*Chapter X*

# THE SEROTONERGIC SYSTEM OF ZEBRAFISH: GENOMICS, NEUROANATOMY AND NEUROPHARMACOLOGY

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## Abstract

The serotonergic system is highly conserved in vertebrate species, including zebrafish. In spite of a genome duplication in teleost fish (which altered the number of genes encoding proteins related to synthesis, transport and signaling within the serotonergic system), the expression pattern of these proteins in zebrafish is similar to other vertebrates. Pharmacological manipulations of the serotonergic system also produce similar behavioral and neuroendocrinological effects in zebrafish and mammals, suggesting that zebrafish represent a promising model for drug discovery and translational research focusing on central serotonergic mechanisms.

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## Introduction

Zebrafish (*Danio rerio*) is a small cyprinid species whose importance in evolutionary developmental biology is rapidly increasing due to the continued development of molecular tools, today representing an established model organism for biological psychiatry research. Recently, zebrafish have gained visibility in the neurosciences (Fig. 1A), mainly due to several key advantages: 1) Low cost; 2) Easy housing/husbandry; 3) Rapid reproductive cycle; 4) Ease of breeding in the laboratory; 5) Suitability for studies of anxiety and stress, 6) Extensive homology with mammals at the genetic, neural and endocrine levels; and 7) Utility for high-throughput screening and physiologically complex phenotyping (Fig. 1B). This chapter will summarize what is known about the neuroanatomical organization of the serotonergic system in zebrafish, as well as the effect of various serotonergic drugs on behavioral and neuroendocrinological parameters in larval and adult animals.

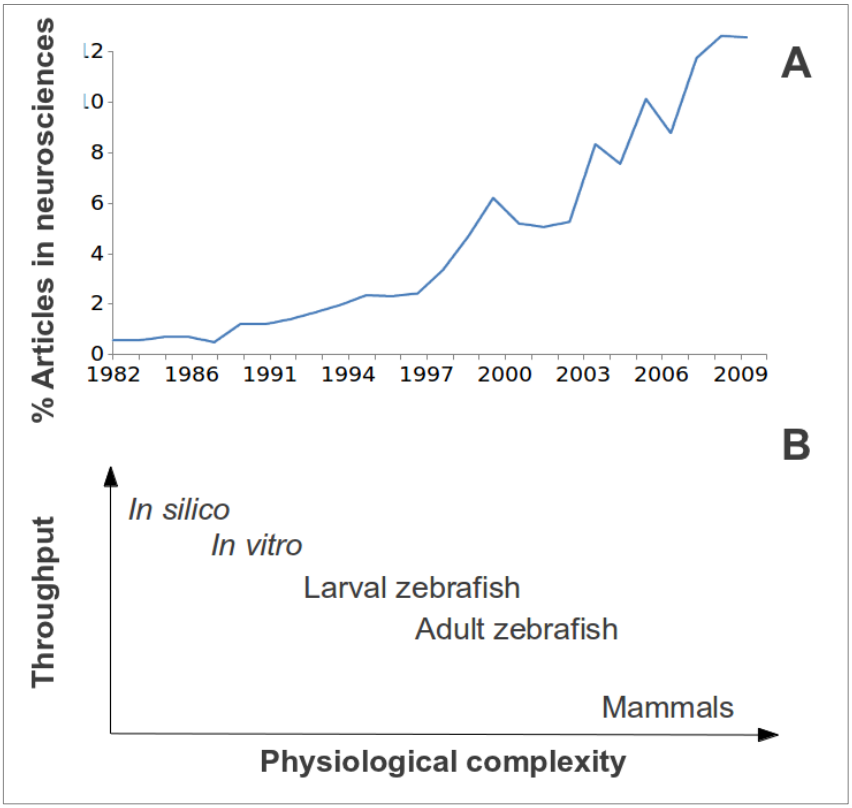


**Figure 1.** The growing use of zebrafish in neuroscience research. (A) A quick search in ISI Web of Knowledge (http://apps.webofknowledge.com/) reveals a steady increase in the number of articles using zebrafish in neuroscience and associated fields from 1980 to 2012. (B) Schematic relationship between throughput and physiological complexity of popular preparations in neuroscience and associated fields; notice that zebrafish represent a compromise between throughput and physiological complexity.

## Genomics of the zebrafish serotonergic system

The serotonergic (5-HT) system of zebrafish shows both similarities and differences with the mammalian serotonergic system [1]. Due to a genome duplication event that occurred at the base of the teleost radiation [2,3], zebrafish possess three copies of the *tph* gene encoding tryptophan hydroxylase (the rate-limiting enzyme in serotonin synthesis) - *tph1a*, *tph1b* and *tph2* [4]. *Tph1a/b* genes are expressed in the pineal gland, retina, hypothalamus, and spinal cord [4], while *tph2* is expressed in the raphe, reticular formation and pretectal area [5]. Similarly, the serotonin transporter (*sert*) gene has also been duplicated in zebrafish [6,7], and both its isoforms (*serta* and *sertb*) are expressed in a complementary fashion, generally following the expression of the *tph* isoforms [6,7]. This transporter shows a 35-fold increased affinity for imipramine and desipramine in relation to the human SERT, while showing approximately eightfold decreased affinity for cocaine [8]. In whole-brain homogenates of zebrafish, serotonin uptake is inhibited by desipramine and citalopram with $IC_{50}$ values of approximately 7 and 9 nM, respectively [9].

An important finding is related to differences in the effects of monoamine oxidase (MAO) inhibitors on zebrafish behavior. While *tph1* and *sert* duplication was retained in zebrafish, a different process occurred with *mao*, the gene encoding MAO. While in mammals two distinct isoforms are present (MAO-A and MAO-B), zebrafish have only one isoform, zMAO [10,11]. zMAO's affinity profile is tyramine > kynuramine > serotonin > phenethylamine > MPTP > dopamine [11,12,13]. MAO-B inhibitors are generally more efficacious in the inhibition of zMAO in brain homogenates than MAO-A inhibitors [13]. However, when the enzyme is purified and expressed, most MAO-B reversible inhibitors are rather ineffective, catalyzing the oxidation of benzylamine analogs more similarly to human MAO-A than to human MAO-B [13]. Moreover, sequence comparisons of zMAO shows that its substrate binding domain is identical to human MAO-A, while the flavin binding domains show 80% identity with both MAO-A and MAO-B [12,13]. Histochemically, the highest levels of zMAO activity are detected in noradrenergic and serotonergic cell groups, with low to moderate activity also found in dopaminergic cells [11].

Two genes encode zebrafish serotonin 5-$HT_{1A}$-like receptors, *htr1aa* and *htr1ab* [7]. The expression patterns of the *htr1aa* and *htr1ab* genes are similar, as mRNA for *htr1aa* is found in the anterior part of the parvocellular preoptic hypothalamus, periventricular gray zone, centroposterior thalamic nucleus, ventral and dorsal parts of the pretectal diencephalic groupings, periventricular nucleus of the posterior tuberculum, diffuse nucleus of the inferior lobe, paraventricular organ, dorsorostral tegmental nucleus, and in the superior raphe [7]. The *htr1ab* gene presents the same pattern, with extra expression in the ventral telencephalon, periventricular hypothalamus, and central gray [7]. Binding of the radioligand [$^3$H]-8-OH-DPAT is displaced by the 5-$HT_{1A}$ receptor antagonist WAY 100,635 with an inhibition constant of approximately 1000 nM in zebrafish whole brain homogenates, and the partial agonist buspirone displaces 8-OH-DPAT with an inhibition constant 3 orders of magnitude smaller (1.8 nM) [14].

## Neuroanatomy of the serotonergic system

While in mammals most serotonergic cells are localized within the hindbrain, zebrafish possess at least three clusters of serotonergic neurons (Fig. 2), among which only the cell groups located in the hindbrain (superior and inferior raphe, medullary cells) are also found in tetrapods, including mammals.

## Raphe nuclei

Due to the homology between the serotonergic neurons in zebrafish and mammalian raphe nuclei, they represent the most studied cell populations in fish [1]. These cells are unique since, unlike hypothalamic and pretectal serotonergic cells, they express the ETS-domain transcription factor-encoding gene *pet1* [15]. In both mammals and zebrafish, the raphe nuclei are composed of two main clusters, the superior (SR) or rostral raphe, and the inferior (IR) or caudal raphe [16,17,18,19]. The SR cluster projects to ascending fibers which innervate most areas of the brain, and, based on topography, homology, chemoarchitecture and electrophysiology data, can be further subdivided into at least 5 sub-regions in mammals [20]. In zebrafish, the superior raphe can be roughly divided into three populations, based on their projection patterns (Fig. 2A): 1) dorsal cells, projecting to the prosencephalon (olfactory bulb and telencephalon); 2) ventral cells, projecting to the hypothalamus; and 3) ventrolateral cells, projecting to the migrated nuclei of the posterior tuberculum ([15,21].

The dorsal and ventral populations appear in rhombomeres 1 and 2 at approximately 25 h post-fertilization (hpf), as assessed by *pet-1* and *tph2* expression [15]. These cells originate in the midbrain-hindbrain boundary (MHB) progenitor pool [15], and Fgf receptors from the MHB are necessary for the differentiation of raphe neurons [5]. There is a tendency for neurons projecting to the dorsal portions of the telencephalon (Dp and Dm, putative homologs of piriform cortex and basolateral amygdala) to be located more dorsally, compared to neurons projecting to the lateral telencephalon (Dl, putative homolog of hippocampus) [21]. In the early juvenile stages (5 to 7 days post-fertilization [dpf]), the ventrolateral population appears [21]. This population is located nearest to nucleus interpeduncularis and is interspersed with DOPA decarboxylase-immunoreactive neurons [22]. Although it has been tempting to homologize this ventrolateral population with the mammalian supralemniscal serotonergic cell group (B9), the specificity of the projections of these cells to the posterior tuberculum suggests otherwise [1].

The targets of *pet-1*-positive projections from the superior raphe (SR) are not homogeneously distributed in the telencephalon or mesencephalon. Within the telencephalon, the dorsolateral portion (putative hippocampal homolog) receives the heaviest innervation, followed by the dorsomedial (putative basolateral amygdala homolog), postcommissural ventral (putative central/medial amygdala homolog) and ventroventral (septal homolog) portions [21], where their density more or less agrees with monoamine oxidase activity [11] (Fig. 3). In the mesencephalon, a high degree of innervation is observed in caudal and dorsal zones of the periventricular hypothalamus and in the periglomerular complex, followed by the nucleus of the medial longitudinal fascicle, the torus lateralis and the central nucleus of the inferior lobe. Low innervation is found in the rest of the mesencephalon, with no innervation observed in the optic tectum [11].

Other chemical phenotypes in the raphe appear concomitantly or after the appearance of serotonergic neurons. For example, between 40 and 55 hpf, the nitric oxide synthase I mRNA is expressed across the hindbrain [23,24,25]. It is unclear whether these cells also express serotonergic markers, similar to both serotonergic and non-serotonergic cells that express NOS-I in rodents [26]. At 28 hpf, corticotropin releasing factor CRF-positive cells appear in the first, second, and fourth rhombomeres of zebrafish, where serotonergic neurons will appear. The CRF-positive cells in r1 are intermingled with tyrosine hydroxylase-positive cells, suggesting that they are part of the locus coeruleus [27], and not of the SR. However, there are some CRF-positive cells which are not part of the locus coeruleus (and thus, presumably, are part of the raphe) [27].

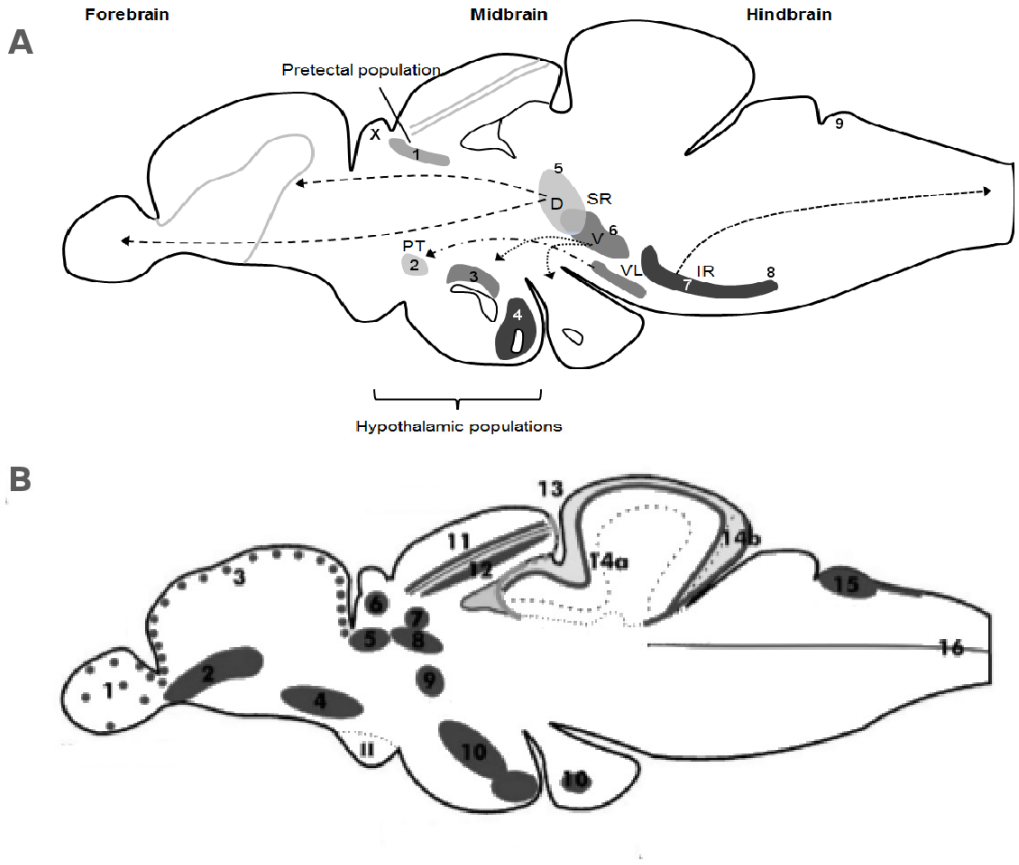


**Figure 2. The anatomy of the serotonergic system of zebrafish. (A)** Serotonergic populations in the adult zebrafish brain, with selected projection patterns from the raphe subpopulations. X – Pineal population; 1 – Pretectal population; 2-4 – hypothalamic periventricular population; 5-7 – Rostral raphe populations; 8 – Caudal raphe population; 9 – Area postrema population. Adapted from refs [1] and [28]. **(B)** Proliferation zones in the adult zebrafish brain, most of which overlap with serotonergic populations and/or their innervation patterns. 1 – Olfactory bulb; 2-3 – Telencephalic proliferation zones; 4-10 – Diencephalic proliferation zones; 11-13 – Mesencephalic proliferation zones; 14 – Cerebellar proliferation zones; 15 – Medullary proliferation zones. Adapted from ref. [29].

The inferior raphe (IR) projects rostrally to the nucleus lateralis valvulae, a precerebellar structure which contains neurons without dendrites and provides mossy fibers to the granule cells of the cerebellum [21]. Importantly, this population projects caudally to motor structures in the hindbrain and spinal cord. The cell bodies and axons from the IR are difficult to separate from another group of cells, described as B1-B2 groups [22], located in the intermediate reticular formation, below the catecholaminergic medial longitudinal fascicle. In zebrafish, these cells express *tph2* and *pet1*, suggesting that they may actively produce serotonin [1,21]. These cells are thought to be homologous to the reticular raphe nuclei [22], and receive projections from rostral IR neurons [21]. Both cell groups depend on the activity of the homeodomain factor IRX1A at 48 hpf, as application of antisense morpholinos for that

homeodomain transcriptor regulator at this stage lead to the disappearance of IR neurons, while sparing SR neurons [30].

The IR extends caudally below the level of the axons of Mauthner cells [31], which are part of a "brainstem escape network". 5-HT-positive fibers are closely aposed to the lateral dendrites of Mauthner cells (Fig. 4). In goldfish, 5-HT can presynaptically facilitate glycine release on the ventrolateral dendrite of the Mauthner neuron [32,33,34]. 5-HT-reactive boutons are also associated with the axon collaterals of another giant reticulospinal neuron, MiD3cm, which is part of this "brainstem escape network" [31], suggesting that the serotonergic projections from the IR modulate the output of reticulospinal neurons onto spinal cells.

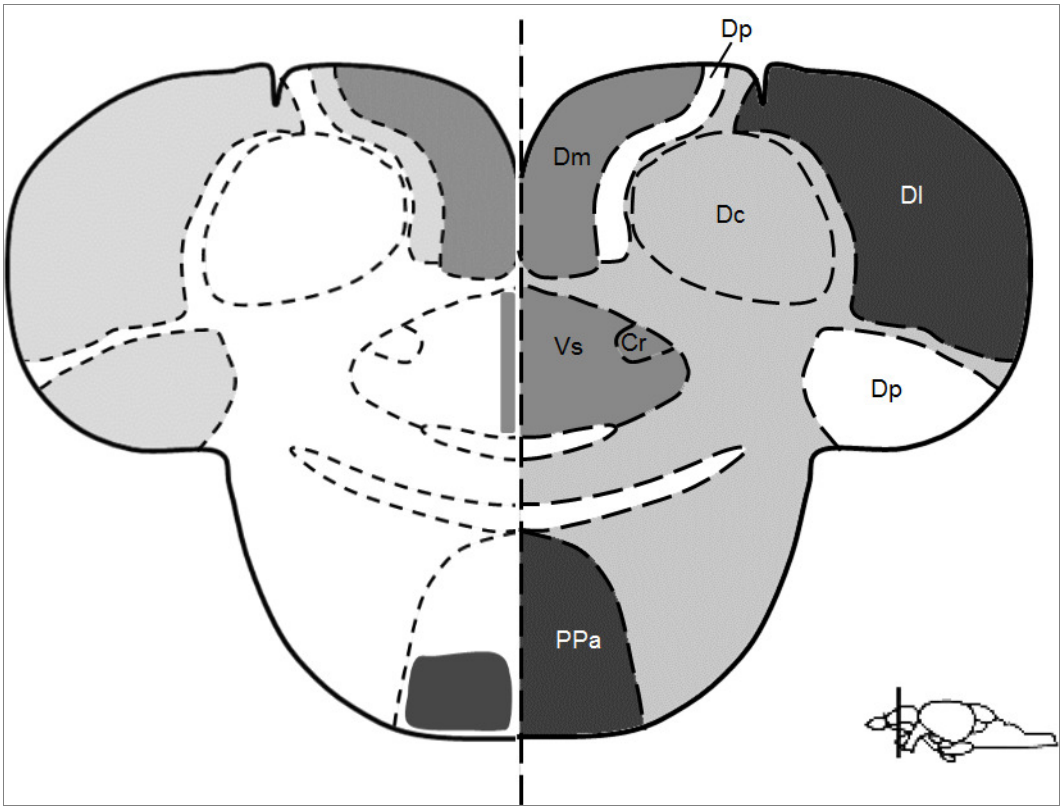


**Figure 3.** Monoamine oxidase activity (Left) and innervation by Pet-1-positive fibers (Right) in the telencephalon of zebrafish at the level of the anterior commissure. Gray levels indicate approximate amount of innervation and/or MAO activity. Adapted from refs. [1] and [11]. Abbreviations: Cr, central ring of subcommissural ventral telencephalon; Dc, dorsocentral telencephalon; Dl, dorsolateral telencephalon; Dm, dorsomedial telencephalon; Dp, dorsoposterior telencephalon; PPa, anterior part of the parvocellular preoptic nucleus; Vs, subcommissural ventral telencephalon.

### Posterior tuberculum/hypothalamic populations

In zebrafish (as well as in amphibians, reptiles, birds and non-eutherian mammals), major clusters of serotonergic neurons exist in the posterior tuberculum and hypothalamic populations [22]. These cells express both isoforms of *tph1*, as well as the other mature markers for serotonergic neurons, such as L-aromatic amino acid decarboxylase, monoamine oxidase, the b isoform of the serotonin transporter, vesicular monoamine transporter 2, the b isoform of the 5-$HT_{1A}$ receptor, and serotonin immunoreactivity [1]. The serotonergic cells are primarily located opposite to the paraventricular hypothalamus, and are grouped in three populations: 1) the anterior cluster, located medially and in the anterior portion of the paraventricular organ (PPa), 2) the lateroposterior cluster in the intermediate portion of the paraventricular organ (PPi), and 3) the posterior cluster in the caudal zone of the periventricular hypothalamus (Hc) [1,22]. In the terminology of Panula et al. [28], these clusters correspond to serotonergic populations 2-4. From the point of view of neurodevelopment, these populations appear 12 h later than those cells in the raphe clusters [35], and their expression is controlled by *too few/fezf2*, mutations of which eliminate noradrenergic and serotonergic cell clusters in the tuberculum and hypothalamus, but not other regions [36,37]. These cells contact the blood vessels and the ventricles via short, thick local processes, and also project profusely to extra-hypothalamic areas. The exact function of these clusters, as well as their projection patterns and targets are currently unknown.

### Pretectal area

The pretectal cluster is the rostral-most serotonergic cell group in the zebrafish brain. Panula et al. [28] identified these cells as the serotonergic population 1, and they seem to appear at 60 hpf [35]. These cells express the mature markers *tph2*, L-aromatic amino acid decarboxylase, *serta*, vesicular monoamine transporter 2, *htr1aa*, and 5-HT-like immunoreactivity) [1]; however, monoamine oxidase activity is absent [11]. Most of the serotonergic fibers in the optic tectum seem to originate from the serotonergic neurons of the pretectal cluster [22], a hypothesis that is reinforced by the observation that no *pet-1*-positive fibers are observed in the tectum [21]. Pretectal nuclei, as well as the optic tectum, have been implicated in the regulation of visuomotor behavior, multimodal sensory integration [38] and escape responses [39].

## Serotonergic innervation of proliferation zones in the zebrafish brain

Serotonin is an important regulator of cell proliferation and maturation in the vertebrate CNS, modulating the structure and function of adult brain areas [40]. Serotonin has been shown to stimulate neurogenesis in the hippocampus of rodents [41,42,43] via 5-$HT_{1A}$ and 5-$HT_{2C}$ receptors [44]. In mammals, adult neurogenesis is restricted to the subependymal zone of the lateral ventricle and to the subgranular zone of the dentate gyrus, whereas in teleost fish, it has been described throughout the CNS [29]. In zebrafish, these cells are located primarily close to ventricular surfaces, in areas which receive dense serotonergic innervation (Fig. 2B). Thus, there is a close association between serotonergic innervation [21], the presence of family 1 serotonin receptors [7], and the proliferative zones (especially zones 1-3 and 10-12) in the zebrafish brain [1].

The functional significance of adult neuro- and gliogenesis in vertebrates still remains poorly understood [45]. Santarelli et al. [42] demonstrated in rodents that blocking

hippocampal neurogenesis abolishes the effect of antidepressants on an anxiety task. These results implicate adult neurogenesis in the efficacy of antidepressant drugs – an aspect that has not yet been examined in zebrafish. Although neurogenesis in rodents is usually reduced by region-specific irradiation, a simple pharmacological approach (e.g.., treatment with the cytostatic agent methylazo-methanol acetate) can be used to assess these aspects in zebrafish. The easy access and abundance of the adult neurogenic zones of zebrafish, as well as the readiness with which one can isolate and produce neural stem cells in teleosts [46] and label cycling cells with 5-bromo-2-deoxyuridine (BrdU) pulses *in vivo* [29], suggests the growing utility of zebrafish in understanding the role of serotonin in regulating adult neuro- and gliogenesis, both *in vivo* and *in vitro models*.

## Behavioral pharmacology of zebrafish serotonergic system

The effects of serotonergic drugs on zebrafish behavior have been studied in larval, juvenile and adult populations. Given the important role of the serotonergic system is affective behavior [20], zebrafish models of anxiety and fear were the main paradigms used in testing those effects (Table 1). In general, the results of these experiments are similar to those observed in mammals, albeit some differences were also present.

As in rodents, the effect of SERT ligands produced some variation. For example, in early juvenile stages (e.g., 5 dpf), serotonin increases the frequency of spontaneous swimming activity without changing the duration of these episodes, while methysergide (non-selective serotonin antagonist) or ketanserin (5-$HT_{2A/2C}$ antagonist) have the opposite effect [47]. In contrast, bath exposure to fluoxetine (1.5 mg/ml) in 5 dpf larval fish for 24 h decreases spontaneous swimming activity, also down-regulates the expression of SERT and 5-$HT_{1A}$ receptors in the spinal cord, but not in the brain [48]. In the bouncing ball assay (Table 1), 2 mg/ml fluoxetine decreases the escape response of 7 dpf larvae, but does not alter thigmotaxis [49]. In adult zebrafish, endogenous serotonin released in the spinal cord decreases the frequency of swimming [50], whereas serotonin reuptake inhibitors (SRIs) produced conflicting results. Acute citalopram treatment decreases zebrafish geotaxis [9], which was unaffected in this test by acute fluoxetine [51]. Likewise, in the light/dark test (Table 1), acute fluoxetine produced no effect [52], anxiolytic-like [9] or anxiogenic-like effects [53], depending on the dose and mode of administration.

**Table 1.** A brief summary of selected popular zebrafish neurobehavioral models

| Model | Rationale | References |
|---|---|---|
| Novel tank test | Conceptually similar to the rodent open field test, this model exposes individual zebrafish to a novel tank (usually, for 6 min), where initial diving response (geotaxis) reflects anxiety-like state. Fish display freezing (immobility) and erratic movements in this test, both indicative of increased anxiety. Anxiolytic treatments generally increase top preference, reducing freezing and erratic movements. Anxiogenic manipulations show the opposite profile in this test. | [54,55,56] |
| Light/dark box | Conceptually similar to the rodent light/dark box test, this model exposes individual zebrafish to a novel light/dark box, where dark preference (scototaxis) reflects anxiety-like state. Anxiolytic treatments generally reduce dark preference and | [52] |

| | | |
|---|---|---|
| | increase activity in the light. Anxiogenic manipulations show the opposite profile in this test. | |
| Bouncing ball test | Based on natural escape-like responses, this model exposes larval zebrafish to a 'bouncing ball' displayed on a screen, mimicking the shadow of a large predator. Larval fish generally swim away from the bouncing ball, turning around to face the stimulus from the distance. Anxiolytic treatments decrease escape-like behaviors in this test. | [49] |
| Open field test | Conceptually similar to the rodent open field test, this model exposes individual zebrafish to a novel tank (usually, for 5-30 min), assessing swimming activity (locomotion) and center:periphery preference (thigmotaxis). Fish also display freezing in this test, together with higher thigmotaxis reflecting anxiety-like states. Anxiolytic treatments generally reduce thigmotaxis and freezing. Anxiogenic manipulations show the opposite profile in this test. | [54,55,56] |

The effects of chronic treatment with SERT inhibitors are more clear-cut, with anxiolytic-like effects observed for fluoxetine on both scototaxis and geotaxis endpoints with different routes of administration and doses [52,57,58]. In line with this, chronic fluoxetine treatment also reduced whole-body cortisol levels in adult animals [58], supporting anxiolytic profile of chronic SSRIs.

In zebrafish larvae, the administration of selegiline (an irreversible MAO-B inhibitor) increases serotonin, but not dopamine or noradrenaline levels in the brain, decreases serotonin-like immunoreactivity in serotonergic neurons, induces ectopic serotonin-immunoreactive neurons in the diencephalon, inhibits spontaneous locomotion, produces postural impairment, and increases heart rate [59]. These effects were prevented by treatment with the tryptophan hydroxylase inhibitor *p*-chlorphenylalanine, suggesting a dependence of these effects on the serotonergic system [59]. In adult zebrafish, treatment with the nonselective MAO inhibitor tranylcypromine decreases geotaxis in the novel tank test [51], while moclobemide (another MAO-A inhibitor) does not produce overt effects scototaxis in the light/dark box [52]. These results are similar to what is observed in clinical settings, in which MAO inhibitors are panicolytic, but have no or little effects on generalized anxiety disorder [60]. This differential pharmacology of geotaxis vs. scototaxis suggests their relevance to different aspects (i.e., panic- vs. anxiety-like states) of zebrafish behavior.

Notably, buspirone is clinically effective in generalized anxiety disorder, but not panic disorder [60]. If a clear-cut pharmacological separation of models was made, buspirone should not have an effect on geotaxis. Nonetheless, buspirone does decrease geotaxis in adult zebrafish in the novel tank test [61] as well as in the group behavior task [62]. A similar effect is observed in the light/dark box in adult [52] and larval [63] zebrafish. So far, it is not known whether this difference in pharmacological efficacy of buspirone between zebrafish and mammals is due to the duplication of the gene that codes for its target, the 5-$HT_{1A}$ receptor [7].

A difference between these tests, in terms of serotonergic mediation, may become more apparent when serotonin release is evaluated. While the geotaxis-based novel tank test did not increase the extracellular content of serotonin in the brain of zebrafish, exposure to the light/dark box does so [53]. Likewise, a positive correlation between extracellular serotonin and the time spent in the dark portion of the light/dark box is observed, with a negative correlation between serotonin and the time spent in the bottom [53]. No effect of acute

fluoxetine was observed in geotaxis [51,52], while a lower dose (2.5 mg/kg) was anxiogenic in the light/dark test, larger doses (5 and 10 mg/kg) were ineffective in this test, and a hyperlocomotor effect found for the 10 mg/kg dose [52,53]. The administration of the 5-$HT_{1A}$ receptor antagonist WAY100,635 produced an anxiolytic-like effect in both tests [53], similar to the partial agonist buspirone [53,61]. LSD, also a 5-$HT_{1A}$ receptor agonist, produced prominent anxiolytic action in zebrafish [54], whereas the 5-$HT_{1B}$ receptor inverse agonist SB 224,289 was mildly anxiogenic in both tests [53] (see similar results in [56] for another serotonergic hallucinogen, mescaline, the pharmacology and clinical effects of which resemble those of LSD).

Interestingly, MDMA (3,4-methylenedioxymethamphetamine, 'Ecstasy') modulating the serotonergic system by blocking serotonin reuptake, evokes an anxiolytic/hallucinogenic-like behavioral profile in zebrafish similar to both LSD and mescaline [54,55,56]. Generally resembling 'anxiolytic' clinical effects of MDMA, but deviating from the hyperlocomotor anxiogenic-like responses typically seen in rodents, this profile supports the notion that the zebrafish is a promising and sensitive *translational* model for serotonergic drug discovery [55,56]. In line with this, mescaline, known to act via 5-$HT_{2A/2C}$ receptors, also exerted anxiolytic effects in the novel tank test in zebrafish [64]. Perhaps even more importantly, the relative efficacy of these serotonergic hallucinogenic drugs in zebrafish is similar to that observed in humans. Specifically, the effective doses of mescaline (20 mg/l [64]) in zebrafish is 200-fold less potent than LSD (0.1 mg/l [54]) and 4 times less potent than MDMA (80 mg/l [55]). This parallels the observations in humans in which mescaline is equally potent as MDMA (200 mg) and >200-fold less potent than LSD (<1 mg). Likewise, MDMA seems to be approximately 800 times less potent than LSD in both zebrafish and clinical literature [55].

Finally, serotonin depletion after the application of *p*-chlorphenylalanine was anxiolytic in the light/dark box, and anxiogenic in the novel tank test. These results suggest a dual role of serotonin in controlling zebrafish defensive/affective behavior [53], albeit the construct validity of both tests may still merit further investigation.

## Where next?

Since serotonergic drugs in zebrafish studies have mostly been administered systemically, our ability to localize and dissect their pharmacological effects remains rather limited. Nevertheless, the future holds much promise in this field, as more robust and precise techniques for functional localization of its action continue to arise. For example, using the expression of c-*fos* mRNA, Lau et al. [65] demonstrated that animals which avoid the light compartment of a light/dark tank show activation of different brain areas than those which do not. Given the effects of serotonergic drugs in this test, *c-fos* expression in these areas (e.g., dorsomedial and commissural ventral telencephali) can be expected to increase or decrease in concordance with the anxiogenic or anxiolytic effect. Another interesting possibility is recording electrical activity in these areas during a behavioral task such as the light/dark or the novel tank tests and correlating this activity with tank location. A technique for these types of experiments is already available for goldfish [66], and could be combined with the administration of serotonergic drugs, as well as with *in vivo* analysis of serotonin content in these regions with differential pulse voltammetry using chitosan-coated carbon fiber microelectrodes [67].

In addition to these techniques, an important molecular “zebrafish toolbox” can also be used to dissect circuit anatomy and function [68]. The Zebrafish Information Resource Center (ZIRC, University of Oregon, OR, USA) provides multiple mutant and transgenic lines, including the ‘*too few’* mutant described before. Besides these already available animals, other knockout and knockdown animals can be generated using tools such as zinc finger

nucleases made by oligomerized pool engineering (OPEN) [69], an approach which can complement targeting-induced local lesions in genomes (TILLING) [70], effectively turning down or off the expression of selected genes of interest. A complementary approach can also be the use of a Gal4/UAS system or a bacterial artificial chromosome (BAC) to drive the expression of a given gene [71,72,73,74,75]. Using this approach, tetanus toxin light chain (TeTXCL) [76], KillerRed [77,78,79], a nitroreductase-metronidazole-based cell ablation system [80], or light-controlled channels or receptors [81,82,83,84,85] can be directed to specific cell groups, such as *pet-1*-positive cells.

Furthermore, transgenic zebrafish lines have recently been developed to differentially express colored fluorescent proteins in neurons to examine their putative targeting of brain structures such as habenula subnuclei [86,87]. Similarly, zebrafish neurons have also been labeled in varying hues through via Brainbow technology, thereby allowing for the multicolor labeling and axonal tracing of the zebrafish sensory system [88,89]. Finally, bioinformatics-based tools are also becoming important for global analysis of pharmacological data, especially since the number of compounds tested in various zebrafish models continues to grow. For example, behavioral cluster analysis performed on the matrix of pharmacological data visualized the effects of the drugs, predicting behavioral outcomes of similar compounds [90,91,92]. We suggest that similar data-intensive approaches can be particularly useful in the search for novel serotonergic drugs affecting zebrafish behavior. For this, the recently developed Zebrafish Neurophenome Project (ZNP) database (http://kaluefflab.com/znpindex.html) [93] represents a useful searchable open-access data repository, specifically dedicated to neurobehavioral and related physiological phenotypes in zebrafish. This database allows researchers to compare the effective doses and relative potency of various serotonergic and other drugs previously tested in zebrafish, providing reference for designing pilot studies using this aquatic model species.

## Conclusions

The mounting evidence of the behavioral, genomic and endocrinological similarities between zebrafish and mammals strongly supports their translational value for neuroscience and biological psychiatry research. Importantly, zebrafish models allow researchers to further identify common conserved serotonergic pathways and biomarkers – especially important for drug screening and design applications. However, while zebrafish are sensitive to a wide array of traditional psychotropic drugs, advances in therapeutics increasingly relies on the identification of novel drug targets [94]. The considerable homology of zebrafish with mammalian gene expression patterns, neuroanatomical organization, and serotoninergic system regulation thereby provides significant strategic benefit in achieving this goal.

Additionally, the conserved serotonergic mediation shared between zebrafish and humans offers the potential to model several clinical pathologies, as well as the drug interactions that underlie various neurotoxic syndromes. Moreover, cross-species comparisons may provide a better understanding of the role of genomic and neuroendocrine mechanisms, as well as the differential engagement of corresponding neuronal pathways, in psychiatric disorders and their potential treatments.

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