds for future testing. These dimensions are: 1) Predisposition to approach partners, 2) Impulsivity and deception, 3) Social recognition and inference, 4) Learning and memory, 5) Communication and levels of investment and 6) Bonding (see Table 2). Building from the information currently available, we present in Table 2, several putative stress-related effects related to each socio-cognitive modules/dimensions, for the cleanerfish system. At this point, more research is needed, not only to continue with pharmacological testing but also to integrate new behavioural studies coupled with new molecular approaches.

5.2. Stress influence to cleaner fish behavioural plasticity: Caribbean cleaning gobies as alternative systems

Advances in the evolutionary understanding of social behaviour have come from systems in which individuals exhibit flexible social phenotypes (Richards et al., 2003). For instance, the existence of behavioural polymorphisms between individuals and populations of a single species may arise in response to ecological or social challenges-constraints, thus imposing on individuals the need to adapt to different contexts (Sih & Bell, 2008; Bergmuller et al., 2010). Such adaptive behavioural correlations usually underline physiological trade-offs that will play a key role in explaining much of animals' performance plasticity to operate within their socio-environmental challenges and which should ultimately bear fitness consequences.

The role of behavioural plasticity in the context of cleaning behaviour emergence, and more specifically, the proximate mechanisms that underlie its adaptive expression is yet to be discovered. In this regard, the Caribbean cleaning gobies *Elacatinus* spp appears as a good model candidate. Indeed, in the *Elacatinus* clade, the absence of cleaning appears to be associated with a sponge-dwelling habitat or, conversely, the presence of cleaning is associated with living on substrata other than sponge (Rüber et al., 2003; Taylor & Hellberg, 2005). That is the case of the Barbadian broadstripe cleaning goby *E. prochilos*, which occurs on both sponge and live coral (Whiteman & Côté, 2004b), and its foraging mode and social systems differ between the two substrata. Coral-dwelling *E. prochilos* are active full-time cleaners (Arnal & Côté, 2000; Whiteman & Côté, 2002), are found living alone but mostly in pairs (male-female couples; Soares et al., 2009) or in small groups (Whiteman & Côté, 2002) and feed mostly of fish ectoparasites, while sponge-dwelling *E. prochilos* occur in large, highly aggressive dominance-structured groups and feed predominantly on polycheate worms that burrow within sponge tissues (Whiteman & Côté, 2004a; Côté & Soares, 2011).

Nevertheless, it has been suggested that occupiers of both habitats retain foraging and behavioural plasticity: coral dwelling gobies do not rely exclusively on client-gleaned items while sponge dwelling ones appear to engage opportunistically in cleaning (Côté & Soares, 2011). The risk of predation becomes a crucial factor here: sponge dwellers particularly those occupying inner positions in the sponge (dominants) seem to have a more protected live style (White et al., 2007). Seemingly, it is solely amongst those that are “forced” out from the inner and richer areas (less competitive individuals) that opportunistic cleaning is observed (Whiteman & Côté, 2002). Cleaners, while protected by conspicuous color stripes, chemical signals and specific behaviour, which serve to attract cooperative clientele (Lettieri & Streelman, 2010) and help clients recognize cleaners (Stummer et al., 2004), are nevertheless increasingly exposed to potential predators. There has even been a suggestion that adopting a cleaning lifestyle is making the best of a dire situation, based on the observation that the growth rates of juvenile and survival rates of adult sponge dwellers are higher than those that live on coral and clean for a living (White et al., 2007).

Stress-related mechanisms should most certainly be crucial mediators of this system. As mentioned above, cleaning gobies exhibit distinct preferences towards some client species over others: e.g. they express these biases by cleaning a greater proportion of visiting individuals of some species over others (Soares et al., 2007), spending more time inspecting these species (Arnal & Côté, 2000), and by attending to these preferred visitors most quickly (Soares et al., 2007). Notably, piscivorous clients are hardly made to wait (Soares et al., 2007) as cleaners’ fast takeover of the situation helps to mitigate potential harmful consequences (see Soares et al., 2012). However, these tests were done with *E. evelynae*, which are solely observed as cleaners and never as sponge dwellers. In Barbados, both species – *E. evelynae* and *E. prochilos* – are reported inhabiting coral reefs, but only *E. prochilos* is observed to have a dual strategy: cleaners and sponge-dwellers. We decided to extend the tests done by Soares et al (2012b) to *E. prochilos*, wondering if cortisol response mechanisms could be related to this species increase in socio-ecological challenges (for methodology, please see Soares et al (2012). Thus, specimens from both species were collected in similar habitats (coral heads) and all were identified as cleaners (aka, none the gobies were collected in sponges). In trials to validate the hormone assay, cortisol immunoreactivity in holding water varied significantly over time and in interaction between treatment and time for *E. evelynae* (for complete results see Soares et al. (2012b)). *E. prochilos* individuals have a significantly wider physiological range when it comes to cortisol secretion (Figure 2). Visual inspection of Figure 2A. shows the amplified range of cortisol response by *E. prochilos* by the second hour but also the ability to sustain that response up to the fourth hour, when it starts to decrease. The wider level of response to the ACTH challenge contrasts with *E. prochilos* response to its putative heterospecific partners: in comparison to a close species of cleaner (*E. evelynae*), *E. prochilos* individuals were not found to react to predatory risk significantly (Figure 2B).

The difference between these two species of cleaning gobies, one referred to have an exclusive cleaner strategy and another with the potential to exhibit an alternative behavioural choice, presents an interesting twist: *E. prochilos* can respond more strongly to stressful contexts but these individuals may not have the finer mechanisms that enables them to react to smaller trophic differences between clients. This robustness of *E. prochilos* response may confer them with the ability to be part of an aggressive, structured group as well as being a cleaner, if the opportunity arises or if necessary due to a shortness of available food-patch (sponges). However, the relative bluntness in reacting to clients may expose them to a greater predatory risk while similarly reducing their cleaner-specialist status in comparison to their sympatric competitors, *E. evelynae*. Some of these fundamental features of cleaning gobies' development ecology warrants for additional studies, as to better evaluate the validity of these assumptions. For example, additional data of *E. prochilos* collected within sponges, according to their status (dominant or subordinate), gender (female, male), breeding status and whether these are paired or not, would be extremely informative. That would then be compared to gobies collected at coral heads (aka, cleaning stations), in accordance with those same variables. behavioural data would also be relevant: for instance, comparing learning abilities in the lab and in the natural conditions would relevant to understand the differences in clientele assemblages between species and behavioural phenotypes.

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5.3. A cleaner is not always a cleaner: Stress involvement in life history changes

Until now, we have focused either on the fine-tuning/ activational variations of individual differences in cleaning behaviour, or to the prevalence of alternative-mix strategies (plasticity) in some species of cleaners. This focus is solely relevant for the category of the so-called obligatory cleaners, i.e. those species that by being fully specialized, clean during their entire life span. However, these species occur solely in 2 of the most speciose groups of teleost fish (Labridae and Gobiidae; Vaughan et al., 2016) while the remaining majority of the cleaner identified species are categorized as facultative, i.e. clean solely during a particular life stage (mostly during their juvenile phase). Hence, cleanerfish species differ tremendously in life histories, and stress-mechanisms may well be involved in the regulation of these transitions between life-history stages, knowing that these vary in relevance when it comes to social engagement. Basically, most of the facultative cleaner species, at some point (frequently during adulthood), stop interacting with heterospecifics to focus exclusively on their conspecific networks. In comparison with obligate cleaners (with some exceptions), most facultative cleaner species try to avoid dealing with dangerous clients, which may be an indicator of a different risk appraisal (Francini-Filho & Sazima, 2008). Predator inspection is perhaps one of the greatest characteristics of obligate cleaners (see section 5.1.) and that reflects on their choice of clients; which is the case of the Brazilian cleaning goby *E. figaro*, referred to prefer piscivorous clients (Francini-Filho & Sazima, 2008). But so far, no study has focused on the mechanisms underlying the neuro-cognitive background of facultative cleaners’ life-history transitions, and how the mechanisms of stress may be implicated.

There are some insights coming from tests done on obligate cleaners, focusing on the role of the arginine vasotocin (AVT) system. The nonapeptide arginine vasotocin (AVT), homologous to mammalian arginine vasopressin (AVP), is involved in many aspects of fish physiology but most deeply in the responses to stress (Balment et al., 2006). Studies have shown that AVT mediates the hypothalamic-pituitary-interrenal (HPI) axis activation (Backström et al., 2011; Gesto et al., 2014). In obligate cleaners' *L dimidiatus*, the increase of AVT levels, with exogenous peripheral infusions, cause them to simply cease all mutualistic activities, but not conspecific behaviour (Soares et al., 2012a, 2012c). These results contrast with those found with cortisol treatments, with cleaners altering strategic tactics towards their clientele (see section 5.1 above, and Soares et al. (2014) and Binning et al (2017)). Thus, we are in view of different stress response magnitudes, producing significant behavioural responses: smaller variations of cortisol producing new metabolic-dietary demands which justifies a change in behavioural pursuit (Soares et al., 2014) and the mediation of AVT on the structural, life history changes between cleaning and non-cleaning, which ultimately underlies a tremendous switch in social and cognitive output (Soares et al., 2012a, 2012c, Cardoso et al., 2015a, 2015b).

5.4. Stress, monoamines and cleaning: an emergent mixture mediating social complexity

At this point we can surely state that stress plays a relevant role in this system: it works to fine-tune cleaners' behavioural states, hence contributing to strategic changes, but overall it helps cleaners to cope with socio-environmental challenges, being the most remarkable, the way they deal with predatory vulnerability but also being involved in life history changes. While cleaners dispose of behavioural and other structural variables to cope with stress, other physiological mechanisms are set to regulate its impact.

For instance, stress factors are known to induce brain dopamine activity in fish (Chabbi & Ganesh, 2015). Naturally, because the functions of the dopaminergic (DA) system are multiple, involving decision-making, learning and reward mechanisms (for example, Messias et al (2016a, 2016b); Schultz (2002, 2006); Soares (2017)), the relationship between dopamine and stress will also be complex, contextual and brain-region specific. As such, the DA system should respond differently to distinct types of stressors as it confers to animals the ability to discriminate between change-related stimuli (Pani et al., 2000). Thus, if DA hyperactivity could lead to a higher propensity to develop addictions, compulsive behaviour, and novel-seeking behaviour, on the other hand, DA depletion may lead to tremendous cognitive impairments and pathologies (for instance, Brozoski et al. (1979); Cools et al. (2001)); while both extreme cases should underlie unbalanced states of individual stress response. Exogenous manipulations made to cleaners' DA system revealed disproportional behavioural changes due to impairment: the treatment with D1 and D2-like receptor antagonists made cleaners interact more but not to forage, rather to almost exclusively provide tactile stimulation to clients (Messias et al., 2016a). This costly investment in clients, when prolonged (in the case of D1 influence) should work as an omission of the predicted reward (Messias et al., 2016a) and may elicit an increase of stress response. Moreover, D1 blockage seems also to be related to an increase of novelty seeking behaviour in cleaners (Soares et al., 2017b). On the other hand, the increase of DA activity seems to enable cleaners learning abilities (Messias et al., 2016b), being that this so-called motivational increase to learning new tasks could be coupled with an increase of stress levels, which at this point is purely speculative. Interestingly, when signal and reward differ (in time and space), a scenario that in natural conditions would occur when a situation of an observing bystander client, DA blockage reduces cleaner impulsiveness towards the sign (sign-tracking response, see Soares et al (Soares et al., 2017a)), which suggests that stress-control mechanisms should also be in place.

The serotoninergic system has also a crucial modulator role on animal stress response and on helping animals to cope with stress. For instance, social stressors are known to increase brain serotonin turnover in fish (Winberg & Nilsson, 1993; Winberg et al., 1997; Dahlbom et al., 2012; Teles et al., 2013), indicating that animals make use of available serotonin to cope with stress effects. Cleaners, whether engaging or not in stressful interactions (depending on the client inspected), need to cope rapidly if they want to continue to forage. The effect of the so-called "serotonin activity facilitators", both fluoxetine and 8-OH-DPAT, motivated cleaners to interact more frequently and become more likely to provide physical contact to clients (tactile stimulation; Paula et al., 2015). On the other hand, the action of serotonin blockers was mostly observed by the significant reduction in the willingness to clean but also, in the rise of confrontational attitude in relation to other smaller conspecifics (Paula et al., 2015) and in delaying the learning competence of cleaners (Soares et al., 2016). Whether this exogenous blockage is eliciting an increase of cleaners' stress levels, beyond what they can normally cope while cleaning, its yet to be discovered.

6. A way forward: Integration between stress and sociality in fish

While there are consistent structural differences in the aversive, social behaviour, and mesolimbic reward networks between fish and mammals, the degree of behavioural conservation in functions of aversive behaviour/stress and sociality between fish and mammals appears striking. For example, an important role for dopamine in shoaling (Buske & Gerlai, 2011; Scerbina et al., 2012; Saif et al., 2013a) parallels the role of this neurotransmitter in the mesolimbic reward-SBN interface. Indeed, shoaling appears to be a motivated behaviour (Al-Imari & Gerlai, 2008) that leads to dopamine release (Saif et al., 2013b). Nonetheless, it is now clear that teleosts do not possess a dopaminergic mesolimbic projection *per se*, having no ventral tegmental area nor a nucleus accumbens (Yamamoto & Vernier, 2011). Instead, a functional analogue (the posterior tuberculum) produces dopamine that is released in a partial homologue (the dorsal nucleus of the ventral telencephalon) (Rink & Wullimann, 2001, 2004; Tay et al., 2011). Thus, a paradox of function vs. structure is observed when fish and mammals are compared (Figure 3). This underlying theme – of conserved function without conserved structure – resonates throughout all monoaminergic systems in teleosts (Figure 3A; Herculano & Maximino, 2014; Maximino et al., 2015b, 2016); indeed, at least in the serotonergic system, it is the mammalian state that appears to be derived (Herculano & Maximino, 2014).

What, if any, are the shared functions of monoamines in stress and sociality in fish? The work reviewed here gives a few hints. First, 5-HT contributes to passive behavioural responses in unstressed animals, increasing avoidance of potentially dangerous places but decreasing avoidance of certain threat (Figure 3B); in stressed animals, however, 5-HT appears to act differently in proactive vs. reactive animals, with increased 5-HTergic signaling possibly promoting active responses to reduce stress and/or eliminate aversive stimuli. DA appears to have a similar role on anxiety and fear, with the activation of D1 receptors increasing anxiety, and the activation of D2 receptors promoting stress-related cortisol release. However, DA and 5-HT appears to have opponent roles in modulating sociality, with D1 and D2 receptors inhibiting cleaning mutualisms but promoting shoaling, and 5-HT$_{1A}$ receptors promoting cleaning and shoaling (Figure 3B).

The research reported in this review suggests that the same systems which modulate aversive behaviour and stress also participate in sociality in fish. Moreover, it is now clear that social interactions are also stressful for fish – especially in the case of mutualistic species, in which every interaction carries the potential for predation. However, stress tends to increase shoaling and decrease social preference (Giacomini et al., 2016). Is it possible that cortisol is the mediator of D2-like effects on cooperation (Figure 3C)? What are the roles of this receptor on shoaling? What, if any, are the roles of D1-like receptors on shoaling? These are all open questions that should clarify the interaction between monoamines and stress in fish sociality.

The relationship between stress coping and sociality is also of interest here. Reactive coping is associated with submissive behaviour and higher reactivity to social encounters, both of which can make social interactions highly stressful. As a result, reactive animals should be less adapted to fluctuating social interactions, showing lower social competence. The suggestion that reactive coping, in fish, is associated with higher stress-induced 5-HT neurotransmission in the Dl (part of the mesolimbic reward system) and lower stress-induced increases in 5-HT signaling in the Dm (part of both the mesolimbic reward system and the aversive behaviour system) suggest that 5-HT tends to inhibit social approach after stress in the Dl and promote it in the Dm.

These observations on the relationships between coping, monoamines, and social behaviour also hint to applications to modeling psychiatric disorders in teleost fish. It has been suggested (Trower & Gilbert, 1989) that individuals with social anxiety disorder suffer from alterations in mechanisms that select socially appropriate behaviour, always acting “as if” they are in a subordinate position. The emergence of fish as model organisms in biological psychiatry (Kalueff et al., 2014; Soares et al., 2017c) positions the findings reported in this review in this larger context. An example is the reported application of social behaviour in zebrafish models of FASD, which shows the potential of these approaches in the discovery of targets and basic mechanisms involved in brain diseases. The rapid increase in the use of fish in biological psychiatry and psychopharmacology (Kalueff et al., 2014) suggests the utility of such approaches.

The direction pointed by this review suggests novel avenues for understanding stress and sociality in fishes in at least two levels: the level of taxon-specific innovations, and the level of what is conserved with vertebrates. For example, understanding fish stress and sociality at the functional neuroanatomical level is probably going to pinpoint solutions which were found by this specific taxon, but will also be able to reveal “deep homologies” between fish and mammals. At the functional level, many of these conserved neurobehavioural functions are currently being used to investigate human diseases, employing fish behaviour (and its modulation by monoamines) as behavioural models in biological psychiatry and psychopharmacology. To reconcile the level of divergence with the level of similarity, neuroanatomy, pharmacology, behavioural analysis, and ecology studies conducted in the lab and in nature need to add to each other and enhance our understanding of fish behaviour and ultimately how this all may translate to better model systems for translational studies.

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Figure captions

Figure 1. (A) Intersections between structures involved in the defensive behaviour network, social behaviour network, and mesolimbic reward network in the vertebrate brain. Structure names refer to mammalian nomenclature. (B1) The mesolimbic reward network in the teleostean brain. (B2) The social behaviour network in the teleostean brain. (B3) The aversive behaviour network in the teleostean brain. (*A)* Adapted from Soares et al. (2017c); (*B1)* and (*B2)* adapted from O'Connell & Hoffman (2011b). Abbreviations in (*A)*: NAcc: nucleus accumbens; VTA: ventral tegmental area. Abbreviations in *(B*): aTN: anterior tuberal nucleus; Dl: dorsolateral telencephalon/lateral pallium; Dm: dorsomedial telencephalon/medial pallium; GC: griseum centrale/central gray; POA: preoptic area; Vd: dorsal nucleus of the ventral telencephalon/dorsal subpallium; Vl: lateral nucleus of the ventral telencephalon/lateral subpallium; Vs: supracommissural nucleus of the ventral telencephalon/supracommissural subpallium; vTN: ventral tuberal nucleus; Vv: ventral nucleus of the ventral telencephalon/ventral subpallium.

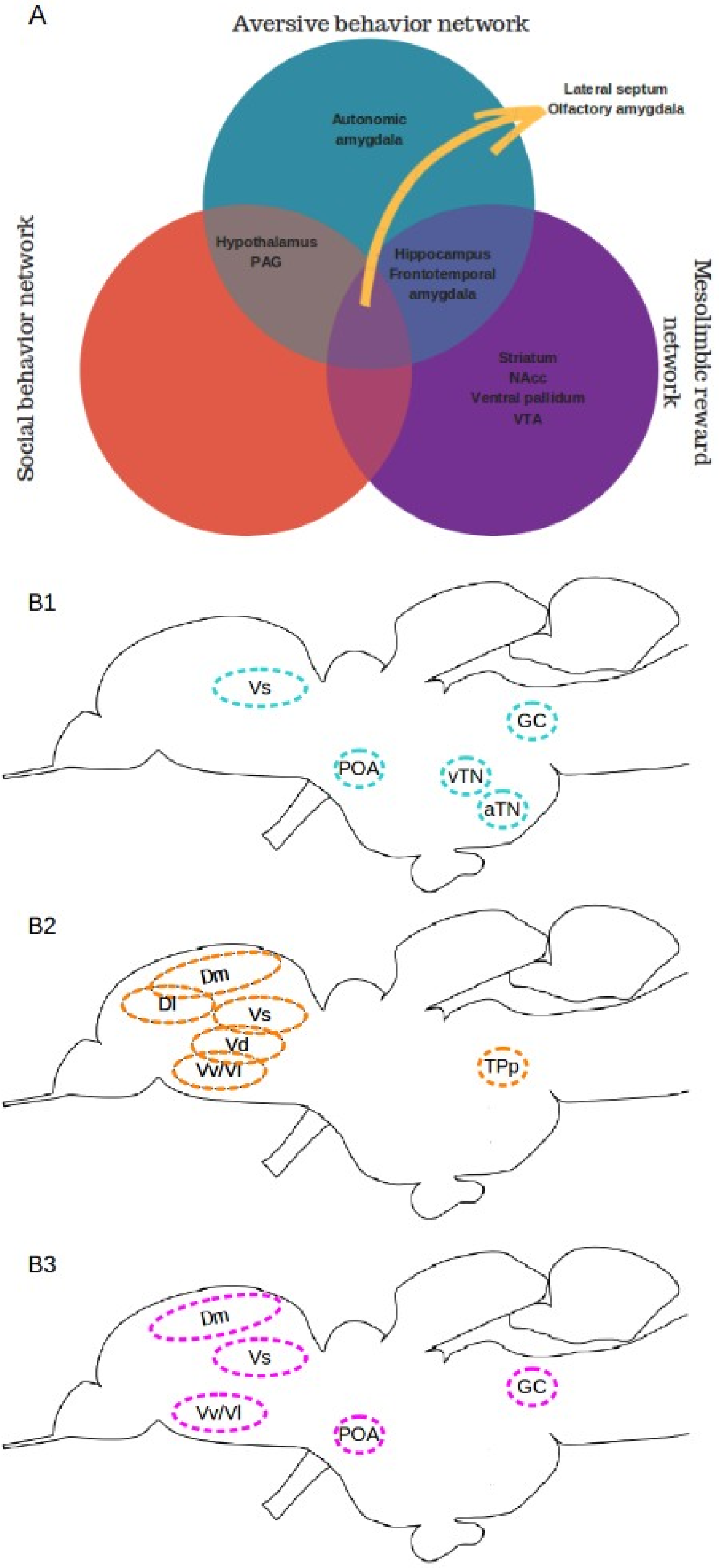

A
Aversive behavior network
Lateral septum
Olfactory amygdala
Autonomic amygdala
Social behavior network
Hypothalamus
PAG
Hippocampus
Frontotemporal amygdala
Mesolimbic reward network
Striatum
NAcc
Ventral pallidum
VTA
B1
Vs
GC
POA
vTN
aTN
B2
Dm
Dl
Vs
Vd
Vv/Vl
TPp
B3
Dm
Vs
GC
Vv/Vl
POA

Figure 2. (A) Temporal variation of cortisol levels in holding-water of cleaning gobies (*E. evelynae* and *E. prochilos*) individuals challenged with an intra-peritoneal injection of porcine ACTH (red line) or Ringer's solution (black line). Interaction effect: 2-way RM-ANOVA, F3,27 = 9.83; p=0.008(B) Same individual response variations of holding-water cortisol to either harmless (H) and/or predatory (P) client stimuli. Variation in hormone levels are relative to baseline levels (control). P values refer to pairwise T tests (*, 0.05; NS > 0.05). Error bars represent ± 1 SEM. N = 7 for *E. evelynae* and N=10 for *E. prochilos*. M. C. S., unpublished data.

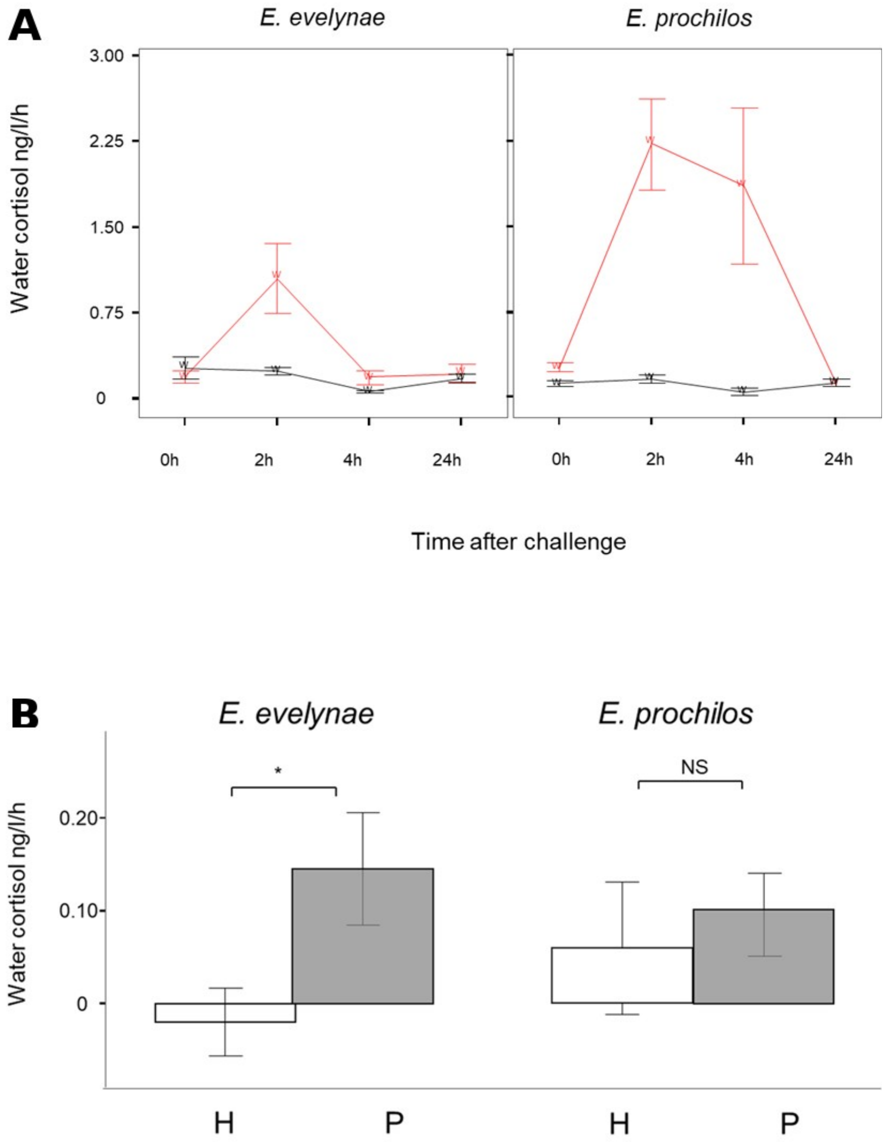
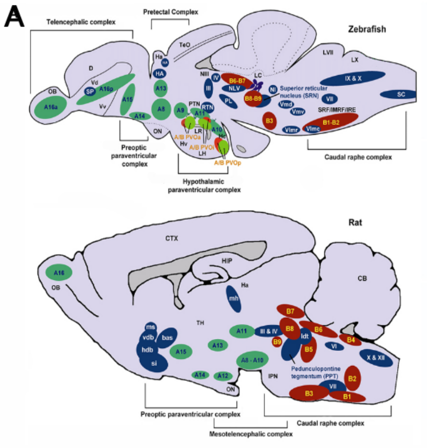


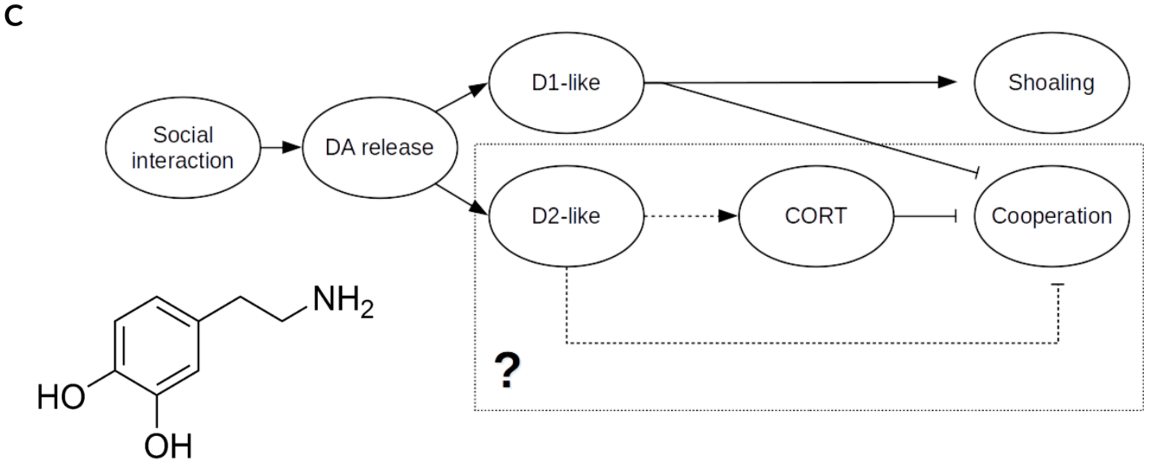
Figure 3. (A) Lack of neuroanatomical conservation of main monoaminergic nuclei in teleosts in relation to mammals. In teleosts (upper panel), monoaminergic centers proliferate throughout the brain; while rodents (lower panel) concentrate 5-HTergic neurons (red) in the raphe complex, teleosts have extra nuclei in the hypothalamus and pre-tectum. Likewise, teleosts have extra DAergic nuclei in the telencephalon, and no ventral tegmental DAergic neurons. Adapted from Parker et al. (2013) and Maximino et al. (2015b). (B) Differential roles of dopamine and serotonin receptors on social and aversive behaviour in teleost fish. (C) Putative pathways for dopaminergic mediation of shoaling and cooperation in teleosts. Social interaction (with conspecifics, in the case of zebrafish, and heterospecific clients, in the case of cleaner wrasse) is known to increase DA levels in the brain. The activation of D1-like or D2-like receptors inhibit cooperation in cleaners, and the activation of D1-like receptors promote shoaling in zebrafish. Inside the box marked with a question mark, a mediation by cortisol of the D_2 receptor response is proposed. Figure made on Piktochart (https://create.piktochart.com/output/7200807-untitled-infographic).

Monoaminergic modulation of fish social and aversive behavior



B

	Social behavior	Aversive behavior and stress	Other
D1R	Promotes shoaling Inhibits cleaning interactions	Promotes anxiety-like behavior	Inhibits novelty seeking Promotes learning
D2R	Inhibits cleaning interactions	Promotes stimulated CORT release	
5HT1AR	Promotes shoaling Promotes cleaning interactions	Inhibits stimulated CORT release Promotes anxiety-like behavior Inhibits fear-like behavior	Promotes learning
5HT1BR		Promotes anxiety-like behavior	
5HT2R		Promotes anxiety-like behavior Inhibits fear-like behavior	



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