


# Social zebrafish: *Danio rerio* as an emerging model in social neuroendocrinology

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## Abstract

The fitness benefits of social life depend on the ability of animals to affiliate with others and form groups, on dominance hierarchies within groups that determine resource distribution, and on cognitive capacities for recognition, learning and information transfer. The evolution of these phenotypes is coupled with that of neuroendocrine mechanisms, but the causal link between the two remains underexplored. Growing evidence from our research group and others demonstrates that the tools available in zebrafish, *Danio rerio*, can markedly facilitate progress in this field. Here, we review this evidence and provide a synthesis of the state-of-the-art in this model system. We discuss the involvement of generalized motivation and cognitive components, neuroplasticity and functional connectivity across social decision-making brain areas, and how these are modulated chiefly by the oxytocin-vasopressin neuroendocrine system, but also by reward-pathway monoamine signaling and the effects of sex-hormones and stress physiology.

## KEYWORDS

cognition, motivation, neuroendocrine, social, zebrafish

## 1 | INTRODUCTION

Social interactions provide key fitness benefits across the evolutionary scale, including better growth, survival and reproductive success.<sup>1,2</sup> In vertebrates, these benefits are facilitated by more nuanced phenotypic repertoires supported by the greater complexity and flexibility of their central nervous system and its interaction with endocrine mechanisms.<sup>3–5</sup> This transversally translates to three key components that enable the formation, organization and function of social groups: social dominance, affiliation (aka social bonding) and social cognition. In particular, dominance hierarchies organize group leadership, determine collective response, and resolve resource allocation; stronger affiliations enable group formation, effective cooperation and mating; while cognitive processing underlies the use and appraisal of social information for improved competitiveness, threat detection and stress management.<sup>6,7</sup> Over the past decade or so, our research group has been examining the neuroendocrine mechanisms underlying these key components in zebrafish, an archetypal model for the evolution of vertebrate sociability, where precise mechanistic tools can be

combined with the detailed quantification of phenotypic expression.<sup>8–11</sup> The aim here is to provide an overview and analysis of our findings, but also to examine where these findings place zebrafish as a model for the study of the neuroendocrinological drivers of social behavior.

Social behavior relies primarily on motivational components, related to the drive to approach and engage others, and cognitive components based on the collection, appraisal, memorization and use of social information. The interplay between these components determines interindividual and group dynamics, and zebrafish are an ideal model to test this because both these social dynamics are central to their response to fitness challenges.<sup>12–15</sup> For instance, dominance within zebrafish groups determines their response to predation and resource-seeking, and it is established by dyadic contests that involve the motivation to engage with opponents and the assessment of their aggressiveness and prior dominance status. Also, the formation of groups relies on the motivation to affiliate with others and the recognition of novel from familiar conspecifics, and social learning requires associations of social cues to rewarding or aversive outcomes

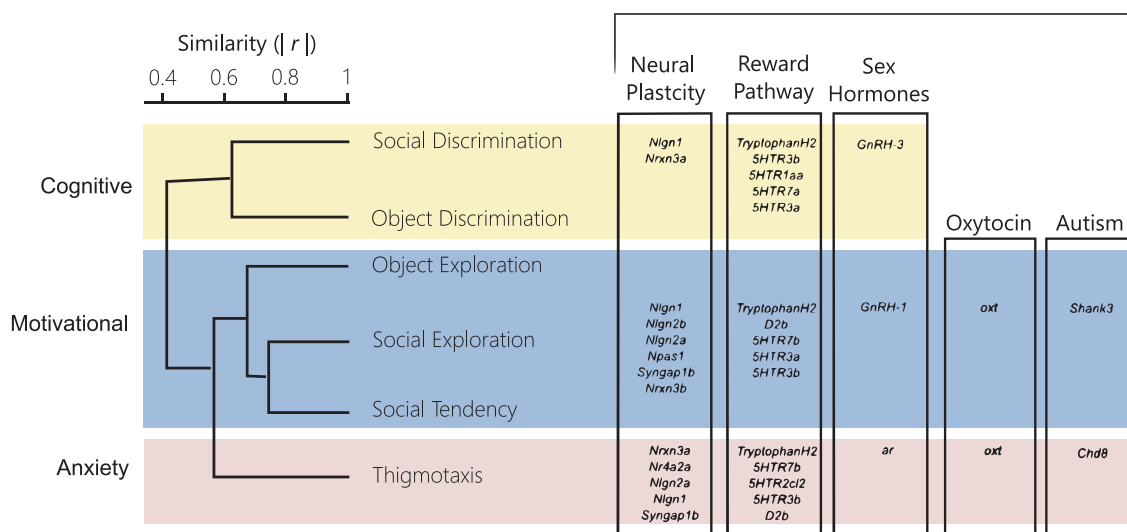
influencing motivation. The role of these components raises a central question about social behavior: whether it is a result of specialized functions and dedicated mechanisms, or whether it relies on general-domain motivational and cognitive components co-opted for use in social contexts.<sup>16–19</sup>

To address this, we recently took an exploratory approach to identify phenotypic components of zebrafish behavior across social and non-social contexts, and to quantify their association to genetic polymorphisms (i.e., single-nucleotide polymorphisms, SNPs) across a list of leading candidate social genes. Although we focused on common lower-order components, such as interaction and exploration tendencies, recognition abilities, and anxiety levels, we found their organization to be as complex as in mammalian vertebrates. In particular, across six widely used wild-type laboratory strains, we found that the memorization and recognition of familiar individuals is strongly correlated with that of familiar objects, and that the motivation to interact with others is linked with the tendency to explore objects and individuals alike.<sup>20</sup> This demonstrates that, similar to support for general-domain mammalian learning and affective mechanisms,<sup>3,21–23</sup> basic cognitive and motivational phenotypes in zebrafish are also generalized for social and non-social contexts. The two phenotypes also cluster separately, with a moderate association of  $r \leq 0.4$ , and share only few SNPs in key social-genes, which suggests they are also partly independent of each other. Moreover, compared to the ability to discriminate familiar from unfamiliar objects and individuals, the motivation to explore and interact with objects and individuals was more strongly related to anxiety behavior in an open field; motivation and anxiety components shared strong associations to polymorphisms in

the oxytocin gene, but also implicated polymorphisms in leading autism genes (Figure 1).

Anxiety is defined by an individual's responsiveness to potential risk, and the effect of exaggerated responsiveness in social contexts can explain the genetic associations with drivers of autism, a pathology generally related to social motivation deficits and social anxiety.<sup>24</sup> It is also reasonable that anxiety influences more the motivation to explore novel stimuli and interact with conspecifics, than the memorization of social cues, and that this involves the oxytocin system given its well-established anxiolytic and prosocial functions.<sup>25,26</sup> Indeed, in our body of work we found oxytocin (CYISNCPIG-NH<sub>2</sub>, aka isotocin; please see Theofanopoulou et al.<sup>27</sup> for a normalization of the term oxytocin across vertebrates) to modulate the motivation to interact with novel conspecifics, and also perceptual and developmental elements of social interaction.<sup>28–30</sup> Although anxiety-inducing social contexts may also implicate elevated oxytocin signaling as a result of anxiogenic functions, their proposed dualistic function is enabled by effects to the saliency-network whereby oxytocin influences the perceived intensity of sociobehavioral cues, either positive or negative.<sup>31</sup>

Together with arginine vasotocin/vasopressin (AV) oxytocin belongs to the evolutionarily conserved nonapeptide system. Due to their structural similarities each of these peptides is able to activate the other's receptors, hence engaging in a so-called “cross-talk” between the two systems.<sup>32,33</sup> Contrary to oxytocin, human and rodent AV is mostly known to drive anxiogenic effects by promoting stress-hormone production and to drive aggressiveness, particularly in males and nurturing mothers.<sup>34–36</sup> However, pharmacological evidence in male mice suggests that while the inhibition of vasopressin



**FIGURE 1** Clustering of social and non-social behavior across three main phenotypic components and their association to genetic polymorphisms. The diagram summarizes findings from a study of six common zebrafish wild-type strains.<sup>20</sup> Social tendency, measured by the proportion time spend near others, clustered with exploration times both when exposed to conspecifics and objects, in an arbitrary motivational component. The ability to discriminate between familiar and novel individuals, as ascertained by local preferences, clustered with the same ability towards objects under a separate ostensibly cognitive component. Both components were discrete from measures of anxiety measured by edge orienting in a novel open arena, although anxiety more strongly associated with the motivational component. All components related to single nucleotide polymorphisms (SNPs) in neural plasticity, reward signaling and gonadocorticoids, but most polymorphisms were different between components. Notably, motivational and anxiety phenotypes related to the oxytocin system and leading autism candidate genes.

signaling (by antagonists) reduces aggressive behavior, treatment with oxytocin also does so.<sup>36,37</sup> In line with these findings, our work in zebrafish also shows that aggressive interactions involve changes to both nonapeptides, with vasopressin levels being greater in more brain areas in winning animals and oxytocin being elevated in the diencephalon of losing animals.<sup>38</sup> This is most likely due to feedback-related antagonistic effects, particularly in interaction with the stress-hormone system.<sup>39–42</sup> This is suggested by the fact that winners, which present elevated plasma cortisol, also exhibit forebrain increases in AV levels coupled with decreases in oxytocin levels.<sup>38,43</sup> Thus, despite their contrasting functions, the two nonapeptides can be effective in the same social contexts, which suggests the involvement of the nonapeptide system in social behavior as a whole.

Our SNP analysis also revealed that genes for neuropeptides and neurexins, and those involved in the dopamine and serotonin pathways, were also involved across the generalized motivational, cognitive and anxiety modules. Variants in these genes are also joined by polymorphisms in gonadotropin-releasing hormone genes for the cognitive and motivational components, and androgen receptor genes for anxiety (Figure 1). Together, these results indicate that neuroplasticity and reward-related signaling are key shared neural mechanisms across social phenotypes, whereas neuroendocrine modulation is performed by the oxytocin-vasopressin nonapeptide system, and the case-specific involvement of the sex-hormone system (i.e., the hypothalamic–pituitary–gonadal axis, HPG) and the stress-hormone system (i.e., the hypothalamic–pituitary–inter-renal, HPI, or hypothalamic–pituitary–adrenal axis, HPA, in mammals). In the following sections we discuss how these collective mechanisms contribute to complex social dominance interactions, to fundamental processes for social affiliation, and to different social cognition abilities.

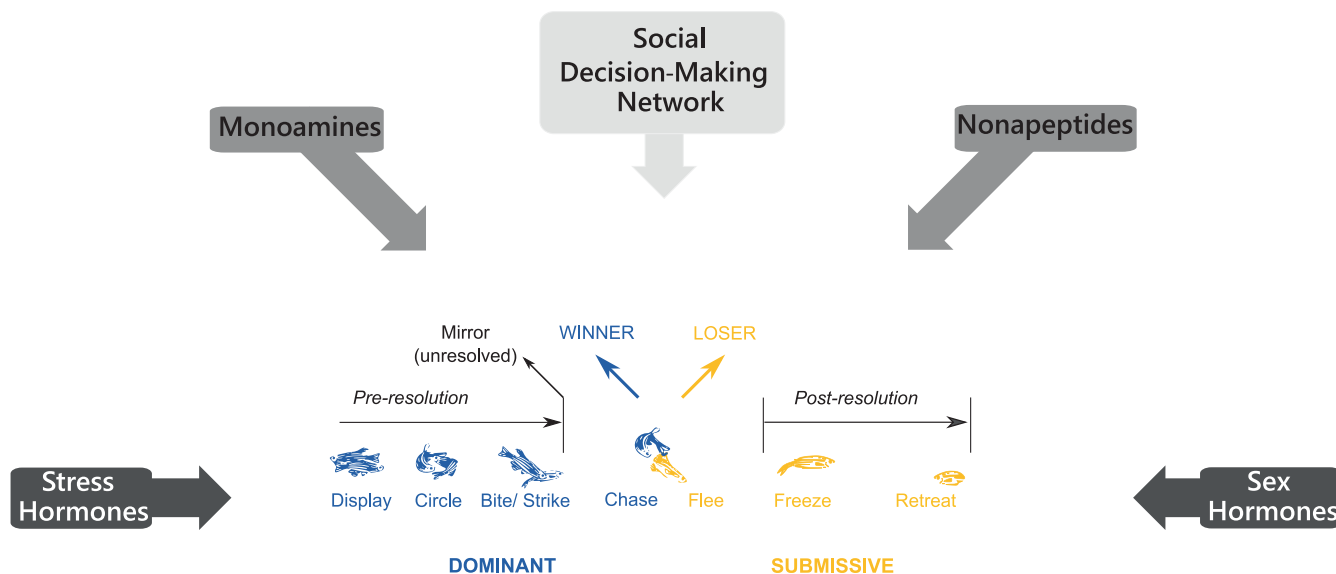
## 2 | SOCIAL DOMINANCE: WINNER-LOSER DYNAMICS AND ASSESSMENT MECHANISMS

The detailed characterization of zebrafish social dominance phenotypes allows the study of the neuroendocrinology of aggression in a model for which a genetic tool kit for the visualization and dissection of socially activated neural circuits is available. The use of zebrafish also has further advantages in terms of their small size, large number of progeny, external fertilization and transparency during development, which facilitate the study of the development of different structures and systems. In our group we started by establishing a behavioral paradigm under which male zebrafish consistently express fighting behavior, characterizing the temporal structure of this behavior during dyadic contests, and identifying effects of previous experience in terms of winning and losing.<sup>44,45</sup> To this end, two size-matched naïve males are first isolated for 24 h and then allowed to interact for 30 min, to clearly identify a winner and loser, and finally the process is repeated for both the winner and loser by pairing each with a novel naïve opponent. This paradigm enables the induction of aggressive interactions even in the absence of a limiting resource

(i.e., the 24 h isolation) and dyads present temporally organized and structured fighting behavior that facilitates quantitative analysis (Figure 2). This behavior comprises a sequence of aggressive acts that determine dominance, initiating with lateral displays and circling, escalating first to bite and strike attacks, and ultimately chases. Escalated attacks have recently been analyzed kinematically for the use of automated-tracking machine-learning pipelines, demonstrating the state-of-the-art in methods that can provide standardized, objective and precise measures of behavior in zebrafish models.<sup>47</sup> Submissive acts include stereotyped fleeing and freezing, but also retreating/quitting behavior, providing the opportunity to identify the timing of the fight's resolution and determine winners and losers. We also demonstrated that a fight's outcome has a very strong effect on subsequent interactions, where loser effects have a higher impact than winner effects as described in other species.<sup>48,49</sup> In particular, winners go on to win 85.71% of second interactions, whereas losers only win 4.55%. More recent work was able to show that this effect is short lasting, except when individuals face the same opponent and the effect can persist up to 24 h later.<sup>50</sup> The pending question is which mechanisms might be implicated in these effects.

### 2.1 | Neuromodulation of social dominance: Monoamines, nonapeptides and hormones

Given the risk–reward trade-offs between winners and losers, monoamine signaling systems are a leading candidate mechanism for social dominance. However, evidence on the effects of monoamines, serotonin and dopamine, on the regulation of aggressive behaviors has been mixed. For serotonin, some studies demonstrated that it inhibits vertebrate aggression, whereas other studies show increased serotonergic activity in specific brain regions during aggression, suggesting that serotonergic regulation acts as a function of environmental context.<sup>51,52</sup> For dopamine the scenario has been somewhat similar, where its activity in the prefrontal cortex and nucleus accumbens not only relates to the initiation of attacks, but also to defensive and submissive responses when being attacked.<sup>53</sup> Together, this evidence suggests that the two systems are heavily involved in agonistic social interactions, similarly across different escalating and de-escalating or submissive behavior. Thus, the high diversity and plasticity of social behaviors in teleosts, such as zebrafish, makes them an excellent system to test the role of these mechanisms. To this end we used our established male zebrafish dyadic-contest paradigm, from which winners and losers could arise, together with interactions with a mirror, where fish experienced an unsolved interaction, and a control group experiencing no interaction.<sup>46</sup> Immediately after each treatment, brains were collected and divided in macro-areas to quantify levels of monoamine and their respective metabolites. During real-opponent interactions males expressed distinct behavioral profiles, with losers exhibiting exclusively submissive behaviors and winners only aggressive behaviors. Those interacting with their mirror image exhibited only aggressive behaviors with a frequency similar to the winners group. Literature has since amassed some criticisms and observations



**FIGURE 2** The agonistic phenotype contributing to zebrafish social dominance and the underlying neuroendocrine mechanisms. Preresolution phenotypes are determinant of the winner's dominance whereas submissive behaviors by the loser determine fight outcome. Fights against mirror images cannot be resolved due to the lack of submissive responses to escalated attacks. The experience of winning or losing relies on local activity and functional connectivity across a network of brain areas involved in social decision-making and which implicates nonapeptide (oxytocin and arginine vasotocin/vasopressin) and monoamine (dopamine and serotonin) signaling, as well as contributions from sex hormones and stress physiology (figure contents adapted from own published work<sup>46</sup>).

regarding mirror tests that may apply to these effects, but in summary the prevalent view is that mirror tests can capture individual aggressiveness but do not faithfully represent real-opponent strategic decision-making, that is, escalation versus submission.<sup>54–56</sup> Nevertheless, we were successful in producing four types of social experiences: winning, losing, non-resolved aggressive interaction (Figure 2) and no interaction (control: social isolation). Quantifying the monoamine levels of groups exposed to either experience, we found serotonergic activity (5HIAA/5HT ratio) to be significantly higher in the telencephalon and olfactory bulbs of winners and in the optic tectum of losers, whereas no significant changes were observed in mirror-fighters compared to the control group.<sup>46</sup> These results suggest that variations in the specialized activity of serotonin is specific to outcome and not the social interaction itself. In contrast, dopaminergic activity was significantly higher in the telencephalon for winners, and higher in the optic tectum for both losers and mirror-fighters. For winners, the increased dopaminergic activity is in line with expectations for increases in social rank and its specific role in the telencephalon may reflect social reward. The increased activity in the optic tectum of losers and mirror-fighters suggests that what is driving this activity is what they observe, that is, the aggressive behavior of the opponent, rather than the behavior they express.

As in monoamines, the role of nonapeptides in the regulation of aggressive behavior has been shown to be varied and species dependent.<sup>57</sup> The differences between species can also arise from differences in regulatory processes, for instance, differences in the number and size of neurosecretory cells, the sensitivity of target tissue (receptors), and the local availability at synaptic points.<sup>58</sup> So far, few studies have measured local peptide concentrations at regions of interest in

the brain in order to link it with the expression of different social behaviors. Using the four contest experiences described previously, we addressed this in zebrafish and found that acute aggressive interactions are associated with rapid changes in nonapeptide levels across the brain and depend on the experience.<sup>38</sup> Compared to non-interaction controls, losers presented higher AV levels in the forebrain (telencephalon and diencephalon), optic tectum, and brainstem; higher oxytocin levels in the diencephalon and lower oxytocin levels in the cerebellum. Winners exhibited increased AV levels in the forebrain, and reduced oxytocin levels in the olfactory bulbs. Mirror-fighters showed increased levels of AV only in the telencephalon. Overall, AV seems to be more involved in the response to acute agonistic interactions than oxytocin, which is in line with results in mammals. This was further supported by the use of discriminant analysis where AV levels were able to better classify individuals into the different social-experience groups than oxytocin levels. The effects of the oxytocin-AV system might involve up- or downstream interactions with other endocrine systems, given the two nonapeptides are linked with the regulation and feedback control of both the HPA axis (and HPI axis in fish) and the HPG axis.<sup>59–61</sup>

The most prominent involvement of the HPG axis is via the effects of androgen responses on male–male territorial competition,<sup>62,63</sup> which may potentially differ in gregarious species, such as humans and zebrafish, given their high tolerance for same-sex conspecifics. However, aggressive interactions comprise a social challenge that imposes stress within the group and may also activate the HPI axis.<sup>63</sup> To examine this, in zebrafish we quantified 11-ketotestosterone (11-KT) and testosterone to identify endogenous androgen responses, and cortisol levels were used as an indicator of elevated endocrinal stress response.<sup>64</sup>

Compared to non-interacting controls, 11-KT levels increased only when encountering real opponents, and thus resolving the contest, irrespective of whether animals won or lost. Compared to the absence of effects in unresolved fights, when animals faced a mirror image, the results suggest that increases in 11-KT are a response to fight resolution, rather than to any specific outcome. In contrast, testosterone levels only increased in losers, which suggests that it is an acute response to this outcome. Cortisol was elevated only in winners, compared to controls and mirror-fighters, suggesting that winning incurs higher social stress. In a separate study, we were further able to identify that this effect persists for up to 2 h after the interaction without returning to basal levels.<sup>43</sup> Together these results demonstrate that hormonal responses to aggressive interactions and their result in terms of social dominance relies on the perception of information on fight resolution and outcome. The social decision-making network (SDMN) has been proposed as a multimodal sensory information network that feeds information to individuals about their social environment. Therefore, social decisions and the consequent behavioral outputs are expected to rely on multiple neural circuits, rather than being controlled by one specific brain region.

## 2.2 | Functional connectivity and neuroplasticity responses to agonistic interactions

Although the SDMN has been proposed on functional grounds, most of its current support is based on structural evidence, that allow the establishment of homologies of its constitutive loci across taxa, as well as on patterns of reciprocal neuronal connections, that confirm the occurrence of structural (anatomical) connectivity among the different nuclei.<sup>65,66</sup> In zebrafish we carried out a functional validation of the SDMN hypothesis by analyzing how social information is mapped in the brain by testing two operational definitions used in systems neuroscience: the functional localization hypothesis, where specific functions are relegated to specific areas, and the functional connectivity hypothesis, where a function can arise by patterns of connectivity between areas.<sup>67,68</sup> To achieve this, we characterized neuronal activity across nodes of the SDMN of zebrafish males, using the expression of two immediate early genes (*c-fos* and *egr-1*) as transient markers, and examined how this related to the outcome of aggressive interactions.<sup>69</sup> Functional localization in individual nuclei revealed the increase of mRNA *c-fos* levels across all five targeted brain regions of the SDMN for all types of agonistic interactions, that is, winners, losers, and non-resolved mirror-image fights, relative to non-interacting controls. Despite contrasts in their behavioral profiles, aggressive winners and submissive losers exhibited similar expression levels across brain regions, suggesting that dominance elicits no localized activation differences. However, by examining coactivation between nuclei, we found that winners and losers exhibit different patterns of functional connectivity, where (1) different regions occupy the central position in the network of each state, (2) different connection densities and (3) clusters (subnetworks) are present in each state, and (4) coactivation correlations between regions are not associated

between the two states. Thus, dominance state depends on between-region connectivity within the SDMN, rather than any localized activity at any specific regions. This provides clear support for the functional connectivity hypothesis in controlling social dominance effects, similar to emerging trends in humans.<sup>70</sup> Nevertheless, downstream neuroplasticity effects in the SDMN remained underexplored.

To address the knowledge gap of changes in the downstream neuroplasticity we examined a set of genetic markers across different nodes of the SDMN, including the: brain-derived neurotrophic factor (*bdnf*), implicated in synaptic plasticity, particularly excitation-induced changes in synaptic strength; neuronal PAS domain protein 4a (*nps4*), involved in homeostatic changes responsible for inhibitory synapse responses to excitatory signaling; neuroligin1 (*nlg1*) and neuroligin 2 a/b (*nlg2*), as indicators of synaptogenesis; and neuronal differentiation 1 (*neurod*) and wingless-type MMTV integration site family, member 3 (*wnt3*) as neurogenesis markers.<sup>43</sup> Our results show that each social-dominance behavioral state is characterized by a specific neuromolecular pattern. Relative to non-interacting controls, winners presented the most distinct phenotype with increased expression of neurogenesis genes (*wnt3* and *neurod*) in the dorsomedial telencephalon, and of one of the synaptogenesis genes (*nlg1*) in the ventral telencephalon, but also a decreased expression of the other synaptogenesis gene (*nlg2*) in the supracommissural nucleus. Although losers were also characterized by a decrease in the expression of the synaptogenesis gene *nlg2* in the supracommissural nucleus, they also exhibited an increased expression of the *wnt3* neurogenesis gene in the ventral telencephalon and increased synaptic plasticity (*bdnf*) in the dorsolateral telencephalon. These increases were shared with animals experiencing unresolved fights by facing a mirror image, suggesting that the changes may be linked to non-rewarding fights, but not necessarily to the cost of losing.

## 2.3 | Sex-dependent effects

The rewards of a contest often rely on the individual's sex, where males and females have evolved to express aggressive behavior under different ecological contexts. In particular, mating opportunities determine much of male–male contests, whereas females often exhibit aggressiveness as part of their parental care repertoire, that is, maternal defense.<sup>71–73</sup> As a consequence, the control of aggression in vertebrates by the SDMN often exhibits sex differences, implicating areas co-opted for sexual behavior in males and parental behavior in females.<sup>74,75</sup> Therefore, we examined whether behavior and associated neuronal activation in the SDMN during aggressive interactions differed between male and female zebrafish.<sup>76</sup> At the behavioral level, males took longer to resolve fights, expressing more displays and more strikes, more submissive behavior and more retreats. Females, on the other hand, resolved fights faster by relying mostly on antiparallel displays. Non-interacting control fish exhibited no sex differences in activation, as quantified by the number of cells being positive for ribosomal protein S6 (*pS6*), a hallmark activation marker. Both males and females exposed to agonistic interactions expressed higher



numbers of *pS6* cells than the non-interacting control fish in most of the brain areas. Winners and losers of both sexes relied largely on areas homologous to hypothalamic regions and the ventral tegmental area. Aggressive behavior in male and female winners also incorporated the supracommisural nucleus, a homolog to the medial extended amygdala, which is consistent with the predictions by Goodson based on evidence from rats and mice.<sup>75</sup> However, female winners also exhibited activity in ventral telencephalic areas homologous to the lateral septum and nucleus accumbens that were inactive in male winners. Although this is consistent with evidence in rodents of regions involved in maternal aggression and the effect of aggressive experiences,<sup>77,78</sup> the differences in reproductive and parental strategies between fish and mammals suggest that this consistency in fish is likely due to the underlying effect of conserved sex-hormone mechanisms, such as estrogen signaling.<sup>66,72</sup> Conversely, male winners exclusively exhibited activity in dorsal telencephalic areas homologous to the hippocampus and the basal amygdala, and in preoptic areas. The preoptic area is one of the most conserved regions of the SDMN that is also involved in male sex behavior. In turn, basolateral areas of the amygdala have long been implicated in male mammalian aggression and recent evidence for the involvement of the hippocampus suggest regulatory effects from AV signaling.<sup>79,80</sup> For submissive behavior in losers, females maintained use of the fish homolog to the medial extended amygdala (i.e., the supracommisural nucleus), but males shifted to activation in the lateral septum. In terms of functional connectivity, the dynamics of the excitation and inhibition subnetworks, assessed against non-interacting control fish, were different between the sexes and in particular for winners. Whereas winning males show increased excitation and no changes in inhibition, winning females show a decrease in both excitation and inhibition. For losers, both males and females show a decrease in both excitation and inhibition, but inhibitory changes were greater in females. Overall, we find that zebrafish exhibit sex differences in aggression seemingly related with brain area specializations analogous to those in mammals, and their predicted dimorphic function, but also demonstrate connectivity changes that can elucidate in greater detail the complexity of neuro-mechanistic drivers.

## 2.4 | Mechanisms of assessment

Together, the evidence on brain activity and neuroplasticity highlights the importance of the social experience, whether this is winning, losing or unresolved contests, which directly implicates the assessment of information during fights. According to game-theoretical approaches for the study of animal contests, the decision of losers to quit can rely on either of three forms of assessment: (1) self-assessments of fight ability, energetic reserves and injury thresholds, (2) opponent assessment of size, weaponry and behavior, or (3) on mutual assessment of own ability compared to the opponent's.<sup>81,82</sup> Although recent meta-analyses indicate self-assessment as the most widespread strategy, changes in internal state and behavior are expected to rely on more accurate and delayed assessment

mechanisms such as mutual assessment.<sup>82</sup> To examine this we compared the brain transcriptome profile between animals with different fight experiences, given these experiences are linked also to behavioral differences, that is, winners exhibit aggressive behavior similar to mirror fighters which do not experience fight resolution, but losers exhibit submissive behavior.<sup>83</sup> We found that status-dependent changes in internal state and behavior rely on the assessment of fight outcome. Divergent changes in the brain transcriptome profile were observed between winners and losers, which parallel the changes in behavioral states. However, mirror fighters, which do not experience fight resolution, but express aggression levels in par with those of winners and receive aggression similar to that of losers, exhibit transcriptome profile differences with both winners and losers. Therefore, neither self-assessment (where mirror-fighters are expected to resemble winners in their transcriptome responses) nor opponent-only assessment (where mirror-fighters are expected to resemble losers in their transcriptome responses) can explain these results. Moreover, the single module of coexpressed genes identified in mirror fighters was not shared by winners or losers, and, notably, winners and losers did not share any gene modules either, which was an assumption of our hypothesis. This demonstrates that mutual assessment of fight outcome is required to activate transcriptomic responses, which provides some clear mechanistic evidence of how social dominance influences internal state, and likely later social interactions. Importantly, this identifies a complexity of strategy rarely seen in non-human purely behavioral models,<sup>81,82</sup> which argues for the use of mechanistic coupled approaches when attempting to model sociocognitive assessment in animals.

## 2.5 | New focal mechanisms and the case for integrative approaches

So far, the evidence in zebrafish provides insights on the interplay between discrete neuroendocrine systems, but there are gaps in the understanding of functional and casual links in the interaction between these systems. The need to address these knowledge gaps comes with increasing evidence of the interaction between systems in other animal models and with newly implicated neural circuitry of social behavior, beyond the nodes of the SDMN, and which relies on these interactions. For example, there is growing evidence of the complementary role of the habenula, which receives nonapeptide inputs, regulates SDMN midbrain monoaminergic pathways and its regulation of agonistic interactions extends to sex-specific aggressive phenotypes.<sup>84</sup> The role of this region in zebrafish agonistic interactions has been recently demonstrated via the antagonistic effects by two dorsal habenula subregions, where silencing the lateral subregion reduces winning chances and silencing the medial subregion increases them.<sup>85</sup> The implication of these habenular subregions is not limited only to the experience of winning and losing, but also to how that experience influences subsequent fights. As the authors of that study note, the circuitry includes axons from these subregions to the interpeduncular nucleus that pass through areas containing the putative

homolog to the mammalian periaqueductal gray. This is a conserved SDMN region implicated in aggression and that exhibits activation via sex hormone controls, but it has been more specifically linked to the context of social communication and the assessment of social signals.<sup>65</sup> Therefore, via this connectivity the habenula exhibits a functional link with assessment mechanisms and hormonal controls, as well as the regulatory role it has on nonapeptide and monoaminergic neurotransmission. Although the implication of the habenula in zebrafish requires further study, as a social-dominance regulatory region it presents a case for the interplay between the different neuroendocrine systems. This also emphasizes the need to further develop mechanistic zebrafish models that examine the interplay between neuroendocrine systems in a targeted manner, using molecular, genetic, neuroanatomical and pharmacological tools.<sup>8,84</sup> Similar benefits may also be provided by mechanistic approaches to understanding the development of perceptual components involved in this and other social phenotypes, such as affiliative interactions.

### 3 | SOCIAL AFFILIATION: PERCEPTUAL MECHANISMS, DEVELOPMENT AND GENE ENVIRONMENT INTERACTIONS

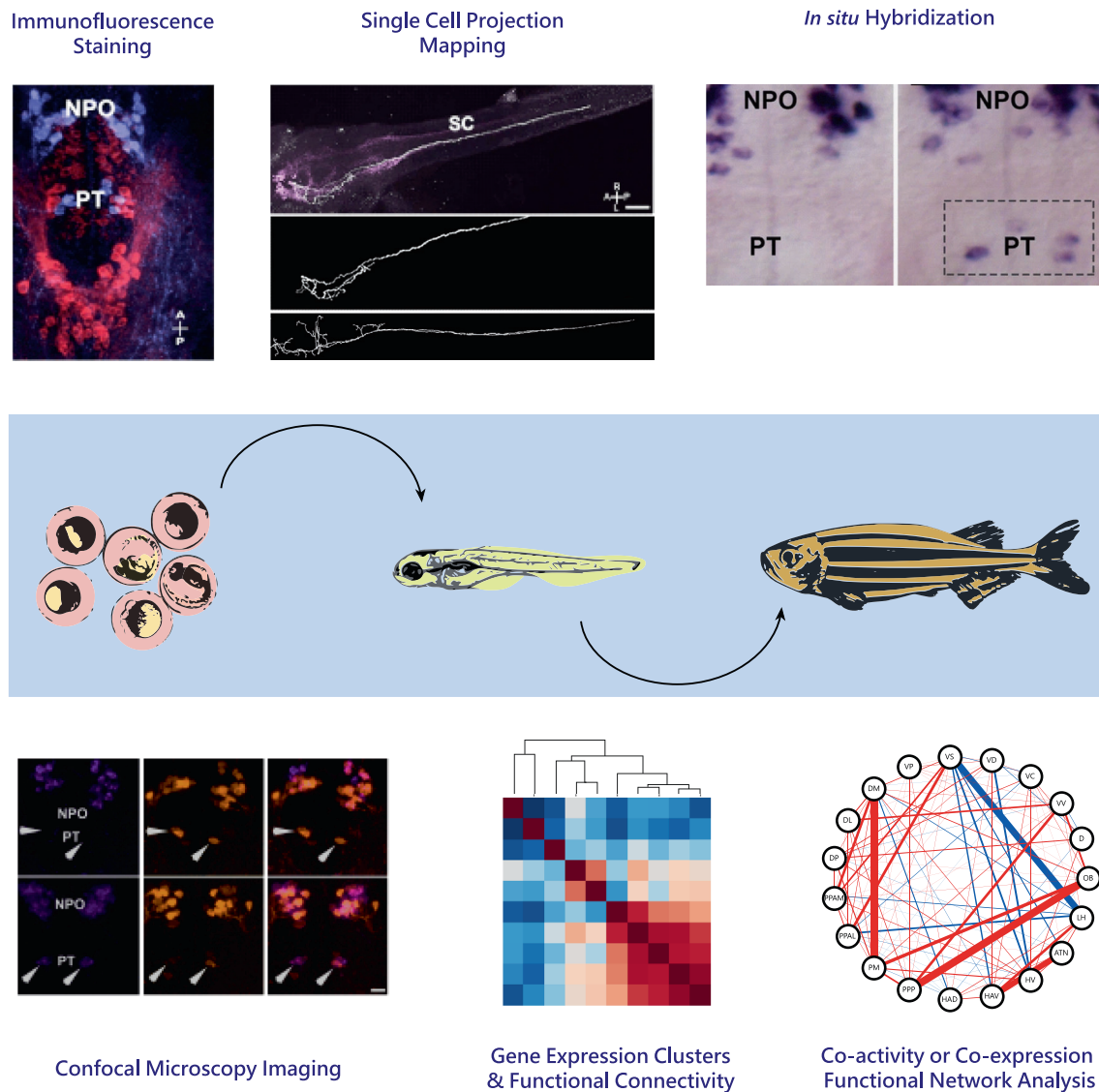
During the formation of social affiliations, the motivation to approach and interact with others relies largely on the perception of particular social cues and their saliency (i.e., their strength as a signal in contrast with background environmental noise). For visual systems, this is mediated by conspecific form/ shape and biological motion, which humans and many other vertebrates strongly rely on to identify others.<sup>86–89</sup> In a recent study of visual perception in zebrafish, biological motion has been identified as a key feature in the early-life development of social affiliation competences.<sup>90</sup> This study used a virtual reality assay for fish aged 10–30 days post fertilization (dpf), where the position of fish in a separate arena is analyzed and used to digitally produce a real-time projection of dots expressing the same motion. The study revealed that zebrafish exhibit shoaling with dots projected from below, only when the dots express the motion of a real fish, and that this response emerges around the second week of life.

#### 3.1 | Oxytocinergic regulation of affiliative response to sensory cues

In zebrafish, the way by which perceptual mechanisms utilize visual signals to modulate motivated interaction with others is facilitated by molecular tools that enable the identification of localized activation in the brain, neuronal projections from one area to another, receptor binding and gene expression, as well as analyses for the identification of interactions via colocalized and cell-specific activity, and functional connectivity patterns between areas (Figure 3). A hypothesis to explain this visually guided social affiliation is that the control of affiliative response towards visual cues may rely on signaling circuits across different visuomotor and social decision-making areas that in

concert elicit attraction to others (Figure 4A: Motivational). Consistent with this, a study of zebrafish neuronal mechanisms of attraction to biological motion, using two-photon imaging and *cfos* labeling, identified an involvement of the optic tectum, pretectum and dorsal thalamus.<sup>92</sup> One cluster in the posterior tuberculum was selectively active during response to bout-like movement and firing neurons during this response were mostly located in the optic tectum and dorsal thalamus. Using electron microscopy, the authors reconstructed the neuronal biological-motion circuit, showing that visual information reaches the dorsal thalamus neurons for bout detection through tectal periventricular neurons, which is then transmitted to the preoptic region and to clusters in the hypothalamus. These same SDMN regions have also been implicated in zebrafish social affiliation by a separate study using *c-fos* expression to map activation changes in animals with isolation-induced social deficiencies and controls, specifically in terms of preference for the presence of social cues (e.g., Figure 4B: Motivational).<sup>93</sup>

In terms of neuroendocrine systems the preoptic region is an area where oxytocin binding and projections are suggested to induce pair and parental bonding, and where oxytocin neurons are evolutionarily conserved. Moreover, some of the hypothalamic areas implicated in social affiliation and bonding are homologous to the paraventricular nucleus where oxytocin is produced.<sup>94,95</sup> Therefore, following the processing of visual information, oxytocin is a likely candidate for the social attraction response organized towards the perceived attributes of visual cues. To address this, we investigated the involvement of oxytocin in the way by which zebrafish integrate biological motion with conspecific form.<sup>29</sup> This was tested using video playbacks where focal fish were exposed to two screens on either side of a corridor and, after habituating to projections of empty tanks, were allowed to explore the arena while two different stimuli were shown in each screen (Figure 4C: Motivational). These stimuli included either a dot or a scaled image of a fish, programmed to produce biological motion in one screen and non-biological motion in the other, based on elementary animacy changes in acceleration and self-propulsion. By quantifying local preference to interact with either of the conflicting projections (Figure 5A), we found that both biological motion and conspecific form are used for social attraction. On the one hand, animals exhibited preference for the image of the fish, compared to the dot, both when stationary and when exhibiting biological motion. On the other hand, animals also exhibited an overall higher preference for biological than non-biological motion, both when observing fish and dots. By comparing response to biological motion and conspecific form between wild-type animals and their mutant siblings that did not express the primary oxytocin receptor (*oxtr*), we found that deficits in oxytocin signaling impose decreases in preference towards biological motion in dots, but not towards zebrafish images.<sup>29</sup> This suggests that conspecific form compensates the decreased perception of biological motion when the oxytocin-signaling deficiencies are in place. Overall, the regulation of affiliative responses seems to be strongly dependent on biological motion and this relies on visual-information relays from the optic tectum and dorsal thalamus, to areas of the SDMN where oxytocin signaling gains control. However, the early-life ablation of



**FIGURE 3** Illustration of molecular tools used to characterize social interaction mechanisms across zebrafish developmental stages. This includes: localized activation and cell quantification in the brain via antibody-coupled immunofluorescence staining; single-neuron projections via the labelling of cells in genetic models; the localization of target DNA or RNA sequences in a tissue using *in situ* hybridization; the quantification of specific cell activity, for example, how many active cells are inhibitory, or of colocalized signals on the same site via confocal microscopy; and the quantification of functional connectivity patterns using correlation matrices and cluster analyses of gene expression, or functional network analysis from the expression of genetic markers or molecular markers of activity (figure contents adapted from own published work<sup>28,61,91</sup>).

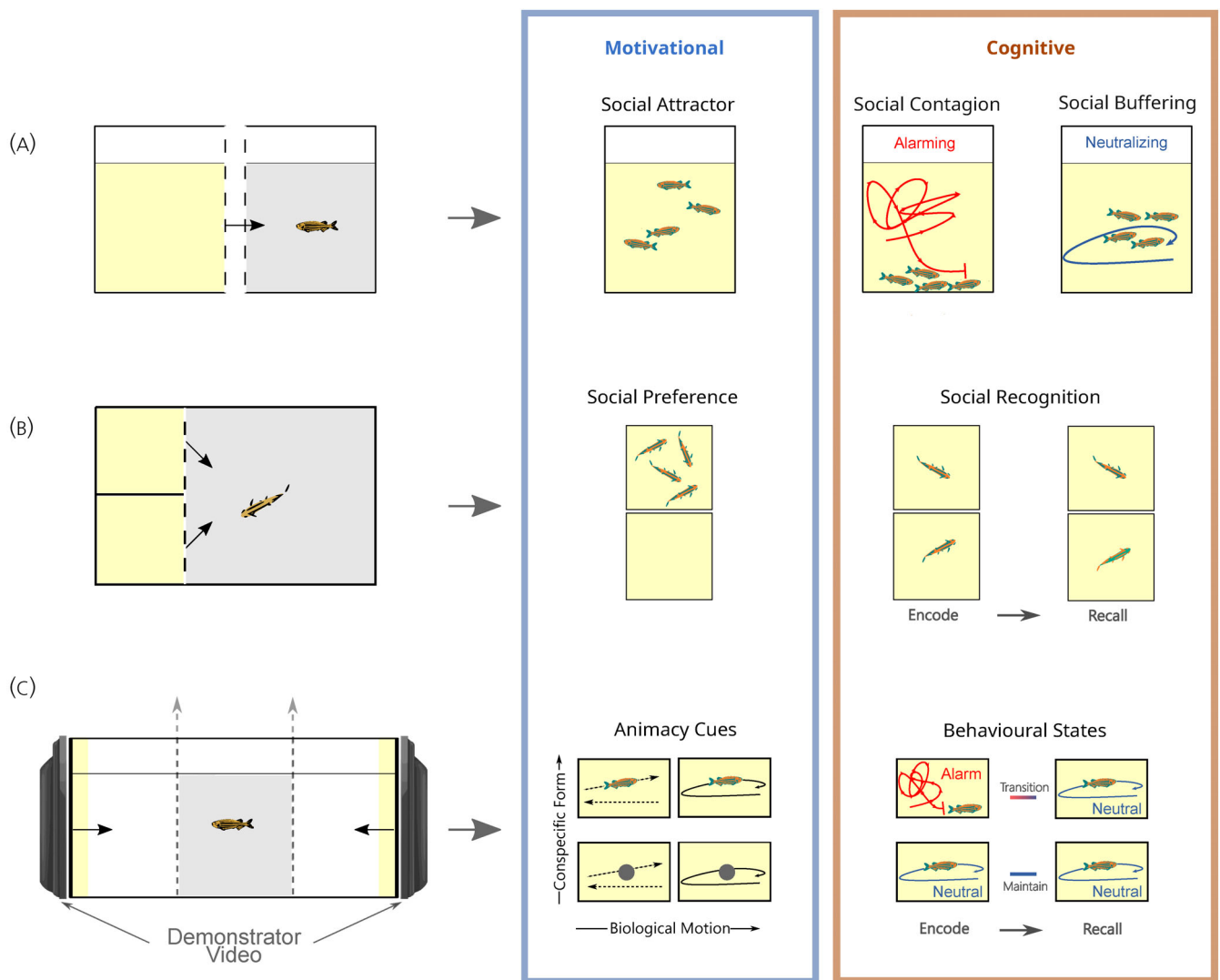
oxytocin neurons in transgenic fish also reduces the number and density of pretektum dopaminergic cells (i.e., TH positive cells),<sup>28</sup> suggesting an organizational effect also at the level of visual perception.

### 3.2 | Gene–environment interactions and developmental regimes

Although the work on the oxytocinergic effects on affiliation provides compelling evidence for the neuroendocrinological control of social cue perception, the effect of these genetically induced changes may rely on interactions with the social environment. In particular, given that genetic oxytocin deficits can elicit different phenotypes, regular

interaction with others that also present these deficits may intensify the effects. Conversely, consistent interaction with others that present no deficits may at least partly ameliorate deficits in those exhibiting them. To investigate these effects, we ran a study where *oxtr* mutants and their wild-type siblings were housed from 4 dpf to adulthood with either conspecifics of their own genotype or with those of the opposing genotype.<sup>96</sup> Compared to wild-types, adult mutants were unable to recognize others independent of their social environment. Although the implication of oxytocin in recognition was not captured in our SNP analysis,<sup>20</sup> that analysis included only the ligand gene, whereas here we targeted the specific effect of the dominant receptor whose expression elicited the same effects in a separate study we conducted without the environmental manipulation.<sup>30</sup> This

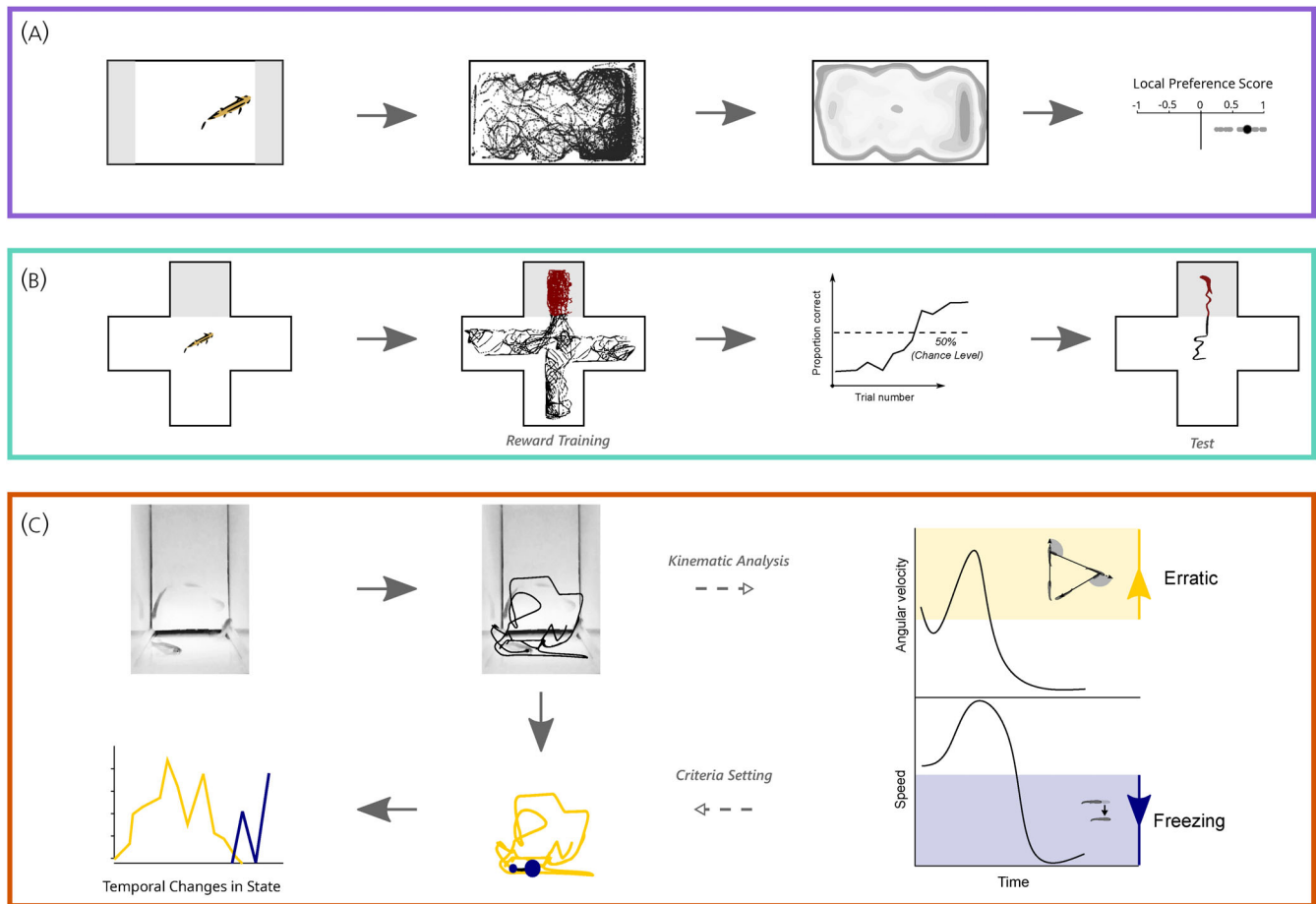




**FIGURE 4** Experimental set-ups used for quantifying motivational and cognitive components of zebrafish social behavior. (A) Social behavior in focal animals can be in response to the simple presentation of conspecifics, which may act as an attractor or can elicit complex responses specific to the behavioral state of others, subject to their recognition. (B) Two alternative choices can present focal animals with the decision to approach areas where others are present, or given the choice between individuals, they may recognize those that remain and become familiar from those that are replaced and are novel. (C) Conflicting video presentations can be used to deconstruct visual elements of social cues, such as motion and form/shape, to identify their perceptual value for motivated approach, while the presentation of conflicting behavioural states at one instance may elicit later responses when no contemporary differences in state are presented, an indication of the cognitive ability to recognize and memorize conspecific states.

may be an indicator that mechanisms underlying recognition are based on regulation and not oxytocin release. However, *oxtr* mutants also exhibited reduced shoaling abilities, namely integration and dispersal, only when housed with other mutants.<sup>96</sup> Thus, in interaction with the social environment, oxytocin's otherwise independent effects on social information use can have carry-over effects on within-group affiliative dynamics. This suggests that the phenotypic impact of deficiencies in the oxytocin mechanism can in some cases be exaggerated by prolonged interaction with others also exhibiting similar deficiencies. Given these effects were imposed from 4 dpf onwards, which includes a key zebrafish social development period (6–21 dpf<sup>97</sup>), it is reasonable to predict that oxytocin function is involved in the developmental processes underlying social affiliation.

A recent systematic review, discussed the developmental effects of oxytocin on human social affiliation, including how early-life impact on oxytocin pathway formation can later affect social information use and integration in groups.<sup>98</sup> The authors, noted that studies are sparse, limited to correlational evidence and use unreliable methods for measuring oxytocin and inferring changes in signaling. Much of this is due to limitations of the tools available for human studies, which highlights the benefit of non-human models.<sup>5,99</sup> So far, much of the work in animals has garnered fundamental evidence, such as the identification of age dependent changes in oxytocin receptor binding following maternal separation in rats.<sup>100</sup> Although often the work relies on pharmacological treatments, more precise genetic tools are available in rodent models. However, vertebrate models with the



**FIGURE 5** Measuring response across different tests of social behavior and cognition. (A) The decision between alternative choices can be used to quantify discrimination or recognition of social stimuli, where an individual is tracked over the duration of the test and their ability quantified by the degree to which time spent near one choice exceeds that spent near all choices, that is, their local preference. (B) Training in complex arenas, like plus mazes, requires animals to associate a cue with a reward at a location, where learning is indicated by increasing success over time and the reaching of a plateau that consistently exceeds chance level, that is, > 50% correct, and where this is performed correctly at later unrewarded probe trials. (C) The transmission or contagion of behavior to others can be quantified by fine tracks of movement that are first analyzed kinematically for identifying thresholds determinant of the occurrence of a specific response and used for its automated quantification.

same availability in toolkits, but also easier handling and better group-living capacities, such as zebrafish, are also well suited. For instance, it has been recently demonstrated that mutation to oxytocin receptors in zebrafish elicits deficits in social affiliation under isolation rearing, but not under social conditions.<sup>101</sup> In addition, mutants can reach peak sociality a week faster than wild-types, where mutation to the non-dominant receptor (*oxtrl*) does not affect the maintenance of this peak throughout development, but mutation to the dominant receptor (*oxtr*) induces periodic decreases in sociality until week 8. This suggests that the effects of oxytocin signaling deficits are dependent on interactions with the social environment during development and may only produce phenotypic changes later in life. Consistent with this idea, in the same study, shoaling behavior in mutants did not differ from wild-types during development, but at 8 weeks mutants became less cohesive. Therefore, oxytocin signaling seems to affect affiliation differently depending on age and social context.

The interaction between development and oxytocin function may rely on epigenetic effects, where early-life experiences can drive

changes in adult brain and behavior.<sup>102</sup> Indeed, epigenetic effects on the oxytocin pathway have been repeatedly noted in humans, with the oxytocin gene (*oxtr*) consistently showing higher levels of methylation than the receptor gene (*oxtrr*), and this effect relies on early-life extrinsic stressors and parental care.<sup>103–105</sup> In addition, different levels of *oxtr* methylation in humans can lead to different social phenotypes, including affiliative and empathic behaviors towards others.<sup>106</sup> Although it remains unknown whether the impact of such epigenetic effects depends on developmental stage, inducing epigenetic-like deficiencies in the oxytocin system at specific life-stages is an option largely reserved for animal models. Using a transgenic zebrafish line, we were able to ablate oxytocin neurons early in development and examine their impact on brain activation, dopaminergic signaling and social affiliation later in adulthood.<sup>28</sup> The oxytocin neurons of transgenics express a nitroreductase protein that can be reductively activated by nitroheterocyclic metronidazole (MTZ) to produce a cytotoxin, hence ablating the oxytocin neurons. Fish were treated with MTZ either as larvae or as adults (>3 months) and tested

behaviorally across different life stages for their social affiliation tendency in terms of preferred approach towards conspecifics. Fish that had their oxytocin neurons ablated during the first weeks of life, but not during later developmental stages, exhibited a deficit in adult social affiliation, a decrease in dopaminergic clusters in the pretectum and posterior tuberculum involved in visual attention gating and reward, respectively, and an altered neuronal activity in the preoptic area and lateral septum homolog (ventral nucleus of the ventral telencephalon). We also identified a recovery of early-life ablated oxytocin neurons in adults, demonstrating that despite the persistent effects of its deficiencies on brain activity and phenotype, the oxytocin system can also be resilient to stress during development.

### 3.3 | Stress-hormone and reward-signaling effects on motivated interaction

The neuroendocrine functions of oxytocin in zebrafish, resulting in the regulation of motivated interactions with others and the formation of social affiliations, likely involve established interactions with stress-hormone and reward-signaling systems. This is because its effects seem to be largely on the development of tendencies to approach others, either individuals or groups, and particularly in the preference for interacting with novel than familiar others,<sup>30</sup> where the perception of social rewards and the dampening of anxiety induced by novel conditions is pivotal. On the one hand, the collective evidence on the anxiolytic effects of oxytocin point to feedback interactions with the HPA system. In particular, corticotropin-releasing hormone (CRH) is the primary hormone regulating the production of glucocorticoids, effective for the HPA physiological stress response, where its primary receptors are dedicated to anxiogenic functions and its secondary receptor to also anxiolytic functions as part of the systems negative feedback controls.<sup>42,107</sup> One frequent site for the receptors with the anxiolytic properties is oxytocin neurons, which shows that oxytocin signaling has been co-opted as an anxiolytic. On the other hand, one of the primary downstream interactions that oxytocin has is with the dopaminergic system, a role played by oxytocin as a so-called “modulator of modulators”.<sup>108</sup> In humans, dopamine signaling has a primary role in promoting social reward and social motivation, a function that we have also validated in zebrafish by our association of the exploration-sociability motivational phenotypic component to polymorphisms in dopamine precursor and dopamine receptor genes.<sup>20,109,110</sup> We have further demonstrated the effect oxytocin can have on the development of the dopamine system, where early-life oxytocin-neuron ablation reduces the number of dopamine cells in specific dopamine clusters later in life.<sup>28</sup> Therefore, it is reasonable to predict that social motivation, such as the drive to affiliate with novel others, relies on anxiolytic activities of oxytocin recruited by the HPA system, which is effective by modulating dopaminergic signaling. More recently, it has been demonstrated that mutant zebrafish for the immediate early gene *egr1* reduced their approach and orienting to social cues, without effects on other sensory or motor behavior, and this change implicated a reduced

expression of the dopaminergic marker tyrosine hydroxylase in the parvocellular preoptic area.<sup>111</sup> Importantly, this area is a central hub of oxytocin neurons, and a homolog to the mammalian supraoptic nucleus of the hypothalamus, where CRH type-2 receptors are coexpressed on oxytocin neurons.<sup>39,66</sup> In a separate study, it has been demonstrated that the expression of neuropeptide Y, which is involved in cell growth and homeostasis, can also negatively impact social interaction tendencies in zebrafish.<sup>112</sup> The deficiencies were recovered by the herbal medicine Ninjinyoeito, but this recovery did not induce changes in oxytocin levels, but it similarly affected CRH and dopamine precursor genes, suggesting that the activity of neuropeptides other than oxytocin also implicates the two systems. Nonetheless, the question for the anxiolytic oxytocin controls on dopaminergic signaling as a candidate system for the regulation of social motivation remains an open question.

### 3.4 | Emerging mechanisms and future approaches

The prominent role of oxytocin, and its interactions with other neuroendocrine systems, does not preclude other social affiliation mechanisms. Our analysis of motivation-associated genetic polymorphisms also suggested an involvement of sex-hormones and neuroplasticity genes.<sup>20</sup> Indeed, recently, a study using genetic zebrafish models, implicated the synaptic plasticity protein *Neuregulin 1* and the neurogenesis protein *DISC1* on social motivation.<sup>113</sup> Added effects can be also induced by epigenetic activity, a result of experience effects on other mechanisms of phenotypic regulation. For instance, the development of social affiliation also relies on changes in epigenetic modification (*PRC2* and *H3K27me3*) induced by DNA-topoisomerase-2a (*Top2a*), but depletion of *Top2a* selectively downregulates autism genes.<sup>113</sup> In line with our SNP analysis, motivation was the only social phenotype to relate with leading autism gene polymorphisms, and particularly with the *shank3* gene (Figure 1). A mutation in the *shank3b* gene in zebrafish elicits autism-like social affiliation and locomotion deficiencies.<sup>114</sup> Similarly, knockout mutations to the autism candidate genes *grin2b* and *katna2* also elicit deficiencies in approaching and interacting with others.<sup>115,116</sup> Therefore, the control of social motivation may rely strongly on oxytocin, but the phenotype is most likely compositely regulated via interactions with other endocrine systems, genetic factors of sociobehavioral pathologies, as well as with socio-cognitive mechanisms.

## 4 | SOCIAL COGNITION: RECOGNITION, LEARNING AND HIGHER-ORDER FUNCTIONS

Social interactions across vertebrates are dependent on three fundamental cognitive abilities, (1) the perception of social sensory information (2) the assessment of that information based on previous experiences and current conditions, and (3) the use of that information to form associations and organize a response. These abilities are integral to different social behaviors, such as the aforementioned

agonistic and affiliative interactions. For instance, the outcome of social dominance contests relies on previous experience in observing and winning contests, and on the assessment of current opponents, such as their size or aggressive intent.<sup>117,118</sup> In turn, affiliative behavior relies on the perception of social information, such as biological form and motion,<sup>29,90</sup> but also on the appraisal of behavioral states in others (e.g., voles increasing allogrooming towards those in distress<sup>119</sup>). Although the cognitive abilities needed for each context present differences, they all depend on the recognition of social cues.

#### 4.1 | Neuromodulation and sensory mechanisms of social recognition phenotypes

Fundamentally, social recognition is the ability to use memorized social information to discern perceived features in others. This ability can scale up from the memorization of individuals based on their repeated behavioral or morphological traits, to the more nuanced recognition of specific behaviors and the internal state they indicate, such as expressions of alarming behavior, distress, or neutral behavior (Figure 4A: Cognitive).<sup>120–122</sup> Social recognition is crucial to group living animals that are bound to exhibit repeated encounters with others and thus plays a significant role on how social behavior is optimized (i.e., social competence).<sup>123</sup> For instance, the recognition of familiar individuals can strengthen affiliations, enable pair bonding, appropriate response to known opponents during agonistic interactions and facilitate the formation of social hierarchies by identifying specific conspecifics or classes of conspecifics.<sup>124–126</sup> Indeed, social recognition can extend beyond individual recognition to the categorization and recognition of species, age group, sex, social role, kinship and reproductive status.<sup>127</sup> Thus, social recognition can be more broadly defined as the ability to categorize social sensory information into different classes and to recall that information during the appraisal of individuals and their behavior in the future. This relies on recognition memory, which is generalized for the social and non-social domain and encompasses the discrimination of familiar from unfamiliar stimuli, based on the encoding and recall of information from previous encounters with those stimuli.<sup>20,128,129</sup>

Our group was able to demonstrate both short- and long-term social recognition in wild-type zebrafish, with animals discriminating familiar from unfamiliar others either these are presented at 5 min or 24 h after familiarisation.<sup>130</sup> In both cases, individuals were first exposed to a single acquisition phase involving the presentation of two novel conspecifics that could be approached and inspected. Following this phase, fish were kept individually and then presented with one of the previously inspected conspecifics and a new individual to test their recognition ability (Figure 4B: Cognitive). While during acquisition animals spent equal time interacting with either novel conspecific, during the recognition test they spent markedly more time with the novel individual, demonstrating their capacity to recall information on the familiar fish that enables them to discriminate between it and the novel individual. In summary, the ability to memorize social information during acquisition enables the discrimination of familiar

from unfamiliar conspecifics later on. To test the involvement of oxytocin signaling on social recognition, we tested zebrafish *oxtr* mutants on the social recognition task. Contrary to wild-type animals, these mutants showed no preference for novel over familiar conspecifics.<sup>30</sup> Moreover, we found that the effect of these genotypic differences are independent of any interactions with the social environment, where mutants exhibit decreased recognition capacities both when housed with other mutants and when housed with wild-type animals.<sup>96</sup> Therefore, oxytocin receptors are necessary for recognition, without any added impact from the social environment.

The recognition of others relies on the use of multiple sensory cues, including visual and olfactory cues that can be detected from a distance. These cues can have different levels of effect on behavior, often based on how reliable the information is perceived by observers. This depends on the level of dominance of each sensory modality and the type of information it is adapted to extract.<sup>131–133</sup> In zebrafish, vision is a dominant modality in social contexts, used both for the detection of biological form and movement.<sup>29</sup> Another dominant modality is olfaction, or chemosensing, and its value in zebrafish social situations is two-fold. On the one hand, it can be used to identify the presence of others, as well as their identification and recognition. This involves early-life learning processes for kin imprinting specific to olfactory-cue exposure at 6 dpf and which involves major histocompatibility complex peptide-signaling and genotypic differences in this system.<sup>134</sup> On the other hand, it is central to the social signaling of threat or danger, where injury of a fish releases an epidermal alarm substance that when sensed triggers a stereotyped behavioral repertoire consisting of erratic movement and immobility/freezing.<sup>135</sup> A consequence of their function is that the two senses have been enlisted together in social recognition and depending on the situation may have additive or contradictory effects. For example, in our work we identified that olfactory cues of unstressed conspecifics can alone provide anxiolytic effects, but that the combined smell and sight of others can markedly improve these effects. We also demonstrated that where the smell of alarm substance induces stress response, the simultaneous sight of unstressed others reduces the negative effect of the olfactory cue.<sup>136</sup> Evidently, these combined effects of the two senses are used by zebrafish to recognize the presence and state of others, particularly in the context of social contagion and buffering. These effects can persist at least up to 30 min after exposure and rely on activation and communication between a specific set of key social cognition brain areas. In particular, by examining activation reflected in gene expression (*c-fos*), together with coactivation patterns, we show how sensing the injury-induced alarm substance of others amplifies activation in some of the most evolutionarily conserved brain regions, including the preoptic area and putative homologs of the central amygdala and the lateral septum (dorsal and ventral telencephalon<sup>66</sup>). However, it also reduces the connectivity between these regions, which suggests that much of the increasing activation may involve inhibition of outgoing neuronal signals. This triggers the behavioral effects of the alarm substance, but it is partly mitigated by the recognized presence of unstressed others (i.e., social buffering<sup>136</sup>). Importantly, these conserved regions are also

involved in the recognition of social olfactory and visual cues released under threat in humans and other mammals, while also most highly conserved across this network is the expression of the receptors of the similarly implicated oxytocin system.<sup>66,121,137</sup>

## 4.2 | The neural circuitry and oxytocinergic control of social memory and learning

The memorization of social sensory information from others, as demonstrated in the examples of recognition, can be extended to facilitate social learning. Although short-term conspecific and object recognition appear to share some genetic mechanisms and phenotypically cluster together (Figure 1), the question remains whether more complex memory and learning capacities are to any extent the product of mechanisms employed both during social and non-social contexts. To test this, we trained zebrafish in a spatial learning task to associate a food reward with images of either a conspecific form or a circle in a plus maze, representing a complex four-choice decision-making paradigm (Figure 5B), and examined whether the expression of the immediate early gene *c-fos* indicated differences in the neural circuitry involved with social and non-social associative learning.<sup>138</sup> While learning performance was similar between the two contexts, the brain regions involved in each learning type were distinct. Social learning was associated with elevated *c-fos* expression in the olfactory bulbs, ventral zone of ventral telencephalic area, ventral habenula and ventromedial thalamus. In contrast, non-social learning was associated with a decreased *c-fos* expression in the dorsal habenula and the anterior tubercular nucleus. Network analyses also show that each learning type elicits a specific functional connectivity pattern across brain regions. However, four segregated submodules of the network related to different functions across the two learning tasks, namely generalized attention, visual response, social stimulus integration and general learning. Therefore, despite localized differences in brain activity, social and non-social learning in this paradigm share common functional connectivity modules, but an additional module specific to social-stimulus integration is recruited during social learning.

However, social learning can occur on different levels and each may enlist different neurobehavioral components. On one level, familiarization with repeatedly or constantly encountered social cues can over time dampen innate responses towards them, resulting in habituation which is the commonest form of non-associative learning. In zebrafish, we identified that habituation to the mere presence of others, in terms of preference between familiar and novel conspecifics, relies on gene–environment interactions. In particular, as few as two sequential exposures over a 24 h period can elicit habituation in mutant fish presenting deficiencies in oxytocin signaling only when those fish are also housed with those of the same genotype (*oxtr*<sup>-/-</sup>).<sup>96</sup> Because of the previously discussed social behavioral deficits of *oxtr* mutants, this suggests that frequent encounters between individuals with low sociality drive faster habituation. On another level, behaviors or individuals may be associated to specific situations, conditions or outcomes, the memorization of which enables simple

associative learning. For example, both the subcutaneous alarm substance released by injured zebrafish and the sight of alarmed conspecifics are social cues that signal threat, eliciting an innate behavioral response of evasive erratic movement and freezing.<sup>136,139</sup> Repeated events may condition individuals to link these signals to specific independent cues or stimuli, for example if the presence or behavior of conspecifics is repeatedly presented together with another cue, then that cue may in turn be associated to risk and elicit avoidance. In one such paradigm, we trained zebrafish to associate chemical or visual information to a cue by exposing them to a light paired with either alarm substance or the sight of an alarmed conspecific (i.e., trained demonstrators expressing an alarm response to the light).<sup>139</sup> Although the training enabled associations to be formed where animals later responded towards the light alone, the timing of the response was improved only when associated with the chemical and not the visual cue. We found this to involve heightened RNA expression in the olfactory bulbs specifically for the *npas4* and the *egr1* genes, which are leading transcription factors involved in learning and memory, and respectively control inhibitory synapse plasticity and epigenetic reprogramming during long-term neuronal plasticity. In addition, changes in connectivity between social decision-making forebrain areas were also implicated based on coactivation patterns in immediate early genes, with the density of gene expression for nerve and synapse growth factors induced by the alarm substance being lower than control conditions. This suggests that the recognition of distress, particularly by innate olfactory signals triggered by injury, enlists network-wide and local changes.

## 4.3 | Emerging neuroendocrine models for complex sociocognitive phenotypes

The ability to perceive and recognize social cues, and associate them to an outcome, can be jointly used during scaled-up sociocognitive phenotypes. The question is whether the neuroendocrine mechanisms modulating lower-order cognitive abilities also modulate these complex phenotypes. To address this question, we recently examined whether the oxytocinergic signaling involved in individual recognition in zebrafish is also involved in the recognition and transmission of distress behavior, as it does in humans and mice.<sup>121,140</sup> To this end, we used another social recognition paradigm where visual cues can enhance the transmission of the alarm substance signal, that is the social contagion of distress behavior (see review by Pérez-Manrique & Gomila<sup>141</sup>). Similar to the recognition of facial expressions of fear in humans and primates, the zebrafish recognition of distress behavior in others could drive their social contagion capacities (i.e., their ability to replicate alarm induced erratic or freezing bouts,<sup>142,143</sup>). Recently, we found that the expression of oxytocin-producing neuronal cells, and both of its receptors (OXTR and OXTRL), are necessary and sufficient for social contagion (Figure 4A: Cognitive) and for the underlying recognition of distress in others (Figure 4C: Cognitive).<sup>91</sup> Specifically, mutations eliminating ligand production or either of the receptors, also eliminate the increase of freezing when freezing in others is



observed. Furthermore, intraperitoneal administration of oxytocin recovers this phenotypic deficit in ligand mutants when compared with saline injection control groups. We found zebrafish oxytocin receptors to be expressed across the conserved brain areas in fish of the SDMN, but most prominent were projections to the subpallium from the preoptic area. Within this region, ventral telencephalic areas that are putatively homologous to the lateral septum and striatum exhibit reductions in activation during contagion (*p56* positive cells; immunostaining). Conversely, activity in these two areas increases in mutants with deficits in oxytocin receptor expression. This suggests that oxytocin elicits decreases in inhibitory signaling, the absence of which is responsible for phenotypic deficits in mutants. This was confirmed via colocalizations of a neuronal activity marker (*p56*) in transgenic fish with fluorescent markers for excitatory (glutamate) and inhibitory (GABA) neurons. Analyses of excitatory and inhibitory coactivation patterns across the brain network also showed that oxytocin elicited some increased excitation under contagion, while the absence of contagion in mutants was paralleled by a segregated connectivity with overall greater excitation in the brain network than wild types. However, oxytocin signaling not only controls the ability to recognize distress, but also the direction of its effects. On the one hand, in contrast to wild-types preferring faster and longer interactions with distressed others, mutants without either of the receptors exhibit absolute deficits in recognition, showing no significant preference for either neutral or distressed others. On the other hand, mutants without oxytocin ligand production exhibit recognition but with a reversal in preference, interested more in neutral than distressed animals. This suggests that the oxytocin system not only controls the ability to recognize a behavior, but also the downstream neurocognitive responses that follow, such as the interpretation of the observed erratic-freezing repertoire as either local danger or conspecific distress. In rats, oxytocin receptors are colocalized with type 2 CRH receptors involved in anxiolytic effects in hypothalamic and brain stem neurons,<sup>42</sup> revealing a feedback loop between the oxytocin and the HPA system, which can elicit oxytocin-controlled decreases in the HPA axis activity.<sup>144,145</sup> As such, much of the control of these downstream processes, particularly the motivation to approach than avoid distressed others, is most likely an extension of the anxiolytic functions of oxytocin.<sup>20,146,147</sup>

The interplay between anxiety and social behavior is a prime example of how composite conditions, where threat detection or stress coping involve social communication, require a greater complexity of cognitive functions. This complexity is defined by the cascade of abilities from directed attention to the recognition of behavioral expressions and their association to particular cues or outcomes. One example is the social buffering phenotype we discussed, where individuals perceive safety in the presence of others and exhibit improved coping towards aversive stimuli, an ability we demonstrated in zebrafish and that involves increased connectivity and local activity between social reward brain regions.<sup>136</sup> Another example is social contagion, defined by the replication of observed behavioral expressions, such as facial mimicry in humans and primates, freezing in rodents, or erratic swimming and freezing repertoires in zebrafish.<sup>142,148,149</sup> The zebrafish behavior can be quantified

kinematically and movement measures used to identify thresholds and to formulate criteria for the automated quantification of phenotypic changes (Figure 5C). Often simplified to directing attention to the behaviors of others and repeating them, on the surface this phenotype can be difficult to distinguish from motor imitation or mimicry. Although, social contagion also requires the intermediate ability to recognize expressions of valenced states in others, such as their distress. Using local preferences towards distressed compared to neutral behavior, we identified that social contagion in zebrafish relies on the oxytocin-controlled recognition of distress, similar to the recognition of fear expressions in humans and rodents.<sup>121,150</sup> This elicited the aforementioned motivational shift to approach distressed others instead of avoiding their risky vicinity.<sup>91</sup> In humans and some mammals, such costly other-oriented responses are common and rely on more elaborate emotion recognition capacities that enable scaled up prosocial and empathic behaviors, such as consolation and compassion.<sup>120,151,152</sup> That the mechanistic origins of these complex cognitive abilities can be traced to zebrafish highlights their capacity as a representative model of mechanistic contributions to the building blocks of higher-order sociocognitive functions.

## 5 | CONCLUDING REMARKS

Overall, the use of zebrafish to study the neuroendocrinology of social behavior has progressed tremendously over the last decade. Testimony to this is the now flurry of reviews examining their viability as models for human sociobehavioral mental pathologies, like autism and ADHD,<sup>153–155</sup> and their potential use as models for social health effects.<sup>10</sup> Across the board, what is recognized are the inherent social properties of the species, including its group living and dyadic interactions; their robust behavioral repertoire and well-established, automated phenotyping methods; the availability of genetic and molecular tools that enable the precise investigation of causal mechanisms; its ease housing and handling; and the welfare advantages compared to mammalian models. Here, we presented the case for the use of zebrafish for fundamental research with translational potential, especially regarding the uncovering of mechanisms responsible for social behavior. Unfortunately, social health has not been a priority to human-focused medical research, but this is something that is beginning to change and successful animal models can be useful in this respect.<sup>10,156</sup> The limitations for using zebrafish involve mostly their inability to capture the full complexity of human sociality, particularly cultural aspects, the role of language, and emotional assessments, although the latter may already be shifting following recent evidence of the recognition and contagion of distress states, as well as their amelioration in others.<sup>91,136,142</sup> The most prominent emerging questions from the field so far, especially regarding the neuroendocrinology of social behavior include: (1) the full characterization of complex causal systems, a key example being the HPA-oxytocin-dopamine interactions; (2) identifying whether mechanisms behind social interactions are implicated in the adverse effects imposed by deficits, surpluses and perceived insufficiencies in social interactions, that is, the

health outcomes of social isolation, overcrowding and loneliness<sup>157,158</sup>; and (3) the establishment of homologies that can facilitate the translational potential of the zebrafish model, particularly by comparison to several species across the evolutionary scale. Together with the increasing empirical evidence and tool availability, this calls for further research utilizing zebrafish as neuroendocrine models of social behavior.

## AUTHOR CONTRIBUTIONS

**Kyriacos Kareklas:** Funding acquisition; visualization; writing – original draft; writing – review and editing. **Magda Cristina Teles:** Writing – original draft; writing – review and editing. **Ana Rita Nunes:** Writing – original draft; writing – review and editing. **Rui Oliveira:** Conceptualization; funding acquisition; project administration; supervision; writing – review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors confirm that there are no conflicts of interest.

## PEER REVIEW

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## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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