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The utility of zebrafish as a model for behavioural genetics

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Recent advances in genetic and imaging techniques have established the zebrafish as an excellent model to study behaviour. Their short development time, compact size and ease of imaging deep within the brain have allowed the neural circuits that control behaviour to be mapped. Increasingly sophisticated optogenetic tools and virtual world setups allow larval fish to be manipulated and monitored in real time [1,2••,3,4,5]. Adult zebrafish are also emerging as a powerful model for behaviours including aggression, anxiety, learning, memory and shoaling [6,7,8••,9•]. In this review we will highlight recent studies in which zebrafish have contributed to our understanding of behavioural genetics.

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Introduction

Recent advances in genetic and imaging techniques have established the zebrafish as an excellent model to study behaviour. Their short development time, compact size and ease of imaging deep within the brain have allowed the neural circuits that control behaviour to be mapped. Increasingly sophisticated optogenetic tools and virtual world setups allow larval fish to be manipulated and monitored in real time [1,2••,3,4,5]. Adult zebrafish are also emerging as a powerful model for behaviours including aggression, anxiety, learning, memory and shoaling [6,7,8••,9•] (Table 1). In this review we will highlight recent studies in which zebrafish have contributed to our understanding of behavioural genetics.

Prey capture

Zebrafish larvae start to hunt prey such as paramecia from around 5-days post fertilisation. Prey capture is achieved through a series of stereotyped manoeuvres which are triggered when prey enters the field of view. The first movement is eye convergence followed by a calibrated series of J-turns — flexions of the caudal tail that orientate the fish towards its target. The sequence is completed by a capture swim [4]. Hunting behaviour can be measured by placing larvae in a virtual environment where films are used to trigger tail and eye responses [4]. Small moving objects such as paramecia are detected by the optic tectum which responds visuotopically to moving (but not static) stimuli [10], as has been demonstrated using the genetically encoded calcium indicator GCaMP7a [11•]. GCaMP is a modified version of GFP that increases in brightness upon entry of Ca^{2+} into the cell [12]. The genetic basis of GCaMP7a enables it to be restricted to specific populations of cells. The optic tectum projects to a pair of neurons in the lateral part of the nucleus of the medial longitudinal fasciculus (MLF) called MeLr and MeLc [13]. Laser ablation of the MeLr or MeLc reduces the ability of larvae to capture prey suggesting this behaviour is largely driven by MLF activation [13]. The combination of fixed-loop virtual environments and genetically based calcium indicators permits the investigation of how objects in the visual field are processed at all levels of the central nervous system. This setup could now be used to screen for novel mutants that show aberrant hunting behaviour.

Lateralised behaviour

Lateralisation, asymmetries of body viscera, brain areas and behaviour is a widespread property of many vertebrates including fish. In the brain, lateralisation has the potential to specialise neural circuit function which may give rise to new behavioural phenotypes [14]. In zebrafish the left and right habenulae (Hb) of the epithalamus exhibit prominent asymmetries that are established by left-sided expression of Nodal pathway genes during development [15]. The Hb receives inputs from the olfactory bulb and retina and projects to the periaqueductal grey matter via the interpeduncular nucleus (IPN) [14]. Zebrafish harbouring a naturally occurring polymorphic mutation in the maternally expressed gene *mother of snow white* (*msw*) [16] show parallel alterations in body symmetry and eye usage when biting an object [17]. Similarly, ENU (N-ethyl-N-nitrosourea; a chemical mutagen)-induced *frequent situs inversus* (*fsi*) mutants show concordant left-biased or right-biased

Table 1

Description of the characteristic postures exhibited by zebrafish during different behavioural tasks. Key references describing these behaviours are included.

Behaviour	Behavioural posture	References
Aggression	Biting, thrashing tail and chasing opponent/pushing mirror	[6,37,41]
Developmental alterations to behaviour	Changes to locomotion, freezing, bottom-dwelling and choice accuracy in a learning task	[20,21••,22]
Fear conditioning	Erratic swimming, freezing and learned avoidance	[23–25]
Lateralised behaviour	Eye usage when biting an object, latency to enter a novel compartment, response to odour or light	[15–17]
Learning and memory	Short-term memory, classical and operant learning, associative conditioning	[2••,8••,28]
Social behaviour	Shoaling, preference for conspecifics, kin	[9•,31,33]

localisation of the pineal gland and eye usage, and differences in Hb size [18]. Left-handed *fsi* mutants have a greater latency to enter a novel compartment compared to right-handed animals demonstrating a range of behaviours connected to asymmetry [18]. Laterality is also seen at the neural circuit level. The right lateral dorsal Hb (ldHb) responds to odours and projects to the dorsal IPN whereas the left ldHB is light-activated and projects to the ventral IPN, as shown using the calcium indicator GCaMP5G [19••]. Experimental manipulation of the Wnt signalling pathway (by subjecting tailbud-stage embryos to a short cold pulse or by using the pharmacological inhibitor IWR-1) [20] can force the Hb into a double-right or double-left configuration and trigger loss of brain responsiveness to one of these stimuli [19••]. Intriguingly, odour presentation appears to activate distinct ensembles of Hb neurons that combine with spontaneous neural activity to switch between different types of behavioural output [21••]. In summary, a combination of mutant analysis and cutting-edge tools has begun to unravel the genetic and neural basis of lateralised behaviours, demonstrating a link between asymmetry at the level of brain anatomy and behaviour. Elucidation of the molecular identity of both *fsi* and *msw* would shed further light upon the genetic cascades underlying this process.

The developmental basis of behaviour

Alterations to the early stages of neural development can trigger long-lasting behavioural and neurochemical changes, which may be linked to the expression of some neurological disorders [22]. Comparison of six zebrafish strains has uncovered large variability in locomotion levels throughout juvenile development indicating that behavioural ontogeny is influenced by both genetic and environmental factors [23]. The orphan nuclear receptor NR4A2 plays a role in dopamine (DA) progenitor commitment by regulating the DA synthesis enzyme tyrosine hydroxylase (TH) and controlling the differentiation of DA neurons in the posterior tuberculum, telencephalon, preoptic area and pretectum. *nr4a2* morphant fish (lacking *nr4a2* activity during the first 3–4 days of embryonic development [24]) show persistent hyperactivity, suggesting a critical role for NR4A2 in tuning the neural circuits that control locomotion [25]. In contrast to this, TH morphant fish exhibit normal levels of activity at adult stages, but increase bottom-dwelling and freezing (anxiety-like phenotypes) in a novel environment [26]. Methylphenidate (MPH), a DA and noradrenaline (NA) reuptake inhibitor used to treat attention-deficit/hyperactivity disorder (ADHD), increases the levels of DA and NA at the synapse. Acute MPH exposure during embryogenesis reduces the time adult fish spend at the bottom of a novel tank and impairs choice accuracy in a 3-chamber learning task [27]. These findings indicate that transient early alterations to dopaminergic neurotransmission can trigger long-term impairments in behavioural plasticity.

Fear conditioning

The habenula (Hb) is a part of the epithalamus that projects to brain stem nuclei including the raphe nucleus and ventral tegmentum. The subdivisions of the habenula are similar in zebrafish and other species: the dorsal and ventral Hb (dHb and vHb) of fish correspond to the mammalian medial Hb and lateral Hb respectively [28]. Inhibition of the lateral subnucleus of the dHb by expression of the tetanus toxin light chain (TeTxLC) does not induce changes in locomotion but increases freezing indicating that the Hb is important for the response to fear [29]. Larval zebrafish learn to avoid a light when paired with a mild shock but are unable to learn when submitted to an inescapable shock. Photobleaching Hb afferents or expressing TeTxLC in the dHb can block this avoidance response, suggesting that abnormalities in Hb function may contribute to anxiety disorders [7]. Zebrafish exposed to alarm substance (AS) also show a fear response that includes erratic movements and freezing. Intercranial administration of the neuropeptide Kisspeptin decreases the behavioural response to AS. Furthermore, inactivation of Kiss-Receptor1-expressing neurons using Kiss1 peptide conjugated to saporin, a ribosome inactivating protein, both reduces Kiss1 immunoreactivity and *c-fos* mRNA in the habenula and decreases the AS-evoked fear response reinforcing the role of Kisspeptin in this behaviour [30]. Although these studies have already demonstrated a role for the Hb in fear, a complete description of the genes and signalling pathways that underlie this behaviour now needs to be produced.

Learning and memory

Zebrafish display learning and memory capabilities and both short and long-term memory formation have been evaluated in this species [31,32]. There is evidence that glutamatergic and cholinergic signalling are implicated in the acquisition and consolidation phases of memory processing [31]. Classical and operant learning behaviours can be observed from 3 weeks post-fertilisation reaching maximal performance at week 6 [33]. In addition, associative conditioning learning has been shown to be protein synthesis-dependent and NMDA receptor-dependent using a paradigm developed for larval zebrafish [34•]. Recent work using a genetically encoded calcium-sensitive protein, inverse pericam, has identified an area of the dorsal telencephalon that is activated during long-term memory retrieval [8••]. This functional map changes when the behavioural task is altered, suggesting that memory traces are dynamically modified during the learning process [8••]. In larvae, calcium indicators have been used to image neuronal activity during behaviour. For example, neuronal populations in the hindbrain show activity patterns that correlate with left or right optomotor behaviour. Moreover, neurons in the habenula, pallium, and midbrain respond dynamically to the changing characteristics of an environment [2••]. This approach can now be used to identify neural activation during learning tasks. Although several memory tasks have been developed for zebrafish, few of the genes that control this behaviour have been identified. A mutant line that fails to change place-preference following amphetamine administration (thus demonstrating a learning deficit) has been described, but the mutated gene has not been cloned [35]. Further work is required to uncover the molecular players involved in learning as well as developing novel paradigms to fully probe the cognitive ability of this species.

Social behaviour

Zebrafish have an innate preference to associate with conspecifics. The absence of social interaction appears to be stressful; when tested individually fish show increased cortisol levels and behavioural variability compared to group-tested animals [9•]. Zebrafish begin to shoal between 7 and 87 days post-fertilisation and show correlated strain-dependent changes in DA and 5-HT levels hinting at a neurochemical basis for this behaviour [36]. Kin recognition is an important step in the evolution of social behaviour. Zebrafish larvae exposed to kin at day 5 and 6 days post-fertilisation recognise each other throughout their life, due to a combination of visual and olfactory imprinting. This process involves the major histocompatibility complex (MHC) code, which influences the chemical and visual features that zebrafish display [37]. Zebrafish appear to only imprint upon kin expressing similar MHC class II genes, and this process is likely olfactory based, because MHC peptides can activate a subset of neurons in the olfactory bulb [38•]. Social behaviour can also be influenced by exposure to other chemicals during development. Fish treated with ethanol at early embryonic stages show decreased individual social behaviour and shoaling, increased anxiety and concomitant alterations in the expression level of the genes *hrt1aa* (5-HT receptor 1a), *slc6a4* (serotonin transporter) and *oxtr* (oxytocin receptor) [39]. Adult zebrafish glucocorticoid receptor (GR) mutants have high cortisol levels and show changes to social behaviour including reduced exploratory behaviour, immobility and lack of habituation to a novel tank. Fluoxetine treatment both restores normal behaviour and normalises cortisol levels, making it possible to study the link between the stress axis and emotional behaviour [40]. The abundance of tools available in zebrafish suggests that this model is ideal to investigate the genetic basis of social behaviour.

Aggression

Recent studies have identified novel genes and neurotransmitters that control zebrafish aggression. Animals use aggression to protect themselves and their offspring, fight for resources and establish dominance hierarchies. Zebrafish aggression has a heritability estimate of 0.36 [41] suggesting that environmental influences play an important role in the expression of this behaviour. Aggression can be measured by recording the interaction of a pair of fish or of a single fish with its own mirror image [42,43,44]. Zebrafish display characteristic agonistic postures including undulating body movements, short slaps of the caudal fin and bites directed against an opponent [44]. Aggressive incidents follow a highly structured pattern [43] and they are influenced by similar neurotransmitters in zebrafish and other vertebrates including 5-HT and dopamine [45], histamine [6], 17α-ethinylestradiol [46] and arginine vasopressin/arginine vasotocin (AVP/AVT) [47]. Mutation of *fibroblast growth factor receptor 1a* (*fgfr1a*) causes a parallel increase in aggression, boldness and exploration regardless of rearing conditions [6]. Furthermore, manipulation of the neurotransmitter ependymin alters aggression in both zebrafish and trout implicating a novel signalling molecule in this behaviour [48]. Although zebrafish aggression research is still in its infancy, validation of robust behavioural protocols and the demonstration that single genes can modulate this behaviour suggest that this is a promising area for further investigation.

Conclusion

Studies of both adult and larval zebrafish have brought new insights into the genetics and neurobiology of behaviour. The relative transparency and genetic tractability of zebrafish makes them ideal to link behaviour to neurobiology at different life stages. The approaches used in this research, including genetically based techniques such as calcium indicators, optogenetic tools to manipulate neuronal activity [49], genetically encoded fluorescent-based reporters [50] and the targeted

mutation of genes [51] suggest that the future of this field is bright.

Conflict of interest statement

Nothing declared.

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