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Research report

Using zebrafish to uncover the genetic and neural basis of aggression, a frequent comorbid symptom of psychiatric disorders

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HIGHLIGHTS

- Zebrafish can be used to uncover genes and neural circuits that control aggression.
- Aggressive behavior is species-specific and can be divided into different subtypes.
- Aggression is a common symptom of psychiatric disorders that may hinder treatment.
- Combining behavioral tests may help understand aggression subtypes.

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ABSTRACT

Aggression is an important adaptive behavior that can be used to monopolize resources such as mates or food, acquire and defend territory and establish dominant hierarchies in social groups. It is also a symptom of several psychiatric disorders including attention-deficit/hyperactivity disorder and schizophrenia. The frequent comorbidity of aggression and psychiatric diseases suggests that common genes and neural circuits may link these disorders. Research using animal models has the potential to uncover these genes and neural circuits despite the difficulty of fully modeling human behavioral disorders. In this review we propose that zebrafish may be a suitable model organism for aggression research with the potential to shed light upon the aggressive symptoms of human diseases.

1. Introduction

Aggression is an adaptive behavior that animals use to protect offspring, compete for resources, establish dominance hierarchies and as a form of defense [1]. Aggression can thus be seen as an umbrella term encompassing several related behaviors linked by common postures and outcomes. Aggression is a natural behavior that animals express throughout the course of their lives. However, heightened aggression levels have the potential to cause harm to the self and others, meaning that expression of this behavior must be carefully controlled. In humans excessive violent or hostile aggression is debilitating and can lead to antisocial behavior, delinquency and crime [2]. Human aggression can be verbal or physical and may include anger as a manifestation of underlying hostility. Statistics suggest that violent crime is on the rise in society. For example, the Federal Bureau of Investigation reported an increase in violent crimes (including arson, murder, rape and robbery) to 11,153,016 cases in the United States during 2012 [3]. Increased aggression is also a symptom of several psychiatric disorders including attention-deficit/hyperactivity disorder (ADHD) and schizophrenia. In order to better understand the biological basis of aggression, and to improve the therapies available for human patients there is a need to study the genes and neural circuits that control this behavior.

In this review we will consider ways in which zebrafish can be used to try and identify the genes and neural circuits that control

Abbreviations: 5CSRTT, 5-choice serial reaction time task; 5-HT, 5-hydroxytryptamine/serotonin; 5HTTLPR, length polymorphism in the gene coding for the serotonin transporter SLC6A4; ADHD, attention-deficit/hyperactivity disorder; ChR2, channelrhodopsin2; ASD, antisocial behavior; AVP, arginine vasopressin; AVT, arginine vasotocin; CSF, cerebrospinal fluid; DSM-V, diagnostic and statistical manual for mental disorders; DA, dopamine; CD, conduct disorder; HPLC, high-pressure liquid chromatography; HVA, homovanillic acid; ITI, intertrial interval; LED, light-emitting diode; PET, positron emission tomography; MIA, mirror-induced aggression; NA, noradrenaline; ODD, oppositional defiant disorder; 5-HT, serotonin.

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aggression. Where possible we will compare research conducted in human patients and zebrafish. Finally, we will highlight future directions for research in this field, including comparing multiple behaviors in zebrafish, linking alterations in behavior to neural circuits, looking at the alterations to neural development that trigger aggression and screening for novel anti-aggressive drugs.

2. Subtypes of human aggression

Similar to other behaviors, aggression is caused by the interplay of genes, neurotransmitters, hormones and environmental influences including social interactions. The genes and neural circuits that trigger aggression appear to be largely conserved between species, permitting the comparison of results [4]. In humans, aggression can be provoked by strong emotions including frustration, fear, irritation and even pleasure [5]. Human aggression can be defined as a violent overt behavior expressed with the intention of physically damaging another individual [6]. There are two main subtypes of aggression: controlled-instrumental aggression that is goal-orientated and mediated by higher cortical areas, and reactive-impulsive aggression that is often provoked by anger and involves both hypothalamic and limbic areas [7]. The expression of aggression in different psychiatric disorders highlights the diverse mechanisms that can lead to this behavior. For example, intermittent explosive disorder and post-traumatic stress disorder are related to increased autonomic arousal, elevating the propensity for reactive-impulsive aggression [7]. Conversely, antisocial behavior disorders can involve decreased autonomic arousal [8], suggesting that instrumental aggression may be linked to blunting of emotions [9]. Thus in humans, aggression can be provoked by both high and low-arousal states that may be mediated by different neurotransmission signaling pathways.

The difficulty of comparing aggression research between different animals is increased by the existence of species-specific aggression subtypes. Non-human aggression can be divided into offensive-, defensive- and predatory-subtypes. Human aggression can also be subdivided into adolescent-onset aggression (where transient adolescent rebelliousness is heightened); reactive aggression (that is impulsive or “hot”); or instrumental aggression (non-impulsive, “cold” or pre-planned predatory behavior associated with psychopathy). Each subtype may be mediated by unique neurocognitive systems [10–12]. Impulsive aggression is generally associated with high arousal, impulsivity and uncontrolled behavior which may be mediated by areas of the brain including the amygdala and orbital- and medial-prefrontal cortices [10,13]. In contrast, instrumental aggression refers to goal-directed, planned behavior associated with low arousal that is linked with callous or unemotional traits and decreased amygdala activation [14,15]. The potentially deleterious outcomes of uncontrolled human aggression mean that experiments in animal models are necessary despite their limitations.

3. Zebrafish as an aggression model

The zebrafish is a popular model for behavior [16] which can also be used to study the genes and neural circuits that control aggression [17]. Zebrafish have a short generation time, they are easy to maintain in the laboratory and protocols to measure behavior in both larval and adult fish have been developed in recent years [17–20]. Furthermore, the genes and neurotransmitters that control behavior appear to be conserved across species despite a divergence in the position of neurotransmitter-positive neurons in the brain of zebrafish and other vertebrates [21]. Zebrafish also show high genetic similarity to other species. For example, 69% of zebrafish genes have human orthologs [22] meaning that it is frequently possible to study human disease-related genes in fish. Mutant strains lacking the function of a single gene are available [23] and genetic [24–28], electrophysiological [29] and optogenetic [30,31] tools have also been established, permitting the visualization and manipulation of neural circuits within the intact brain.

Zebrafish use aggression adaptively to monopolize resources such as mates or food, acquire and defend territory and establish dominance hierarchies. The ability to influence social behavior thus has the potential to have a far-reaching impact upon fitness. For example, dominance hierarchies, which are established following agonistic interactions, are important for optimizing reproductive success [32]: dominant males mate more often than subordinates and dominant females produce more eggs when paired with a dominant male than with a subordinate [32]. Similar to other fish species zebrafish express aggression as a series of stereotypic body postures which can easily be measured in the laboratory. These include undulating body movements, short slaps of the caudal fin and bouts of swimming and biting directed against an opponent [19]. Aggressive incidents follow a highly structured pattern [33]. Agonistic bouts start with aggressive display: fish extend their fins, circle and attempt to bite each other. Fights then progress to include more frequent bites, chases and attempts to strike the opponent. The fish which loses the fight then becomes submissive, exhibiting postures that include fleeing, freezing and retreating [33]. The outcome of a fight can have a significant impact on future performance. The winner of a dyadic fight is more likely to win their next encounter, whereas the fish that loses is slower to escalate aggression in the second fight. The influence of past experience on agonistic behavior thus means that repeated aggressive interactions tend to be resolved more quickly than first encounters. Furthermore, neither the size of the fish nor the latency to initiate fighting acts as a good proxy for future fighting performance [33] suggesting that other intrinsic properties influence fighting ability.

Aggression can be measured by either placing two fish in a tank and observing their behavior [33–35] or by recording the reaction of a single fish to its own mirror image [19,36,37]. Dyadic fights between two fish represent a naturalistic setting in which all agonistic postures can be recorded [33]. However, there is the potential that fish may harm each other or be stressed during the interaction, questioning the ethical acceptability of allowing two animals to fight. Mirror-induced aggression (MIA) is simple to record and does not risk damage to the subjects involved. However, MIA may not recapitulate all aspects of an aggressive interaction with another fish [35,38,39]. Aggression levels are similar in both male and female fish when using MIA [36,37,40] whereas males are more aggressive than females when two fish interact [32,41]. Furthermore, MIA stimulates longer bouts of aggression (around 30 min compared to 7 min in a dyadic fight) and never elicits submissive postures [35]. There are also differences in the pattern of brain and hormonal activity activated by these protocols. In the cichlid *Oreochromis mossambicus* interaction with a mirror does not lead to the release of the androgen hormones testosterone and 11-keto-testosterone [39]. High performance liquid chromatography (HPLC) measurements of 5-HT and the 5-HT metabolite 5-HIAA have shown an increase in 5-HT in the telencephalon and 5-HIAA in the optic tectum of fish exposed to a mirror fight. Conversely, the winners of dyadic fights show an increase of 5-HT in the brainstem (i.e. the raphé nucleus) and a reduction of 5-HIAA in the diencephalon [35]. The dopaminergic system is also differentially activated depending upon the type of encounter. Dopamine (DA) is increased in the telencephalon and diencephalon of dyadic winners, whereas MIA leads to an increase of DA in the optic tectum [35]. Taken together, it is clear that the choice of paradigm modifies the type of behavior elicited and that MIA may only trigger some aspects of an aggressive interaction. Nevertheless, MIA provides a simple, easily-standardized and non-invasive method to compare

aggression that shows good correlation with fights between two fish [42]. Furthermore, the use of a mirror to elicit aggression represents a refinement and replacement that may be less harmful for the animals involved, suggesting that its use may be warranted in some experiments.

Similar to other behaviors, aggression can be viewed as an integrative response to environmental stimuli. Although it is tempting to consider behavior as the product of central nervous system activity, aggression will also be influenced by feedback from the autonomic nervous system and periphery. For example, agonistic interactions are stressful, and will lead to activation of the hypothalamus–pituitary–gonadal axis (called the hypothalamus–pituitary–interrenal axis in fishes) [43]. Alterations to the aggressive output of an animal may thus occur at multiple levels: in the processing or assignment of valency to visual or social signals (such as distinguishing between an opponent or a mate), in the activation of appropriate neural circuits to react to a such a signal, or in the motor control of agonistic behavior which may occur at the level of the hindbrain and involve input from the peripheral or autonomic nervous system. For example, application of ethanol to the tank water can alter both aggression levels [19] and social behavior in zebrafish [44], but whether this is due to altered stimulus valency or motor output is not clear. Thus, one of the greatest challenges facing behavioral biologists is to understand the underlying intention that triggers a behavior. Addressing this issue will likely require information from a combination of behavioral paradigms.

4. Modeling human aggression in zebrafish

Several factors complicate the study of human aggression in animals and such research is often treated with suspicion by clinicians and psychiatrists [1]. Aggression is a common symptom of human psychiatric disorders including attention-deficit/hyperactivity disorder (ADHD) and schizophrenia. Nevertheless, aggression is not described as a disease in the Diagnostic and Statistical Manual for Mental Disorders [45] making the treatment of aggressive symptoms in humans difficult. Therefore, studies of agonistic behavior in model organisms could be used to understand the genetic basis of human aggression. In the next sections we will discuss how zebrafish could be used to investigate the neurological and neurochemical basis of human psychiatric disorders that include aggression as one of the symptoms.

4.1. The neurological basis of human aggression

In the human brain, aggression appears to be controlled by a neural network that includes the medial preoptic area, lateral septum, ventromedial hypothalamus, anterior hypothalamus, medial amygdala and periaqueductal gray matter [46]. Neuroimaging studies have shown that frontal cortex activity is inversely related to aggression, with brain damage to this area resulting in increased agonistic behavior [47]. For example, positron emission tomography (PET) studies show altered glucose metabolism in prefrontal areas in individuals who are susceptible to exhibit impulsive aggression [48]. Other brain areas such as the amygdala and hippocampus contribute to the expression of emotions and experience, both of which can influence aggressive behavior [49].

4.1.1. The neurological basis of zebrafish aggression

There are still relatively few studies that have linked specific areas of the brain to the control of aggression in zebrafish. HPLC analyses of mirror-induced aggression (MIA) and dyadic interaction of two fish have demonstrated alterations to dopamine, 5-HT and their metabolites in the telencephalon, diencephalon and optic tectum in response to agonistic behavior (discussed in Section 3 above, [35]). Similarly, Filby and colleagues have identified alterations to the expression levels of genes throughout the brain, without linking them to specific nuclei [41]. Other important brain areas include the preoptic area of the anterior hypothalamus, where vasotocin-positive neurons have been correlated with dominance (and therefore presumably aggression levels, [43]), and the histamine-positive periventricular nucleus of the inferior hypothalamus [36]. However, studies of the neural circuits which drive adult behavior are still relatively scarce, highlighting the need to increase the amount of research done in this important area.

4.2. The genetic and neurochemical basis of human aggression

Aggression is thought to be controlled by catecholaminergic neurotransmitters with modulatory input from glutamate, GABA and neuropeptides [50]. These neurotransmitter systems are often the target of therapeutic treatments such as the neuroleptic drugs used to manage aggressive and violent patients [51]. In the next sections we will briefly discuss the neurotransmitters that have been connected to aggression in humans.

4.2.1. 5-HT

5-HT (serotonin) is thought to have a general inhibitory role in human aggression [52] by stimulating prefrontal cortical areas that send inhibitory projections to the nucleus accumbens. For example, hypofunction of 5-HTergic neurotransmission is thought to represent a biochemical trait that predisposes individuals to impulsive aggression [53]. Aggression may also be triggered by alterations to 5-HT synthesis, which is catabolized from the amino acid tryptophan. Experimentally depleting tryptophan increases aggression in men [54,55]. Moreover, administration of the 5HT agonist fenfluramine significantly increases 5HT metabolism in the prefrontal cortex [56] and this action is reduced or absent in individuals with impulsive–aggressive personality disorder [57,58]. In support of a global inhibitory role for this neurotransmitter, increasing 5-HT levels can reduce aggression in animals [59] and selective 5-HT re-uptake inhibitors show anti-aggressive effects in patients with personality disorders [60]. Aggressive behavior is also correlated with alterations in the concentration of 5-HT metabolites in the cerebrospinal fluid (CSF) [61]. For example, aggressive psychiatric patients show reduced 5HIAA concentrations in the CSF [62,63], with a similar result found in violent, impulsively aggressive men [64,65].

Many genes related to 5-HT neurotransmitter signaling have been connected to the control of aggressive behavior. Mutations in the *TPH2* gene that codes for an enzyme needed for 5HT synthesis have been shown to alter aggressive behavior in mice [66]. Expression of the 1473C allele of *TPH2* leads to higher 5-HT levels and an increased frequency of attacks upon intruders. The 5-HT reuptake transporter-encoding gene *SERT* is also associated with aggression. Knockout of *SERT* reduces agonistic behavior in mice, demonstrated by an increased latency to initiate aggression and fewer attacks [67]. Furthermore, the S allele of the *5HTTLPR* gene is associated with a higher propensity for violence in humans [68,69]. Single nucleotide polymorphisms of the *5HTR1A* receptor gene can also alter aggression. For example, the C1019G variant is associated with increased receptor expression, reduced 5-HT neurotransmission and a reduction in agonistic behavior [70]. Finally, variation in the *5HTR1B* receptor are associated with antisocial behavior in alcoholics [71], with the G861C genotype being associated with elevated aggression in children [68] although this relationship has not been replicated in all studies [72,73].

4.2.2. Dopamine

The initiation, execution, and consequences of human aggression are thought to be under the control of dopaminergic neurons

in the mesocorticolimbic pathways [50,74,75], suggesting that dopamine (DA) may modulate motivational aspects of this behavior. The concentration of homovanillic acid (HVA, a DA metabolite) in the CSF has been found to be inversely related to impulsive aggression, suggesting a role for post-synaptic DAergic receptors [61]. The action of drugs that target DA also supports a role for this neurotransmitter in aggression. Methylphenidate, a psychostimulant used in the treatment of attention-deficit/hyperactivity disorder (ADHD) both increases DA release and reduces aggression in humans [76,77]. Drugs that act as antagonists at D2 DA receptors (such as haloperidol and clozapine) can also be used to manage the aggressive symptoms of psychotic patients.

Genetic studies implicate COMT, an enzyme that metabolizes both DA and noradrenaline in the control of aggression. A single nucleotide polymorphism in COMT has been shown to be related to elevated aggression in a population of schizophrenic patients [78]. The DA transporter gene *DAT1* has also been connected to agonistic behavior; a 10-repeat allele of the 3′UTR VNTR is associated with violent delinquency [79]. Moreover, polymorphisms in the DA receptor *DRD2* have also been associated with aggression in children [80].

4.2.3. Noradrenaline

Noradrenaline (NA) may exert an opposite effect to DA in the control of aggression. Infusions of NA to the lateral ventricles of rats reduce fighting and attack behavior, suggesting that a balance between DA and NA may mediate aggression [81]. Moreover, intraventricular injection of 6-hydroxydopa, which reduces NA levels, has been found to increase shock-induced aggression in rats [82]. This suggests that NA may exert an inhibitory influence on aggressive behavior. However in humans, propranolol, a NA inhibitor, reduces aggression by more than 50% in aggressive patients, demonstrating that the role of this neurotransmitter in agonistic behavior is ambiguous [83]. To date, there have not been many studies of genes related to NA signaling. However, *Dopamine beta-hydroxylase* knockout mice exhibit a reduction of aggression in the resident–intruder aggression paradigm [84]. In summary, NA has not been as well studied in relation to aggression as other neurotransmitters such as 5-HT or DA, and this appears to be an important area for future research.

4.2.4. Monoamines interact to control aggression

Similar to other behaviors, aggression is most likely influenced by the interplay of multiple neurotransmitters. For instance, DA and 5-HT neurotransmission may interact to mediate aggression, with aberrant DA signaling exacerbating the predisposition toward aggressive behavior induced by alterations to 5-HT [53]. For example, dysfunction of 5-HT within the prefrontal cortex is thought to lead to DAergic hyperactivity and a predisposition toward impulsive aggression. Furthermore, drugs that target both neurotransmitters have been shown to influence agonistic behavior. Clozapine is an atypical antipsychotic drug that interacts with $5HT_2$, D2- and D4-DA receptors as well as GABA and glutamate receptors to decrease aggression [85–88]. Other atypical antipsychotics with actions at multiple subtypes of 5-HT and dopamine receptors have also been shown to be effective in reducing aggression, including loxapine [89,90], olanzapine [91,92], quetiapine [93], risperidone [94,95], ziprasidone [96], and amisulpride [97,98]. The atypical antipsychotic drug aripiprazole may also influence aggressive behavior. This drug is a partial agonist at both DA D2- and $5\text{-}HT_{1A}$ receptors, as well as acting on many 5-HT receptor subtypes [99]. Moreover, the main metabolite of aripiprazole is a potent DA D2 receptor antagonist [100]. This drug has been suggested to be effective in reducing agitation and aggression in psychiatric populations [101].

Human genetic studies have also provided evidence for a role of monoamines in aggression. Brunner and colleagues found a disturbance in the regulation of impulsive aggression in a study of eight males from an extended family with a mutation in the *MAO-A* gene [102]. Moreover, maltreated children with high *MAO-A* activity levels exhibit a reduced likelihood to display antisocial and violent behavior compared to maltreatment coupled with low *MAO-A* activity [103]. *MAO-A* is involved in metabolism of DA, 5-HT and NA, demonstrating that multiple neurotransmitter systems interact to control aggressive behavior.

4.2.5. Glutamate and gamma-aminobutyric acid

Research into the role of glutamate in aggression is quite limited, although NMDA receptors may represent a promising target for anti-aggressive drugs [104]. In humans, memantine, an NMDA receptor antagonist, has been shown to significantly improve aggressive symptoms and agitation compared to placebo [105]. Furthermore, AMPA-type glutamate receptor deficient mice are less aggressive than controls [106] implicating the action of glutamate at this receptor in this behavior.

Gamma-aminobutyric acid (GABA) has also been linked to aggression. Psychotic patients exhibiting violent outbursts are often treated with benzodiazepines that enhance GABA function [107–109]. Moreover, alcohol has frequently been shown to facilitate aggression and violence in humans [110,111]. These effects are thought to occur through allosteric modulation of $GABA_A$ receptors [74]. In agreement with this, levels and turnover of GABA are reduced in the more aggressive C57 mice [112]. Furthermore, mice deficient in the GABA transporter subtype-1 (*Gat1*) display reduced aggression compared to wild-type [113], likely due to an increase in GABA at the synapse.

Similar to the combined action of monoamines, both GABA and glutamate may interact to modulate aggression. Topiramate is thought to act as both a positive modulator at $GABA_A$ receptors [114,115] and a negative modulator of glutamate via AMPA receptors [116] and has been shown to reduce aggression and agitation in humans [117,118]. Other anticonvulsants which also modulate both GABA and glutamate have been shown to reduce agonistic behavior, including valproate [119,120] and gabapentin [121,122].

4.2.6. Neuropeptides

There is some evidence that connects neuropeptides to human aggression. For example, a positive correlation has been demonstrated between CSF levels of vasopressin and aggressive behavior in patients suffering from personality disorders [123]. Furthermore, an association has been found between the vasopressin receptor 1B and childhood aggression [80]. In hamsters, the selective vasopressin V1a receptor antagonist SRX251 significantly reduces aggression [124]. Vasopressin receptor 1b knockout mice also show reduced agonistic behavior [125]. Together, this research suggests that the development of selective vasopressin antagonists represents a promising avenue of research for identifying novel ways of treating human aggression [126].

4.3. The genetic and neurochemical basis of zebrafish aggression

Aggression appears to be controlled by similar neurotransmitters, neuropeptides and hormones in zebrafish as in other vertebrates, including 5-HT, dopamine [40], histamine [36], 17α-ethinylestradiol [127] and arginine vasopressin/arginine vasotocin (AVP/AVT) [43]. Recent research has shown that aggression has a moderate heritability estimate of 0.36 suggesting that environmental influences, perhaps including maternal care, also play an important role in the expression of this behavior [128]. Modulation of the function of a single gene can impact upon aggression providing direct evidence for a genetic basis. For example, injection of

an antibody targeting the neurotransmitter ependymin increases aggression in subordinate and reduces aggression in dominant fish [34]. Furthermore, mutation of the gene encoding the fibroblast growth factor receptor 1A protein triggers increased aggression [36]. *fgfr1a* Mutant fish are always more aggressive than control siblings despite an influence of environmental rearing conditions upon basal agonistic levels [36]. Thus, aggression levels are plastic and can be modified by both rearing conditions and environmental complexity [37,129].

In the adult zebrafish brain, 5-HT positive neurons are found in the hypothalamus and pretectal diencephalon as well as in the raphé nucleus [130]. However, 5-HT levels have a similar inverse relationship with aggression as in other vertebrates including humans [36]. Pharmacologically increasing 5-HT signaling levels has been shown to alter aggression in a manner similar to rodents [36,41,42], suggesting a conserved function of this neurotransmitter across species. Furthermore, homologs of the 5-HT pathway genes that have been linked to aggression in humans (*SERT*, *5HTR1A*, *5HTR1B* and *TPH2*) have already been identified in zebrafish [131,132], suggesting that mutant lines lacking the function of these genes could be useful to probe the genetic basis of this behavior in fish.

To date, there are no studies that have been published that directly test the contribution of dopaminergic, noradrenergic, glutamate and GABA signaling toward zebrafish aggression. However, the successful characterization of *fgfr1a* mutant fish, and the identification of a histaminergic neurotransmitter basis of this behavior suggests that further examination of these neurotransmitters is a promising area for future research.

5. Human psychiatric disorders linked to aggression

Increased aggression is a comorbid symptom of several psychiatric disorders including attention-deficit/hyperactivity disorder and schizophrenia. The increase in aggression exhibited by individuals with psychiatric disorders may hinder their treatment. However, each psychiatric disorder may be related to a different subtype of aggression, complicating the development of specific therapies. For example, ADHD is frequently associated with impulsive rather than instrumental aggression [133]. A better understanding of the neurological basis of aggression in these diseases – perhaps driven by research in animal models – may thus help develop improved treatment options for patients. Some aspects of both ADHD and schizophrenia have already been modeled in zebrafish, and so in the next section we will concentrate on these two diseases.

5.1. Attention-deficit/hyperactivity disorder

ADHD is a common psychiatric disorder that is characterized by developmentally inappropriate inattention, hyperactivity and impulsivity expressed in more than one context (such as at home, school or in the workplace). The symptoms of ADHD can be divided into three major subtypes: predominantly inattentive, predominantly hyperactive and impulsive or a combination of both [45]. ADHD affects between 3 and 5% of schoolchildren worldwide regardless of nationality or cultural setting [134,135]. ADHD symptoms may also persist into adulthood in about 50% of cases [136,137]. Co-occurrence of ADHD and other diseases occurs frequently. For example, one study has found ADHD co-occurring with conduct disorder (CD) in 30–35% of cases and oppositional defiant disorder (ODD) in 30% of cases [138].

Data from drug treatments, genetic analyses and neurobiological studies suggest that alterations to multiple interconnected neurotransmitters including dopamine (DA), noradrenaline (NA), and to a lesser extent serotonin (5-HT) and glutamate, can trigger the symptoms of ADHD (reviewed in [139]). The discovery that methylphenidate, an amphetamine-like compound that increases both DA and NA levels in the prefrontal cortex can be used to manage ADHD [140] has focused research onto these neurotransmitters. ADHD patients are thought to have a reduction of dopaminergic signaling in the prefrontal cortex. Other brain areas which have been connected to ADHD include the striatum (caudate nucleus and putamen), the parietal cortex, the vermis and the inferior lobes of the cerebellum [141–144]. Multiple DA signaling pathway-related genes have been linked to ADHD. For example, the DA transporter, DAT1, modulates synaptic dopamine levels by regulating its re-uptake from the synapse. DAT1 is the primary target of methylphenidate and a variable nucleotide tandem repeat polymorphism in this gene has been found to be associated with ADHD [145–149]. The gene that encodes the DA D4 receptor has also been identified as an ADHD candidate [150,151]. Linkage data has also connected NA and 5-HT neurotransmitter signaling pathway genes to this disease (e.g. [152]). Therefore, alteration of the function of many genes may lead to the emergence of a single behavioral disorder.

5.2. Studying ADHD in zebrafish

Zebrafish have already been used to model some aspects of ADHD. Lange and colleagues used morpholinos to knock down the ADHD-susceptibility gene *lphn3.1* and identified a correlation between reduction in the number of DAergic neurons in the diencephalon, locomotor impulsivity and hyperactivity [153]. Levin and colleagues incubated zebrafish embryos in the ADHD-treatment drug methylphenidate (MPH) for the first 5 days of development and then quantified changes to adult behavior. Drug treated fish showed a reduction of time spent at the bottom of a novel tank following 50 mg/l MPH treatment (an anxiolytic-like phenotype) and an increased latency to enter a novel compartment in an aquarium following 12.5 mg/l and 50 mg/l treatment [154]. These behavioral alterations were correlated with an increase of NA, 5-HT and DA in the brain, providing critical information about the effect of chronic methylphenidate treatment on neural development. Finally, the ADHD treatment drug atomoxetine has also been used to study impulsivity in adult fish (see Section 6.1.2 below, [155]). However, there have been no studies that have quantified aggressive behavior in zebrafish ADHD-like models. This would appear to be an interesting area for future research, which perhaps requires the generation of stable mutant lines rather than using morpholino-injected animals.

5.3. Schizophrenia

Schizophrenia is a severe psychiatric disorder that is characterized by positive, negative and cognitive symptoms [45]. Although schizophrenia is thought to be caused by defects in early brain development [156], disease symptoms typically appear between 16 and 30 years of age. Schizophrenia affects around 1% of the adult population in the USA according to the National Institute of Mental Health. Schizophrenic patients can also exhibit high levels of aggression with patients who commit violent acts expressing more positive than negative or cognitive symptoms [157]. The efficacy of D2-dopamine receptor antagonists to treat schizophrenia gave rise to the dopamine hypothesis of schizophrenia. Neurobiological studies have led to the suggestion that schizophrenia is due to a general imbalance in dopaminergic neurotransmission, including hyperfunction of subcortical mesolimbic dopamine projections and hypofunction of the mesocortical dopaminergic system [158]. Genetic studies investigating candidate genes for schizophrenia have also implicated dopaminergic

neurotransmission. Catechol-*O*-methyltransferase (*COMT*) plays a role in the metabolism and clearance of DA from synapses and has been identified as a susceptibility locus for schizophrenia, ADHD and aggression [159,160]. A single nucleotide polymorphism in *COMT*, Ala-72-Ser, may contribute to homicidal behavior in schizophrenics [159,161]. However, although changes to dopaminergic signaling can lead to schizophrenic-like symptoms, they may not trigger the disease per se [158]. Determining the common genetic and neurological changes that can trigger aggression and schizophrenia may permit identification of possible targets for novel therapies; this is a promising area future research.

5.4. Studying schizophrenia in zebrafish

Schizophrenia is a severe psychiatric disorder whose symptoms include mood changes, disorganization of thought, agitated body movements, anhedonia, depression, speech problems and in some cases aggression. Although it is not possible to study all of the symptoms of schizophrenia in zebrafish, the developmental function of schizophrenia-linked genes can be examined. Schizophrenia-related genes that have been investigated in zebrafish include *disc1* [162–164], *rgs4* [165] and *kinesin17* [166]. Furthermore, treatment of zebrafish with the NMDA receptor antagonist MK-801 causes schizophrenia-like behavioral alterations that include changes to social interaction, hyperactivity and amnesia [167–170]. Therefore, fish lacking the function of these genes could provide interesting models to study aggressive behavior linked to schizophrenia.

6. Future directions for aggression research in zebrafish

The recent development of genetic and optogenetic tools for zebrafish has raised the potential to expand our knowledge of the control of behavior at the genetic and cellular level. In the next section we will propose several promising areas for future research.

6.1. Comparing multiple behaviors in zebrafish

To date, the studies of aggression carried out in zebrafish have either measured the interaction of two fish in a tank or mirror-induced stimulation, both of which may be similar to offensive aggression in other animals. It is not clear whether zebrafish can display other types of aggression and paradigms to investigate this have not been established. One way to address this question would be to combine measurements of aggression with behavioral tests that can probe other aspects of attention or social behavior.

6.1.1. Impulsivity

Human aggression subtypes can be classified by the presence (reactive aggression) or absence (instrumental aggression) of impulsivity. In rodents, tests to measure impulsivity are well characterized including the 5-choice serial reaction time task (5CSRTT), the go/no go test and the stop test, each of which may measure different types of inhibition [171]. Zebrafish also exhibit both motor and cognitive impulsivity. Motor impulsivity is characterized by sharp peaks in the locomotion curves of fish as they swim [153]. Cognitive impulsivity can be recorded using an aquatic modification of the 5CSRTT, an approach which has been validated by Parker and colleagues [172]. In the 5CSRTT fish are given a food reward for correctly nose-poking an illuminated yellow light-emitting diode (LED) but not one of its four non-illuminated neighbors. Following a training period in which this behavior is learnt, a 10-second intertrial interval (ITI) coupled to second (green) LED is introduced. Entry into any yellow LED-containing compartment before the end of the ITI is scored as a sign of impulsivity. The fish is then punished with a 10-second time-out and no food reward. Entry into a compartment in which the yellow LED is not illuminated will also trigger the punishment [172]. Importantly, impulsivity appears to be controlled by similar neurotransmitter pathways in zebrafish and other species; application of a low dose of the ADHD-treatment drug atomoxetine leads to a reduction in anticipatory responses in the 5CSRTT [155]. Comparing performance in the 5CSRTT task to mirror-induced aggression appears to be one way in which aggression could be divided into subtypes. Measurements of impulsivity could also be extended by establishing the go/no go test which is currently used in both rodents and humans. Zebrafish could be taught to react with a nose-poke to an LED of one color and to ignore a second colored light. Using several different paradigms to measure impulsivity may help better characterize this behavior.

6.1.2. Stress and anxiety

In humans, instrumental aggression occurs in the absence of activation of the emotional axis [14]. Stress or anxiety is another behavior which has been well-characterized in zebrafish. When introduced into a novel tank a fish will dive to the bottom, only swimming up toward the surface when anxiety levels are reduced [173]. Another measure of anxiety is to measure the time spent on the dark or light side of a two-colored aquarium. Adult fish will avoid the light side of such a tank [174,175], a behavior which can be modified by applying anxiolytics such as diazepam [176]. Therefore, it is quite simple to measure stress/anxiety in fish, and couple it to direct analysis of the stress axis (e.g. by measuring cortisol levels before and after an aggressive episode); this test may thus provide further information about the factors that elicit agonistic behavior.

6.1.3. Social behavior

Zebrafish are social animals that naturally form loose groups called shoals. Shoaling can reduce the risk of predation and improve both foraging and reproductive success [177]. Social group preference can be measured by several different methods. A single fish can be allowed to choose between compartments that are either empty or contain a group of conspecifics (the individual social behavior assay) [44] or the distances between individuals in a group can be quantified [44,178].

Zebrafish show a preference for the presence of conspecifics rather than shoaling with other groups of fish. Wild-type zebrafish with a normal pigmentation pattern will spend more time close to a compartment that contains similar wild-types compared to one containing stripe-less *nacre* pigmentation mutants [177]. However, cross rearing of wild-type and *nacre* will produce fish that shoal with their tank mates; thus social preference appears to be learned during development and may be based upon olfactory or visual cues [177]. Shoaling is also influenced by genetic background. TU strain wild-type fish swim much closer to each other than AB wild-types in a similar group [178]. Furthermore, treatment of zebrafish with ethanol during development both causes fish to cluster less closely together [44,179] and leads to variable alterations in the expression of genes that have been linked to social behavior including *avpr*, *htr1aa*, *oxtr* and *slc6a4* [44]. In contrast to this, acute ethanol treatment does not affect shoal cohesion in adult fish whereas nicotine treatment increases the distance between fish [180], an effect which is likely due to alteration of neurotransmitter signaling.

Similar to the formation of dominance hierarchies, aggression levels may be important in the establishment of shoals. Thus alterations to the behavior of a single animal may lead to changes at the population level. Together, these observations suggest that it would be informative to compare the aggression levels of individual fish and their impact upon shoaling.

6.2. Alterations to ontogeny that have long-lasting impacts on aggression

As well as being triggered by direct alterations to neurotransmitter signaling, changes to behavior can also be caused by more diffuse and variable alterations to brain function. Such alterations may include miswiring of neural circuits, disinhibition of local interneurons or adjustment of normal brain homeostasis [181]. The external development of zebrafish and the ability to compare behavior at several life stages makes this species an ideal choice to address this question. Gene function can be transiently knocked down by injecting morpholinos, short DNA sequences that block translation [182]. Morpholinos are an ideal tool for this since they are only active for around 4 days [182] meaning that gene function will recover over time and adult behavior can be measured. This approach has already been successfully used to examine the behavioral effect of perturbation of dopaminergic neuron development. Zebrafish that have a transient reduction in the function of the gene encoding the dopaminergic synthesis enzyme *th1* show long-lasting alterations to adult behavior including reduced freezing and less time spent in the bottom of a novel tank, an anxiolytic phenotype [183]. Several other studies have also shown behavioral changes linked to dopaminergic neurons. For example, transient abrogation of the function of the DAergic pathway genes *nr4a2a* and *nr4a2b* during development has been shown to trigger permanent locomotor hyperactivity [184].

A similar approach can be used to assay the effect of drug treatment on zebrafish behavior. The atypical antipsychotic risperidone is used to treat aggression in human patients [101]. Acute risperidone treatment during development causes a transient reduction in spontaneous swimming without long-term changes to behavior [185]. ADHD is increasingly being diagnosed in adult patients, giving rise to the possibility that developing human fetuses may be exposed to these drugs via their mothers. Studies of the behavioral effect of drug treatment during zebrafish development provide an excellent opportunity to investigate this question.

6.3. Using optogenetics to define neural circuits

One of challenges that face behavioral geneticists is to link alterations to behavior to changes to the underlying neural circuitry. There are a variety of experimental techniques that can be combined to address this question. For example, rtPCR can be used to identify global alterations to gene expression which can then be localized to discrete brain areas by in situ hybridization or immunohistochemistry. If modifications to specific nuclei can be uncovered, then the related neural circuits can be identified by tracing using substances such as DiI (e.g. [186]). Finally, neural circuits can be manipulated by using genetic tools and quantifying any ensuing changes to behavior. For example, the advent of optogenetic tools to manipulate neuronal activity in living animals [30,31], coupled to the availability of fully transparent zebrafish lines such as *casper* [187], provides an exciting opportunity to dissect the neural circuits that underpin behavior. Studies using this technology have already shed new light upon the control of locomotion in zebrafish [188–190] proving the feasibility of this approach. There are two ways in which aggression neural circuits could be identified. Firstly, previously characterized promoters expressed in defined brain areas can be used as a starting point to interrogate circuit function. A second, perhaps more exciting, approach would be to use Tol2-based transgenesis to randomly integrate a channelrhodopsin (ChR2)-containing vector into the genome. In cases where ChR2 expression is restricted to the brain, illumination with a blue light would allow potential behavioral functions to be uncovered. There are however several possible drawbacks to this approach. Firstly it is not clear whether simply bathing an adult *casper* fish in blue light would be powerful enough to activate ChR2—proof of principle experiments would be needed. Secondly, there is still a general lack of promoters that have been characterized in the adult brain and it is also not clear the extent to which promoters that are active in the larval brain will also work in adults. Nevertheless, the potential to map aggression-controlling neural circuits represents an exciting avenue for future research.

6.4. Screening for novel drugs that modulate aggression

One area in which zebrafish may be particularly useful would be in searching for novel drug treatments for aggression. Zebrafish larvae are a good model system for pharmacological studies since compounds can be diluted in the embryo medium and larvae are transparent meaning that internal organs can be visualized throughout development [191]. Larvae are small, simple to generate in large numbers and easy to manipulate making them ideal for high-throughput work. However, screens that use complex behavior as a read-out are difficult to design and implement efficiently. One way to try and circumvent this issue would be to use mirror-induced aggression (MIA) in juvenile animals. Using a mirror to measure behavior may help reduce variability between subjects despite the drawbacks inherent in using a mirror as a stimulus. Drugs could then be organized by their "behavioral fingerprint," a combination of their chemical structure and their ability to modify aggression, thus permitting the function of novel chemical structures to be predicted [192].

7. Conclusion

Aggression is a common symptom of several psychiatric disorders which can hinder the treatment of these diseases. Comorbidity with ADHD and schizophrenia suggests that common genes and neural circuits may link together seemingly discrete disorders. Research in model organisms including zebrafish has the potential to open up novel avenues for future research. Future experiments should be designed to record several key behaviors – such as aggression, impulsivity and anxiety – with the aim of better understanding the global genetic and neural basis of aggression subtypes.

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