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The Zebrafish Model in Neurological Research: Implications for Clinical Practice

Anubhav Dubey[1*], Mamta Kumari[2], Niladry Sekhar Ghosh[3], Richard Owusu Nyarko[4], Josephine Aba Sackey[5], Edward A. Boateng[5], Francis Asante[6], Vikram Kumar Sahu[7]

[1]Department of Pharmacology, Maharana Pratap College of Pharmacy, Kothi, Mandhana, Kanpur, Uttar Pradesh – 209217, India
[2]Department of Pharmacy, Harcourt Butler Technical University, Kanpur, Uttar Pradesh, India
[3]Faculty of Pharmaceutical Sciences, Assam down town University, Guwahati 781026, India
[4]Ghana Christian University College Accra, Ghana
5Department of Surgery, International Maritime Authority Hospital, Tema – Ghana
[6]Faculty of Health Sciences, Ghana Christian University College, Accra
[7]Department of Pharmaceutics, Maharana Pratap College of Pharmacy, Kothi, Mandhana, Kanpur, Uttar Pradesh – 209217, India

[*]**Corresponding Author:** anubhavdwivedi803@gmail.com

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ABSTRACT

Despite their striking similarities to humans in terms of genetics, anatomy, and physiology, zebrafish (*Danio rerio*) have emerged as a promising alternative model for studies involving the nervous system. Their well-defined neural system, high genetic similarity, and clear embryogenesis make them ideal for studying neurological problems in detail. Neurodevelopmental and neurodegenerative disorders, such as epilepsy, Parkinson's, and Alzheimer's, are best studied using these models because of their practical benefits, which include fast reproduction, minimal maintenance cost, and compatibility with high-throughput screening. They are genetically tractable, which enables accurate modeling of human neuropathology, and they have strong behavioral tests that provide measurable indicators of motor, cognitive, and emotional capabilities. The use of live imaging methods in real-time brain analysis has been greatly enhanced by recent advancements, such as calcium-based neuronal activity monitoring. This review discusses the constraints of translating zebrafish research to mammalian systems, illustrates the advantages of zebrafish as a neurobiological model, and highlights important research results. Taking everything into account, zebrafish serve as an excellent model for studying neurological processes and expediting the development of customized treatments.

INTRODUCTION

Since then, animal model systems have provided a technical way to conduct research that would otherwise be impossible with human participants. They are an essential component of research and offer invaluable information about human physiology and pathology. Furthermore, they enable the development of novel therapies

for human illnesses in addition to more sophisticated pharmacokinetic and pharmacodynamic research. Medical research has frequently employed mice and other animal models due to their well-known physiological and genetic resemblances to humans. Other animal models, however, are demonstrating clear benefits over these traditional models. As a result, this subject has seen a rise in attention in recent years **(Sarasamma *et al.*, 2018)**. Fish appear to be the most intriguing non-mammalian vertebrate model among the animal models that have been researched thus far due to their cheap maintenance expenses and the ability to produce offspring outside of the womb that enable *in vivo* imaging. For example, melanoma has already been effectively studied using Medaka (*Oryzias latipes*) and platy fish (*Xiphophorus*) as models **(Dubey *et al.*, 2022)**. In the early 1980s, Streisinger and associates initially used the zebrafish (*Danio rerio*) as a model for genetic research **(Dubey *et al.*, 2023)**. Small tropical vertebrates, zebrafish are used as models for both forward and reverse genetic research because of their inexpensive care costs. Zebrafish were initially employed as models in forward genetic research, which identifies a certain genotype by observing a particular trait. This scenario occurred in the instance of point mutations created by N-ethyl-N-nitrosurea (ENU)-driven mutagenesis, which was followed by a thorough phenotypic screening. However, this strategy was tedious and time-consuming for forward genetic screening **(Streisinger *et al.*, 1981)**. Thankfully, more sophisticated methods in recent years have made it possible to overcome the difficulties of utilizing zebrafish to create a number of illness models and to extend zebrafish research through reverse genetics, which is the study of the phenotype caused by a given genotype. Furthermore, research has shown that the genomes of zebrafish and humans are quite similar; it is estimated that 70% of human genes have at least one zebrafish orthologue. Given that humans and mice share over 80% of their genomes, these findings are astounding **(Grunwald *et al.*, 1992)**. Furthermore, zebrafish contain orthologues for more than 80% of known human disease genes, including oncogenes and tumour suppressor genes, and numerous pathways—including those linked to carcinogenesis—are also conserved **(Haffter *et al.*, 1996)**. In addition to highlighting their potential for *in vivo* screening for novel treatments—a crucial function in our age of personalized medicine—all these traits and supporting data have increased the use of zebrafish as a model for comprehending human genetic illnesses, including cancer. Therefore, the zebrafish offers a number of benefits and distinctive characteristics that mouse models do not, which explains its auxiliary and supplementary function in medical research, even if mouse models are still the most commonly utilized. Beyond their tiny size and inexpensive care costs, zebrafish have several other benefits when used as models. With the capacity to fertilize around 200–300 eggs every 5-7 days, they exhibit exceptional fecundity. Early physiological and/or pathological development can be observed by *in vivo* direct cell imaging due to the embryos' rapid *ex utero* growth and optical clarity **(Howe *et al.*, 2013)**. Furthermore, the casper zebrafish was developed as a genetic strain specifically designed to preserve

transparency into adulthood, which further reduced the cost of studying the behavior of cancer cells in a live creature **(Bootorabi *et al.*, 2017)**. Phenolic molecule screening in zebrafish may assist in addressing the restricted information provided by cell-based assays for the investigation of the absorption, distribution, metabolism, and toxicity of chemicals and medications. In zebrafish and primate models, similar physiological reactions have been noted when medicines and small compounds are used **(Hason *et al.*, 2019)**. Both the transparency of the embryos, which facilitates the collection of imaging data following treatment, and the high throughput tests, which are enabled by the female's capacity to lay a large number of eggs (about 10,000 eggs annually), are advantageous for drug screening. This implies that laboratory space is the only constraint on the simultaneous execution of imaging, cellular analysis, and sophisticated statistics in an extraordinarily high number of fish **(White *et al.*, 2008)**. These presumptions clarify how the zebrafish may serve as a link between biological validation of a particular drug and cell-based tests. Additionally, the identification of therapeutic targets for the treatment of human illnesses is still a major issue in medical science, and zebrafish may hold the key to the solution **(Dahm *et al.*, 2022)**. Even when the target is unclear, phenotype-guided drug trials may indicate a treatment's efficacy if phenotypic changes occur throughout the entire organism. This method aids in the simultaneous discovery of the molecular pathways underlying the illness that are causing that particular phenotype as well as the creation of novel medications **(Verkman *et al.*, 2006)**. Zebrafish research allows us to examine more phenotypes at lower manpower and financial costs than mice. There are a few disadvantages to this fish model that should be noted despite its great versatility in medical research, particularly in pharmaceutical research. First of all, because zebrafish are poikilothermic, they must be grown in conditions that are at least 28°C in order to thrive. Studies where temperature is a deciding factor are hampered by the fact that this threshold is different from the homeostatic temperature of mammals. However, zebrafish can tolerate a wide range of temperature fluctuations, from 6 to 38°C, for brief periods **(Veldman *et al.*, 2008)**. Second, the existence of genes in multiple copies (paralogs) is a consequence of teleost genome duplication, which might complicate molecular genetic research. Finally, the absence of antibodies specifically targeting zebrafish proteins, along with the technological challenges in producing antibodies against these targets, represents another significant drawback of using zebrafish in research **(Fernandez *et al.*, 2016)**. Since immunogenic glycans on zebrafish extracellular proteins prevent the elicitation of protein-specific antibodies in mammals employed to raise such antibodies, this is particularly important for cell surface and secreted proteins **(Spence *et al.*, 2022)**.

Comparing zebrafish and humans

Danio rerio shares 1,231 genes with humans, mice, and other species, and it possesses 70% of human genes, according to the zebrafish genome sequence. Therefore,

zebrafish embryos that are implanted with disease-causing genes from humans will inevitably have the same illness as the adult fish. For the purpose of assessing new medications and human ailments, the zebrafish model seems promising **(Force *et al.*, 1999)**.

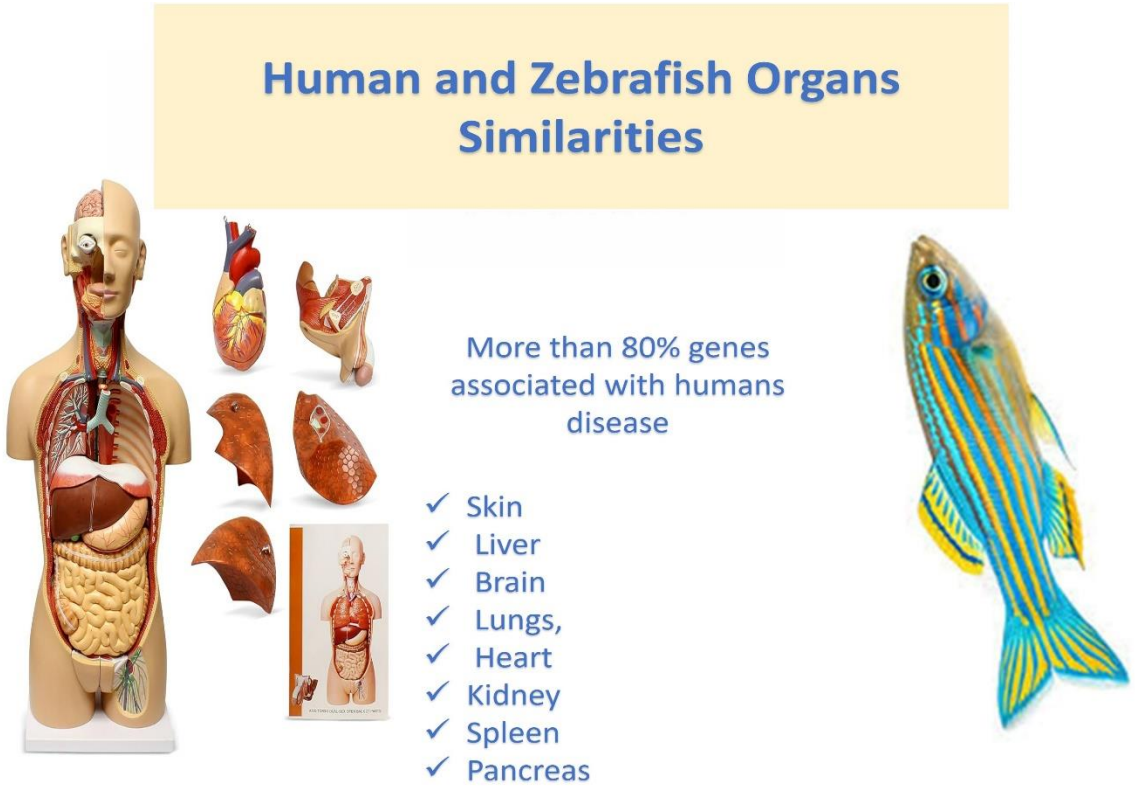

Fig. 1. The physiological systems of humans and zebrafish compared

Zebrafish classification

The zebrafish, scientifically known as Dactinopterygii, belongs to the Cypriniformes order and the Actinopterygii class of fish. The Cyprinidae family includes it, and the Chordata phylum includes it as well. The *Danio* is a species of animal that falls within the Animalia kingdom **(Taylor *et al*., 2003)**.

Depiction and structure

The zebrafish, or *Danio rerio*, is a tiny, fusiform fish with a compressed body shape and a length of three to four centimeters. In addition to a missing lateral line, an upward-pointing terminal mouth, and two blue melanophore and three lighter iridophore/xanthophore bands, these insects have five iridescent pigment stripes. Their

capacity to steer, stabilize, and shove is coordinated by their paired pectoral and pelvic fins, as well as their forked caudal fin (with around 16 rays), anal fin (with one spine), and one or more dorsal fins (with two or three spines) **(Staudt *et al.*, 2015)**. Camouflage and social signals are provided by the five alternating stripes of melanophore and iridophore/xanthophore, which modify the dispersion of pigment. The sensory system consists of a full lateral line of around 30 neuromasts, big color-vision eyes, and inner-ear otoliths that aid in balancing. They change from pronephros to mesonephros in their kidneys, have a two-chambered heart, and possess a swim bladder to help them stay afloat. Males are easily identifiable by their brilliant yellow anal fins, while pregnant females are more easily bred due to their rounded abdomens and genital papillae **(Traver *et al.*, 2003)**.

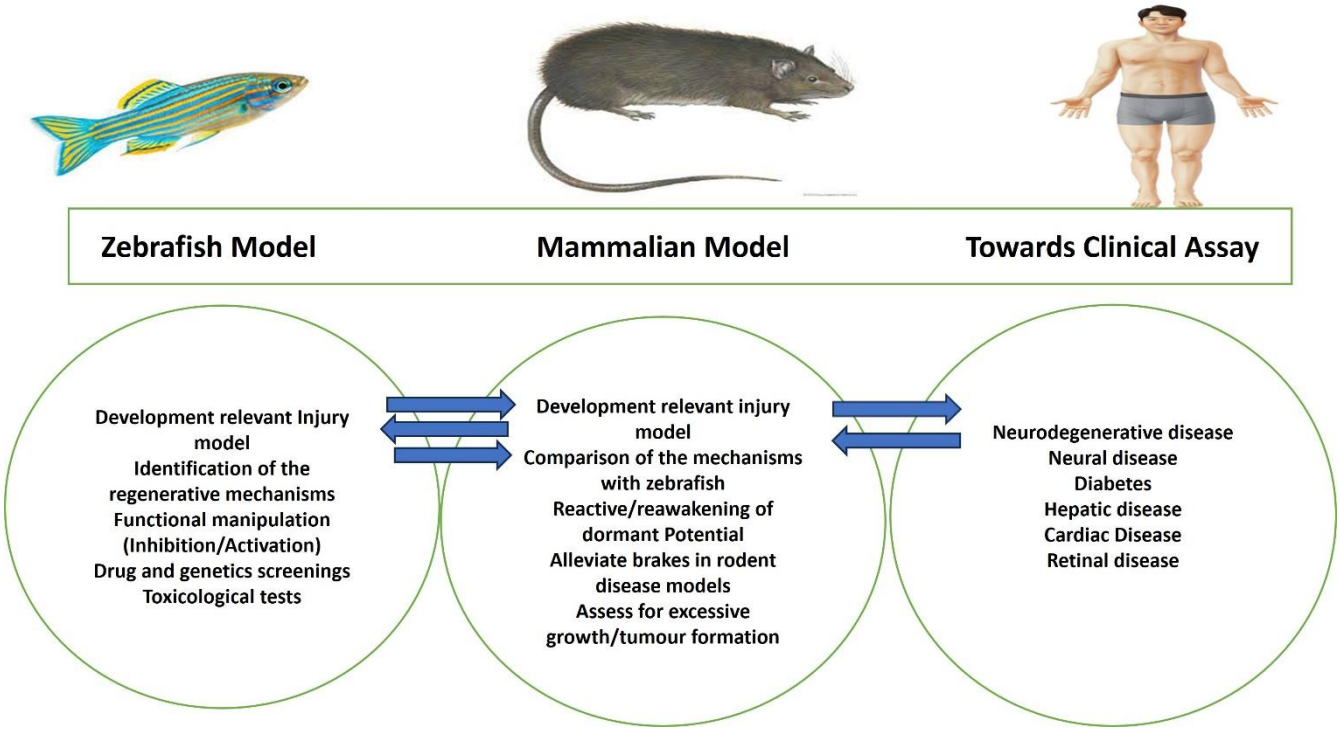

Fig. 2. Disease in rats and zebrafish: A comparative analysis

Zebrafish use as an animal

Using the best animal model, like the squid, for studying nerve impulse transmission is a basic scientific premise in biological research. We need an animal model in order to investigate the workings of the human brain. Various scenarios are analyzed to show the different models used and how the effectiveness of their solutions is dependent on how well they handle the given difficulties. The link between heredity and human illness in fruit flies was strongly proved by Thomas Hunt Morgan's discovery that genes are carried on chromosomes. Subsequent research found that several other animals

maintained or preserved over half of the disease-related genes found in humans **(Fischer *et al.*, 2014)**. A well-known example of a hereditary disorder with therapeutic promise is Friedreich's ataxia, which often results in early death and makes affected individuals incapable of functioning. Since the condition is caused by a conserved faulty gene (FXN), fruit flies having the gene and under control may be utilized for fundamental research. Fruit flies can have their genetic defects fixed, and testing on humans is a practical substitute. Since both mice and humans are mammals, their brains are much more similar in structure and function than fruit flies' and flies'. The fact that a genetic mutation in mice may induce an illness that is comparable to one in humans has led to its usage in several contexts. Several neurological diseases have benefited from this approach. An example of an autistic spectrum condition that mostly impacts females is Rett syndrome. According to what Huda Zoghbi found in 1999, the main reason is a mutation in a particular gene called MECP2. Mice lacking this gene have neurological symptoms similar to Rett syndrome. Returning the missing gene will reverse the neurological disease **(Henrikson *et al.*, 1967)**. These examples show how simple it is to breed mice with a particular genetic mutation, which aids in the hunt for genetic or non-traditional remedies for human diseases. Thanks to its capacity to influence genetic processes in mice, it has also become considerably more successful in understanding how the brain functions. Most notably, the human brain is structurally and functionally comparable to the monkey brain. More and more, pigs are being used as the experimental animal of choice for transgenic brain gene alterations. This research examines the feasibility of inducing neuropsychiatric diseases in pigs by simulating human brain sickness symptoms, events, or structures. We talk about the serious *in vivo* brain investigations that use pigs and the moral and practical problems with them. The review of the current level of knowledge on behavioral processes, including learning and memory, concludes the overview of pigs' appropriateness as an experimental model for a range of human brain diseases. The virtually total elimination of polio is one scientific accomplishment that exemplifies the relevance of these similarities between monkeys and humans. Paralysed victims of the polio virus often need ventilators and iron lungs. Additionally, it impacted regular life by postponing events and banning swimming pools for a number of summers around the globe. Before anybody else, in 1910, Karl Landsteiner and Erwin Popper had found the poliovirus. The fact that the virus was detected in monkeys but not in rabbits, guinea pigs, or mice suggests that the virus may be transmitted between primates and humans. An experimental immunisation was administered to humans in 1935, 25 years after some modest studies were conducted on animals. Although it was not generally the case, immunisation was unquestionably prompted by some polio cases. The problem arose from people not fully comprehending the vaccination and the virus. In 1955, after twenty years of expanded knowledge and the promising results of Jonas Salk's vaccine, the following trials were conducted. The majority of the world's epidemic has been contained. Despite monkeys being the most successful animal model for the disease,

it took over fifty years to acquire basic understanding about the virus and the vaccine. It wasn't until 1935, twenty-five years after a few pilot tests on animals, that vaccination was tried on humans. Although vaccination did not occur in the majority of polio infections, it did occur in a small number of instances. The issue arose from misunderstandings about the vaccine and the infectious disease. Twenty years after Jonas Salk's vaccine showed promise and knowledge had developed, the following immunisation studies were carried out in 1955. Much of a global pandemic has been eradicated. It took almost half a century, but fundamental information about the virus and the vaccine finally emerged when it was decided that monkeys were the best animal models for the sickness. The benefits of using the monkey model to learn the fundamentals of human disease cure are covered in this Colloquium's topics. The degeneration of neural connections in the brain is a major contributor to several of these diseases **(Kim *et al.*, 2017)**. There are studies that have already been conducted on humans and others that demonstrate how research will lead to therapies for human problems. As an alternative to trying to provide a comprehensive explanation, we will focus on a few prominent cases that have resulted in the treatment of brain illnesses, are now undergoing human trials, or are being created for future testing. Also covered are the ramifications of limiting basic research to monkeys and how much is required to understand how the brain works before considering solutions to problems affecting the human brain. The following is a synopsis of the five main types of research that we use. Brains and behavior of humans, monkeys, and mice are compared in the first. Presented before each of the four sections on brain diseases is the perspective of a physician. Throughout these research articles, macaque or marmoset monkeys are used; the terms "monkeys" and "nonhuman primates" are used interchangeably **(Saleem *et al.*, 2021)**.

Intelligence and conduct

Part one delves into the topic of studying human brain diseases using rodents like mice and monkeys. All of the mammals discussed here have brains that are quite diverse from one another. This part differentiates between the four species—mice, macaque monkeys, marmoset monkeys, and humans—discussed throughout the colloquium. Anatomical regions and their connections in the cerebral cortex, the top layer of the brain, vary greatly in quantity and complexity. The brain's fundamental cortical areas serve as a foundation upon which more complex cognitive processes are constructed. According to ***Van Essen et al*. (2022)**, there are a total of 180 zones in a human brain, compared to 117 in a marmoset, 43 in a mouse, and 161 in a macaque monkey's brain. Variations in brain size and behavior among animals are reflected in an increase in the number of cortical areas. In addition, mice lack the anatomical commonalities between these areas that are present in the three monkey species. Animals' unique transmitters, or substances that nerve cells employ for communication, are the focus of Disney and Robert's research

(Singhal *et al.*, 2023). Unlike in mice, they found that a transmitter may not target the same kind of cortical neurons in macaque monkeys. Medication effects on the brain are often mediated via neurotransmitter systems. Thus, it's possible that primates, including humans, won't respond to a medicine that was originally designed for mice, monkeys, or any other species with comparable physiology. The primary area of interest for Luo and Maunsell is the intricate behavioral role of attention in various brain diseases. Their analysis of monkey attention experiments led them to the conclusion that, contrary to popular belief, attention is not a single system but rather a collection of separate brain processes that overlap and involve several circuits **(Choi *et al.*, 2021)**.

The use of zebrafish as a neurological model

Different brain disorders

There are a wide variety of disorders that can affect the brain, including but not limited to Alzheimer's disease, epilepsy, traumatic brain injury, stroke, brain tumours, dementia, encephalitis, neurodevelopmental disorder, migraine, MS, bipolar disorder, intracranial aneurysm, autoimmune disease, cerebral palsy, cerebral hypoxia, Parkinson's disease, antiphospholipid syndrome, anxiety, autism, and many more. The vast majority of brain researchers believe that each human brain has about 100 billion neurones linked by around 100 trillion (1014) synapses **(Saputra *et al.*, 2025)**. The functional interactions between these neurones span several time scales, and their physical distribution varies greatly across different geographical sizes. For this reason, studying the brain and the neural bases of its functions has been a formidable scientific challenge. Clusters of neurones, neural circuits within a function block, or a collection of connections between brain regions may influence brain functions, as we now know, in addition to a single neurone or a single brain region acting independently **(Dang *et al.*, 2025)**. There are few diseases as devastating and incurable as Alzheimer's disease (AD), schizophrenia (SCZ), and Parkinson's disease (PD). Thus, it should come as no surprise that understanding brain network connection is one of neuroscience's long-standing aims. This long-standing goal has lately spurred an unprecedented surge of large-scale initiatives and collaborative projects, such as the China Brain Project and the Human Brain Project. *In vivo* brain illnesses may be studied for structural and functional abnormalities using neuroimaging methods, which can be used by both scientists and physicians **(Rayfield *et al.*, 2025)**.

Mental illness

Brain and neurological diseases are among the most serious global health problems today. These disorders impact people's ability to move, think, and feel. Worldwide, 50 million individuals suffer from epilepsy and 100 million from Parkinson's

disease; stroke is a leading cause of disability and mortality in this population. The emotional and physical toll that this disease takes on patients and their loved ones is immense. For example, dyskinesia, tremor, stiffness of the muscles, and imbalance are symptoms of Parkinson's disease, a neurodegenerative disorder that develops gradually over time. Reduced mobility due to these symptoms may have a significant influence on patients' quality of life and day-to-day functioning. The neurological disorder epilepsy may cause recurrent seizures. Twitching muscles in the limbs, changes in perception and thought, and loss of consciousness are all signs of these seizures. The unpredictability and potential danger of seizures may have a negative impact on patients' daily life and social activities. In most cases, a blocked or burst artery is the immediate cause of a stroke, a sudden cerebrovascular disease. Mortality, hypoxia, brain damage, and, in the worst-case scenario, paralysis, a loss of language and cognitive abilities, and death may all result from a stroke. Brain and nervous system illnesses not only cause obvious physical symptoms, but they also have a significant influence on patients' mental health. Anxiety, despair, and cognitive decline are among the mental health problems that patients may encounter, along with a decline in their social skills and overall quality of life **(Rock *et al.*, 2022)**. For many decades, zebrafish have been used as an experimental fish. The major emphasis at first was on developmental biology because of the zebrafish's apparent advantages, such as its big clutch size, transparent embryos, and in utero embryonic growth. Cancer was one of numerous adult zebrafish disorders discovered by scientists when rearing the fish in a controlled environment **(Sutkus *et al.*, 2025)**. Neurological illness studies on the latter revealed that zebrafish are capable of developing almost any kind of cancer on their own. The ultimobranchial gland, peripheral nerve, thyroid, liver, stomach, and testis are the most common tissues to be targeted by spontaneous neoplasia. It is less common to target tissues such as blood arteries, brain, gills, nasal epithelium, and the lymph myeloid system. Research on zebrafish brain disorders and an overview of the species' benefits and drawbacks are included in these reviews. Zebrafish, or *Danio rerio*, are valuable model organisms for studying physiological function and have many other applications outside just "fishy" topics **(Wang *et al.*, 2022)**. Zebrafish are often used in developmental biology due to their established scientific importance. Its potential as a model for research into hereditary and congenital diseases in humans is starting to emerge. Researchers interested in the functioning of whole species or organs have not found this model organism to be as beneficial. When applied to the complex integration problems that would concern an organ physiologist or regulatory physiologist it showed great potential **(Gaspary *et al.*, 2018)**.

The sensory neuronal network

Stuffer, alligator, and macho are the three known mutant lines that exhibit either a completely absent or significantly reduced withdrawal reaction to touch. Ribera and Nucleon-Volhard investigated the electrical membrane properties of mutant and wild-

type embryos using whole-cell recordings from mechanosensory Rohan Beard neurones in semi-intact preparations of 2- to 4-day-old embryos. Scientists have connected a spike in activity in these neurones to the beginning of the behavioral response. The touch-insensitive mutant fish exhibited reduced sodium current amplitudes and fewer or no overshoots in action potentials. In contrast to macho mutants, whose action potentials and sodium currents remained low, alligator and stuffer mutants had comparable but less pronounced effects. Only sodium channel activity was affected; resting membrane potential was unaffected. As an embryo of a wild-type fish went from being touch-insensitive to touch-sensitive, mechanosensory neurones in the embryo generated sodium current. Sodium current and action potential overshoot did not exhibit any indications of developmental anomalies in males, whereas action potential length was within normal limits. In order for the behavioral reaction to touch to become noticeable, the researchers found that voltage-dependent sodium currents have to increase. Though they have not been found yet, the molecular processes driving the formation of sodium channels in the membrane should be better understood if the gene abnormalities responsible for these three mutants are uncovered. Approximately 40% of the zebrafish that were studied survived the hypoxic treatment and resumed their normal swimming abilities. As they made their way back to normal swimming patterns after hypoxia therapy, the surviving zebrafish kept behaving abnormally, including circling and spinning. These results suggest that the model we outline below could serve as a substitute for other models for evaluating hypoxia-induced brain injury **(Nakajima *et al.*, 2021)**. Researchers have utilized fish in the past to study oxygen depletion. Environmental hypoxia and anoxia, which kill fish all around the globe, have been the focus of a number of studies. But no study has shown that fish or zebrafish can mimic hypoxic ischaemic brain injury, even though zebrafish are often employed as animal models for a wide variety of biological studies, from embryonic to behavioral. But no study has shown that fish or zebrafish can mimic hypoxic-ischaemic brain injury, even though zebrafish are often employed as animal models for a wide variety of biological studies, from embryonic to behavioural. Our findings that 60% of the zebrafish perished and 40% survived following hypoxia treatment provide credence to the study's goals and indicate that this animal model system is receptive for studying hypoxic-ischaemic brain injury and potential therapeutic treatments. We used the following hypoxia treatment endpoint in this study: zebrafish maintained a stationary position with periodic opercular movements for 1 minute. Since zebrafish were able to live without brain damage when exposed to little hypoxia or for too long in the hypoxia chamber and died when exposed to too much therapy, determining an endpoint may be difficult **(Stewart *et al.*, 2015)**.

The zebrafish has many benefits in scientific research

As a model organism for investigating neurological diseases, zebrafish provide several unique benefits. Their short care requirements and short life cycle make them perfect for *in vivo* evaluations of gene function and medication effectiveness, allowing for

large-scale genetic screenings and high-throughput drug screening. Researchers are able to draw significant similarities in neurobiology between zebrafish and mammals due to the conservation of brain growth and function. Zebrafish also have complicated behaviors that can be measured statistically, which helps researchers understand the processes and neuroactive chemicals that cause diseases like autism and epilepsy. The modeling of eye movement problems is made possible by their well-characterized oculomotor system, which further expands their relevance in neurological research. In sum, zebrafish are an incredibly useful model organism for studying the genetic and molecular underpinnings of a wide range of neurological disorders. There are fewer non-mammalian models that resemble humans than this one. *D. melanogaster* and *C. elegans* are two examples. Although zebrafish and human brains are significantly different in size and structure, they have a similar organization. Zebrafish brain regions are remarkably well preserved and may even be linked, in contrast to human brain regions. It is believed, for example, that the human striatum is similar to the zebrafish ventral telencephalon. Similarities between humans and zebrafish extend to their personalities and habits as well. For example, if neurotoxins are administered to zebrafish, they may display abnormalities in their movement that might be misinterpreted for symptoms of Parkinson's disease, such as swimming delays and altered patterns. Research that sequenced the whole human genome also discovered that zebrafish and humans share 70% of the same genetic material. It seems that synteny has been maintained across the two species, since 80% of the genes are found on the same chromosome and in the same order. The zebrafish model's genetics are well-known, and researchers have looked at transgenic versions extensively. Zebrafish embryos are a one-of-a-kind model for studying the embryonic process in real time because of their transparency, rapid absorption of certain chemicals or neurotoxins, and ability to develop outside. Zebrafish outperform other animal models due to their rapid reproduction and short life cycle. Zebrafish have an egg-laying capacity of 200–300 each week. Because a larger sample size allows for more significant findings in each experiment, this is tremendously helpful to this species. Aside from that, zebrafish typically live for three to four years and reach sexual maturity in about the same amount of time. When test design is considered throughout development, a short life cycle may help reduce testing time and money. The methods used to breed and care for zebrafish are more complicated and easier than those used with rodents and other animals. Fish are highly prized as vertebrate model organisms due to their exceptional embryology and genetic manipulation capabilities. There are a lot of reasons why zebrafish embryos are good models for understanding development. The development of the embryo is shown by the chorion, and it is rather large. Embryos are see-through during the first twenty-four hours of their development, when their developing organs are most easily discernible. The embryo also grows rapidly. The typical features of the vertebrate body, including the split brain, become apparent after about two days. the eyes, the hearing, and every organ inside the body. Intestines of larger animals are much larger than those of zebrafish. In

contrast to the tens of thousands of glomeruli seen in human kidneys, a single glomerulus runs the whole length of a larval zebrafish's kidneys. Zebrafish have their life cycle in five days, during which they hatch, swim, feed, and share. Production takes a very lengthy time—two to four months—when compared to other vertebrates. There has been a dramatic increase in the number of tools and methods developed for Serrates in recent years because of the abundance of progenies that allow genetic research **(Fontana *et al.*, 2022)**. One example is the mapping of guinea pigs as a model organism, which is made possible by collecting mutant or recombinant individuals. Everyone is invited to join the exam. The advantageous physiological traits, testing convenience, and depth of information about zebrafish are the reasons for its present and increasing application in human illness research. Protecting zebrafish farms from rats should not cost too much either. The genomes of zebrafish and humans are structurally and physically more similar because they are homologous. Zebrafish and humans have a size-matched phylogeny that diverged around 450 million years ago. In addition to sharing many common expression patterns with humans, zebrafish have orthologs of most human genes. The sequence of amino acid residues is shared by 70% of orthologous proteins. Notably, several zebrafish genes are duplicated, indicating a high degree of gene duplication in the zebrafish lineage. Two zebrafish orthologs may share the same gene's activities (a phenomenon called "sub functionalization"; as indicated by **Lynch and Force (2000)**. It may be more prudent to develop a controlled and study able strain of the illness in zebrafish if one of the two genes fails to function, rather than in humans. To suppress the activation of many genes at once, antisense morpholino oligonucleotides may be injected into zebrafish embryos if needed. Researchers may examine control biological systems objectively, without their own preconceived notions about how a process might work, by doing large-scale random mutagenesis screening on zebrafish **(Garg *et al.*, 2024)**. To study the molecular mechanisms behind hereditary diseases in humans, zebrafish models may be used to search for modifier mutations that either reduce or increase the disease phenotype. Even while the method for manipulating zebrafish genes is less involved than the one for mice, it is nevertheless quite comprehensive. Although targeted mutagenesis ("knock-out") and gene replacement ("knock-in") are not possible via homologous recombination, chemical mutagens like N-ethyl-N-nitrosourea (ENU) may induce very high rates of random mutagenesis. Heterozygosity in the offspring of fish exposed to ENU allows for the detection of mutations in any particular gene. Homozygosity may result from single-mutation cases. Nowadays, zebrafish are used in TILLING, which involves removing an area created from a specific location in the genome. Proteins that can attach to certain genes and cause DNA damage have recently emerged as a means of causing small deletions in those genes. For genomic changes, there has been a lot of work on developing transgenic technology for zebrafish. Sleeping beauty transgenes have dual use: as vectors for transfer and as vectors for input. Injecting endonuclease I-SceI-treated DNA or DNA with empty fragments into fertilization products is another way to modify

transcripts. Gene expression may be activated by heat shock stimulation **(Sun *et al.*, 2024)**.

The zebrafish model: Drawbacks and benefits

As a model for future manipulations, zebrafish provide various benefits. While these embryos and fish are useful for many types of biological study, there are a few that pose challenges. Being non-mammalian is a major drawback of using zebrafish in studies involving humans (such as disease models). However, because zebrafish are thermogenic, they can produce embryos even in situations where space is limited. As a result, the metabolism and potential efficacy of certain drugs may vary when compared to mammals. Exposure of zebrafish embryos to drugs during their development results in direct absorption, regardless of the foetus's or mother's sex. As an example, tricaine methane sulfonate is ineffective in humans but valuable for zebrafish due to its fast breakdown by their homoeothermic metabolism. The genes that determine a zebrafish's gender are not present in humans. Nevertheless, such evidence does not rule out the possibility that oestrogen and other hormones that influence sex also impact zebrafish growth. The effects of environmental levels of steroid hormone analogues on catastrophic development may be studied in zebrafish, which might be useful for environmental toxicity research **(Reichmann *et al.*, 2022)**

Fig. 3. Differences between male zebrafish and females zebrafish. Males have a more stream-lined body with darker blue strips while the females have a white protruding belly.

Clinical trials

Uncontrollable shaking

In the first stage, there is no change in swimming behavior or anything else. The first stage of a zebrafish larva's development is characterized by an increase in swimming activity. In Stage II, the fish swims around the well and delicately brushes up against its edge. This is characterized by quick, swirling well sweeps. Stage III: The fish begins to roll over onto its side as a result of the fast extension and flexion of its head and tail, which causes it to lose its natural upright horizontal posture. Transitions between the two ends of the fish that last between one and three seconds are referred to as stage III type convulsions.

As an epilepsy model, zebrafish provide many advantages **(Du *et al.*, 2025)**. Among the many human-like neurological traits shared by zebrafish are homologues for 85% of the epilepsy genes. When it comes to genetic tinkering, zebrafish are quite accommodating. The developing eggs of zebrafish quickly absorb medications. Zebrafish are perfect for the screening of genes that might increase or decrease vulnerability to seizures since they are easily multiplied in high numbers. Within three to four months of fertilization, zebrafish reach sexual maturity and may produce an egg clutch of fifty to two hundred. This short presentation introduces the neuroscience community to the possibilities of studying epilepsy in zebrafish. So far, the most noteworthy findings include creating a model of acute seizures in immature zebrafish, conducting the first comprehensive screening of zebrafish for characteristics relevant to epilepsy, and identifying a gene (tenascin-m3) that may be associated with seizure definitions **(Prakash *et al.*, 2025)**. Patients suffering with palatogenesis may find relief in the future if gene therapy makes it possible to either target these genes or alter their function. Future research could utilize zebrafish's unique features to investigate the nervous system circuitry responsible for seizure-like activity in vertebrates, create zebrafish knockouts with human epilepsy syndrome-causing single-gene variants, or use mutagenesis screens to identify additional epilepsy. But we think that new insights into epilepsy will emerge from the more sophisticated and contemporary applications of this basic vertebrate. There are, of course, caveats to the offered study. Research centres that use zebrafish models to investigate epilepsy may expand in the future. In order to determine the genetics of epilepsy and its therapy, zebrafish are very useful in high-throughput genetic or pharmacological screening. However, it is not even close to everything in this tiny, see-through vertebrate **(Kumari *et al.*, 2025)**. This article explains, however, that zebrafish research has increased significantly within the previous twenty years. The advent of modern imaging methods has opened up formerly unimaginable fields of study, such as gene expression, organon cerebral vascularization, and behavioral investigations in live animals. More and more is being learnt about the anatomy, physiology, and disease dynamics of the zebrafish brain in relation to those of humans. The remarkable ability of

zebrafish to regenerate their brains after damage is only one example of how a better understanding of these differences might influence other vital areas of study. We think that zebrafish have recently shown promise as a model for epilepsy research, thanks to a number of studies. All vertebrates, including humans, probably share the neurological and developmental pathways that cause epileptic seizures, as similar systems in zebrafish and rodent models further support this theory **(Dubey *et al.*, 2023)**.

Dementia

ApoE is an essential protein that binds to certain receptors to help lipoprotein absorption. Due to its relationship with sporadic Alzheimer's disease, apoe ε4, a substantial risk factor for the condition, is connected to the illness's development. Although the two proteins share 28% of their amino acid sequences, it is feasible to clone the zebrafish ApoE cDNA by loading a part of zebrafish ApoE with basic amino acids to match the lipoprotein receptor binding region of human ApoE. Overall, whole-mount *in situ* hybridization shows that ApoE is highly expressed in the deep cell layer during the blastula stage and later in the brain and eyes, namely in the rhombencephalon, telencephalon, and mesencephalon. Further research is needed to determine the significance of ApoE in the development and maturation of zebrafish, but the fact that it is expressed in the yolk syncytial layer implies that it is essential for their nourishment. In order to learn how the zebrafish apoe works physiologically, we may either knock it down or take it out. By comparing gain-of-function and loss-of-function effects when the apoe ε4 allele is introduced into embryos, this detrimental mutation may be studied. When it comes to vertebrates, the zebrafish is among the select few that permit extensive forward genetic screening. This technique has been used to detect mutants deficient in γ-secretase substrates. A transparent zebrafish is a great tool for studying vertebrates because it allows us to see the spatial and temporal properties of individual cells. It is possible to mimic the knockout process in mice by inducing genetic lesions in specific genes using zinc-finger nuclease-based methods and TILLING. Thus, by combining forward and reverse genetics, we can reproduce disease-related mutations and their symptoms. Zebrafish are the most cost-effective vertebrates for drug screening once the model is available, thanks to their short generation time and high fertility. The newly created tau transgenic zebrafish enables real-time monitoring of harmful changes *in vivo*. The transgenic vector can express additional proteins, such as aβpp, and cause zebrafish to develop amyloid-like disease. However, zebrafish are not often used in chemical testing because of their unusual design, which provides different routes for drug absorption than humans do because of their differences from mammals. Further research is needed to understand how zebrafish distribute, metabolise, and excrete medicines. Investigating the role of zebrafish cytochrome P450 homologues is one part of this process. Unlike the wealth of information regarding zebrafish growth that has been accumulated over the last 30 years, the field of drug screening is relatively new. However, zebrafish uses have spread quickly in transdisciplinary research, including

anything from chemical tests to fundamental genetic studies. The creation of several transgenic zebrafish illness models has raised hopes that the zebrafish system may soon become an important tool in the drug discovery process. The brains of zebrafish and humans are structurally quite similar, according to research (They posses 38 neuroanatomical and 42 neurochemical pathways which are quite similar to humans). The dorsal, medial, and lateral pallium of zebrafish are similar to those of other species' exocortex, hippocampus, and amygdala, respectively. A zebrafish's encephalon is the result of the merging of the diencephalon, telencephalon, and cerebellum. The zebrafish brain has many similarities to the human brain, including muscarinic cholinergic receptors **(Schuttler *et al.*, 2017)**, and the main excitatory glutamatergic and inhibitory GABAergic neurotransmitter circuits. Glutamate, acetylcholine, serotonin, dopamine, histamine, GABA, and serotonin are all neurotransmitters, and these enzymes are essential for their production and breakdown. Comparing human cells to other cell types reveals similarities between them and astrocytes, microglia, oligodendrocytes, myelin, cerebellar Purkinje cells, and motor neurones. According to previous studies on neuronal patterns in the adult spinal cord, the degree to which zebrafish resemble higher-order animals is determined by neural differentiation and the formation of spinal networks **(Knap *et al.*, 2023)**. Researchers have explored non-associative learning in zebrafish larvae by observing their behavioral and cognitive responses to advanced development (AD). Larvae showed a dramatic reduction in startle reactivity when exposed to several acoustic stimuli seven days post-fertilisation. Another group injected aβ1-42 into the hindbrain ventricle of zebrafish embryos 24 hours after fertilization (hpf). Embryos with increased tau phosphorylation in certain gsk-3β locations in the 5 dpf larvae exhibited significant cognitive deficits. Neurobehavioral abnormalities, including changes in photometer responsiveness, tail flexion frequency, and tail apoptosis, were seen in zebrafish embryos exposed to trimethylating chloride (TMT). A new pharmacologic model of Alzheimer's disease was recently created with the inclusion of okadaic acid. The presence of okadaic acid has been shown to increase both tau phosphorylation and aβ plaques. It would suggest that these fish also suffer from memory and learning issues. Zebrafish exhibited Alzheimer's disease-like traits after being exposed to aluminium in an acidic environment; this included reduced learning capacity and poor locomotor activity. Furthermore, it has been shown that administering aβ1-42 intraventricularly to a developing brain leads to an increase in tau phosphorylation, memory loss, and cognitive impairments. This is why zebrafish are becoming more and more popular as a model for studies in neuropharmacology. The zebrafish model for the discovery of neurospecificity medications has been the subject of several efforts to use the embryonic and larval visual acuity of zebrafish to identify potential therapeutic compounds. This model system is ideal for high-throughput drug discovery due to the tiny size of the embryo and larvae. Almost all zebrafish drug discovery trials focus on systems other than the nervous system; the vast majority of these studies focus on other systems. A wide variety of

psychiatric drugs have also been studied in zebrafish. Researchers have proven that both chemicals impact the development of the nervous system. However, zebrafish have revealed that cocaine, amphetamines, morphine, ethanol, and nicotine promote behavior associated with rewards or fear. Interestingly, zebrafish learning paradigms have shown that nicotine enhances cognition. A group of scientists recently found prostaglandin E2, a small chemical that regulates the balance of haematopoietic stem cells. Studies on zebrafish have also looked at tiny drugs that might alter the embryonic heartbeatby inhibiting mutations, help with cardiovascular problems, and prevent cell cycle stoppage. Most crucially, studies have shown that these medications have comparable effects in both people and zebrafish, lending credence to the notion that zebrafish models for drug discovery might potentially generate or uncover therapeutic molecules that are well-suited to human problems. Research on the effects of several neuroactive medications on adult zebrafish has also demonstrated that these chemicals alter the swimming and diving behaviours of the fish when introduced to their aquarium water. Several drugs have been studied in zebrafish, including piracetam, heroin (diacetylmorphine), kynurenic acid, methylenedioxy methamphetamine, a nitrosamine explosive called 1,3,5-trinitroperhydro-1,3,5-triazine, and hallucinogenic compounds such as mescaline and phencyclidine. Another low-cost method for finding minute neuroactive compounds is rapid behavior-based screening **(Quin *et al.*, 2028)**.

Brain attack

` Particularly in the elderly, brain amyloid angiopathy may induce lobar intracranial haemorrhage, which can be deadly. The diagnosis is established histologically when amyloid peptide builds up around brain arteries, leading to fibrinoid necrosis, microaneurysms, and vascular rupture. The precise biology of the illness is unclear, and there are no known therapeutic therapies that alter the disease's natural progression. Endothelial dysfunction due to beta amyloid buildup causes early senescence, according to one of the primary theories for cerebral amyloid angiopathy-related haemorrhage. Recently, researchers have begun using it to learn more about the disease's mechanics. These studies assessed the expression of p21, an inhibitor of cyclin-dependent kinase, and beta-galactosidase activity in whole-mount zebrafish embryos after beta amyloid peptide was diffused into the water using in situ hybridisation. According to these findings, microvascular architecture and function undergo a gradual change as a result of a connection between amyloid accumulation and endothelium ageing. The significance of the zebrafish model for investigating this illness is emphasized most strongly **(Dubey *et al.*, 2023)**. How injecting beta amyloid affects the endothelium is an area that needs more investigation. Screening tiny molecules for potential novel treatment targets in brain amyloid angiopathy, Alzheimer's disease, and other diseases marked by amyloid buildup may be useful, as minute peptides may penetrate zebrafish. Research on ischaemic and haemorrhagic stroke has made use of zebrafish as a model animal. Their usefulness as a

high-throughput screening tool for studying gene function and creating new medications has been shown. We should expect to learn more and develop novel treatments for cerebrovascular disease in humans by studying zebrafish, as many biological pathways of illness are shared throughout vertebrates. We reviewed the most current papers to determine whether zebrafish models had been used in stroke studies. Using the terms "zebrafish model" and "ischaemic stroke" or "haemorrhagic stroke" in an NCBI search turned up original research publications from every period. However, for the sake of this analysis, we limited ourselves to looking at the initial report of each model's use. The models used to produce the results did not specifically address stroke pathophysiology. Hypoxia is the leading cause of ischaemia in zebrafish, whether they are adults or larvae. Submersion in an oxygen-depleted environment may cause brain ischaemia in some animals. According to **Yu *et al.* (2021)**, 60% of adult rats that were placed in a hypoxic chamber for 5–6 minutes perished; those who managed to live were severely affected by bilateral cerebral ischaemia. Although the animals' unexpected eye movements and swimming patterns might be used as a broad indicator of severity, there was no correlation between behavioral results and lesion size. Additionally, no studies have examined the effects of prolonged hypoxia on other organs in the body. In order to further illustrate the notion, Braga et al. reached the third stage of hypoxia by deliberately introducing dissolved oxygen at a concentration of 1.5-7 mg $l^{-1}$for an unspecified duration. The scientists did find that more than 75% of the fatalities happened within 48 hours, which might be due to the zebrafish's inherent regenerative abilities; however, no more research was undertaken to confirm this. To create a hypoxic environment, **Matsumoto *et al*. (2020)** planted larvae (at 4 days post-fertilisation) in an oxygen absorber for 2 hours with 3.0 mg l^{-1} dissolved oxygen. Despite decreased cerebral blood flow and global oedema, 10% of rats managed to survive the hypoxic chamber. Unfortunately, this group was not adequately addressed by the researchers. Possible methods for preventing ischaemic strokes in animals include glucosamine, free radical scavengers, and enhanced autophagy in the brain. Because of the significant mortality rate and the fact that this model of global ischaemia does not represent reality, more research in rodent models is necessary to confirm these results **(Dubey *et al.*, 2023)**. Research on chemically produced hypoxia using $CoCl_2$ has also been conducted. This method is more reliable and efficient than producing hypoxia in a low-oxygen environment, and it may be delivered to larval stages. It was shown that hypoxia has far-reaching effects on the growing baby, including pericardial oedema, spinal deformities, and brain abnormalities. Thrombosis and embolism, which are prevalent in clinical settings, have different causes, but locomotor impairment and hypoxia-induced neuronal death both indicate an ischaemic event, which is undesirable in an ischaemic stroke model because it could impact the stroke's severity and the rate of recovery. Utilizing transgenic reporter lines and live imaging of cranial arteries, blood flow, and neurones, a newly published model explored cerebral ischaemia-reperfusion in the context of brain

injury utilizing the global hypoxia approach. A more accurate model for localized ischaemia occurrences has been developed using the photosensitive dye rose bengal. While these global hypoxia models may present some challenges in terms of specificity for drug research, they are frequently useful for validating results from a more specialized rodent model for effectiveness in another species. Thrombosis occurred in the adult fish after an intraperitoneal injection of rose bengal caused coagulation and damage to brain endothelial cells due to transcranial lighting of the brain. Additionally, proof-of-concept evidence of rt-PA's potential to reverse thrombosis has been shown. Though it takes more time to use than immersion in a hypoxic chamber, the photothrombotic model is useful for study because it may cause brain-specific injury. Drug screening problems in adult zebrafish models are comparable to those in rat models **(Crilly *et al.*, 2022)**. To establish the severity of a stroke in adult fish, it is necessary to remove the brain and measure the extent of the lesion. However, this method has not been implemented yet, and the adoption of a genetically designed, transparent adult strain would be ideal. Modern magnetic resonance imaging (MRI) techniques have also been utilized to capture images of adult zebrafish; however, this technique needs a specific setup to allow anaesthetic and water to pass through the fish's gills. Chemical hypoxia and the hypoxic chamber paradigm both raised mortality rates in adults, and a consistent stroke size and frequency are required for low-throughput drug screening. These zebrafish models are suitable for routine medication testing, but they need further optimisation before they can be considered. Drug development in zebrafish models of stroke is still in its early stages, but it shows tremendous potential for translational success, comparable to what we are seeing in other disease areas. Comparing zebrafish larvae to adult animals, the former are better suited for drug screening with medium-to-high throughput. For the purpose of developing drugs to treat stroke, the most valuable models currently available are zebrafish larval models of spontaneous haemorrhagic stroke. Further improvement is needed to make ischaemic stroke zebrafish models more suitable for drug screening. It would be great if we could create "spontaneous" ischaemic stroke models by creating stable mutant lines. Stroke research may benefit from zebrafish illness models as an additional drug discovery platform; however, a thorough integration of this system into current preclinical pipelines will need time to develop. Persuading the public that zebrafish are an essential part of the preclinical toolbox for translational stroke research will need further work. Maintaining this endeavor is critical for making sure that any potential medications found in zebrafish stroke models are now pharmaceuticals that can be used in humans.

Parkinson's

This work elucidates the behavioral and phenotypic characteristics of the MPP+PD zebrafish model and highlights the potential for the development of drugs that target the HDAC1 and HDAC6 disease isoforms. It seems that therapy with HDAC1 and HDAC6 inhibitors reverses the reduction in TH immunofluorescence and resazurin metabolism that is produced by MPP+. The ability to monitor the effects of toasting A

and MS-275, two inhibitors of zebrafish HDAC1 and HDAC6, *in vivo*. The dopamine agonist apomorphine moderated the effects of MPP+, which included motor deficits and concentration-dependent abnormalities in metabolic and sensory activities. Medicinal inhibition enhances tubulin and histone marker acetylation, and the zebrafish HDAC1 and HDAC6 deacetylase active regions are very comparable to their human equivalents. While neither MS-275 nor lovastatin affected the biogenesis nor function of the mitochondrial complex, they did reverse the MPP+-induced decrease in diencephalic tyrosine hydroxylase immunofluorescence and metabolic activity in whole larvae. In terms of impact on innate motions, only lovastatin and MS-275 are relevant. No improvement in locomotor deficits was seen when tostatin and MS-275 were combined with MPP+; however, tostatin did relieve head impairments **(Cansiz *et al.*, 2021)**. The results of this study provide new therapeutic targets by showing that blocking the zebrafish HDAC1 and HDAC6 isoforms may restore cellular metabolism in a model of Parkinson's disease. On the other hand, the fact that mobility has not improved implies that inhibitors of HDAC1 and HDAC6 alone could not help with Parkinson's disease. Finding components dependent on the zebrafish brain circuit's integrity will be a huge boon to cell-based research, according to this finding. This organism's receptivity to pharmacological targeting of HDAC1 and HDAC6 opens the door to more studies on HDAC inhibitors for other illnesses mimicked in zebrafish. An adult zebrafish model of Parkinson's disease was successfully produced by intraperitoneal infusion of MPTP. Injections into frozen fish may cause noticeable PD symptoms after three or five days. Research, particularly clinical trials for neurological diseases, places a premium on recovery by day 10. However, neurodegenerations may be intentionally generated to treat Parkinson's disease. For example, in a study involving adult zebrafish, the administration of MPTP intraperitoneally effectively caused the illness. Just one injection every three to five days is all that's needed to cause noticeable PD symptoms in frozen fish. Clinical studies involving neurological illnesses place a premium on the recovery period following day 10. Still, neurodegeneration induction offers a potential therapeutic approach to Parkinson's disease **(Dubey *et al.*, 2023)**.

Feeling anxious

Understanding and treating anxiety disorders requires investigation into the underlying process that underlies the formation of brain circuits and their role in behavior control. It is clear from this research how crucial zebrafish are for mice. We outlined the current zebrafish behavioral models and how effectively they detect anxiety symptoms using tests including the novelty tank test, light/dark test, open field test, and social preference test. The glutamate and GABA systems, which are fundamental to zebrafish nervous systems, are the subject of this investigation. More information on gene expression and cortisol levels was found. To improve fishing models for research on human anxiety, the following subjects should be explored. The experiment followed the general outline of the previously mentioned method, with the addition of medications to

water, in the hopes that the behavioral validation of anxiety models would be more thorough and resilient. Due to the fast absorption of pharmaceuticals via the skin and fins, which varies with the activity and surface shape of the fish, these tests cannot exactly alter the dose of drugs. Research on medicinal zebrafish should take precedence in the years to come. It is a fantastic plan to measure the drug's distribution to different organs. Research comparing zebrafish to different vertebrates is essential because neurotransmitter signalling routes may differ in mobility, location, and function. Despite these limitations, research shows that zebrafish may be useful in many areas of neuroscience. The effects of the neuropharmacological baicalein on stress-induced anxiety may be seen in the behavioral traits that are evaluated after stress exposure. Baicalein is helpful for stress and associated mental health issues because it reduces the impact of acute stress and UCS. In order to analyze and demonstrate its mechanism, the GABAA receptor has been chosen as the target for the molecular docking investigation. To identify conventional medications based on their mechanisms of action, greater research into brain neurotransmitter estimates in zebrafish models is required. Mechanism-based docking research confirmed the results of previous baicalein-based investigations. Research into baicalein's potential as a beneficial anti-anxiety therapy is still in its early stages, and despite its high level of safety and efficacy, it has not yet obtained regulatory clearance as a therapeutic medicine **(War *et al.*, 2022)**. Therefore, further research is necessary to fully understand the efficacy and therapeutic benefits of baicalein for the treatment of mental diseases such as anxiety. The molecular basis and expression levels of many transporter genes and synthetic enzymes needed to produce anxiolytic effects remain poorly understood, despite encouraging results to date. To prove that baicalein works in treating anxiety disorders in humans, we need to conduct clinical studies **(Selvaraj *et al.*, 2022)**.

Ataxia

Neuronal loss and/or motor abnormalities resembling human illnesses are seen in the majority of zebrafish models examined for severe ataxia and X/FXTAS-associated faulty genes. However, cancb4a and cancb4b morphants, as well as tbp morphants, do not exhibit any abnormalities in the nervous system or the motor system. The significance of tbp in early development and gene transfer was demonstrated in these studies, even though tbp models derived from morpholino and SCA17 had minimal effects. Mice were made using morpholinos that express cancb4a and cancb4 functions in epiboly, yielding cancb4a and cancb4b, respectively. Despite this, the heterozygous mutant animals more closely resemble the symptoms of episodic ataxia. The majority of zebrafish and morpholino research may provide surprising outcomes, which needs to be kept in mind. Results from RT-qPCR, mRNA recovery, Western blotting/immunohistochemistry, and morpholino control should be used to confirm them. Furthermore, distinct traits, sometimes more subtle in mutant animals, may occasionally be uncovered by comparing a morphant sample with the matching mutant line **(Silva *et al.*, 2025)**. This might be

because of the mutants' ability to retrieve maternal mRNA and undergo genetic compensation. Research on mutants is, therefore, essential to bolster Morpholino's findings. The cerebellum and its connections may be studied by manipulating the central nervous system of zebrafish. Several anatomical features distinguish the brains of humans and zebrafish. For example, zebrafish do not have a direct telencephalic reflex to the spinal cord, and their central nervous systems do not have corticospinal and trans spinal connections. Although there are some drawbacks to using zebrafish as a model for neurological diseases, the benefits outweigh them. One drawback is that 0.24% of the zebrafish genes are lost when the whole ridged ray genome is replicated. This might make model creation more difficult. One of the many benefits is that it may be used as a model to study valuable substances. Here we provide a synopsis of some of the treatments that have been tested and shown to be effective in zebrafish models of fragile X syndrome and severe ataxias. Recent advances in gene sequencing technology have enabled the analysis of full genomes of people with neurodegenerative illnesses, including spastic ataxias and HSP, leading to rapid progress in finding new genes and potentially harmful variations. Because of this, it is crucial to characterize the functions of these new genes and variants. So far, a range of overlapping clinical signs has been identified, including those of HSP, HA, ASS, and other neurological diseases. The associations between HSP and intellectual/developmental impairment or HA are not new, and neither is the connection to the mechanisms underpinning more common neurological disorders such as MS, ALS, Parkinson's disease, and dementias. Researchers have made heavy use of zebrafish as an *in vivo* model system because of the high genetic diversity of ASS and HSP **(Dubey *et al.*, 2025)**.

Ethics

Finally, we need to consider the ethical considerations that come with working with zebrafish. As we delve further into the complex behavioural and cognitive characteristics of animals, our concerns regarding the reliability of our experiments intensify. It is of the utmost importance to assess the cognitive and pain parameters of animal models and ensure that the results accurately foretell potential harm. The topic of fish suffering is divisive. Research conducted at the Roslin Institute by Sneddon, Braithwaite, and Gentle found that neurones in fish respond rapidly to stimuli that cause pain. Furthermore, when they are faced with unpleasant stimuli, they display negative behavior. Rose, who views anguish and suffering as different drives, has been quite critical of the Essendon discovery. Rose argues that fish lack an intrinsic ability to cope with pain and that complex responses to painful stimuli occur subconsciously after pointing out inconsistencies in the way Essendon et al. interpreted their results. Even while any given animal could have an unpleasant reaction to some stimuli, very few of them really feel pain. By comparing the sizes of different animals' brains, with the human brain serving as a proxy for intelligence, we may obtain a clearer idea of how different

species' cognitive skills vary. The brain of an adult zebrafish weighs less than 0.1g, but the brain of a human being is thirteen thousand times larger. While most zebrafish studies focus on embryos, one source suggests 10,000 neurones per click, and no reliable estimate of the number of neurones in zebrafish embryos has been found. Included in this region is around 10% of what is found in honeybees. Zebrafish embryos are believed to have more complex neurological connections than adult zebrafish due to the fact that the first neurones are formed 7.5 hours after fertilization. We need to think about the ethical implications of working with zebrafish. As we delve further into the complex behavior and cognitive characteristics of animals, our concerns regarding the reliability of our experiments intensify. It is critical to assess the potential cognitive and pain experiences of animal models and ensure that test results accurately represent damage. The topic of fish suffering is divisive. Researchers Sneddon, Braithwaite, and Gentle of the Roslin Institute found that neurones in fish respond correctly to pain-inducing stimuli. Additionally, they exhibit atypical behavior in response to stimuli that are supposed to be unpleasant **(Lammert *et al.*, 2025)**. Rose has vehemently disagreed with Sneddon's conclusions, arguing that pain and nociception are two separate but related processes. According to Rose, fish do not have the neural circuitry in their brains to detect pain, yet they are capable of highly nuanced reactions to painful stimuli while asleep. In addition, he points out some discrepancies in the conclusions obtained by Sneddon et al. Even while all animals are capable of reacting to painful stimuli, it is unclear how many of them are conscious of their discomfort. We may learn more about the cognitive abilities of other animals by comparing their brain sizes to the human brain, which is used as a surrogate for intelligence. The brain mass of an adult zebrafish is less than 0.1g, which is thirteen thousand times smaller than that of a human brain **(Dubey *et al.*, 2024)**. While the exact number of neurones in zebrafish embryos is unknown, one source claims that for every click, there are 10,000 neurones. Working with embryos is a big part of zebrafish research. This volume amounts to around 10% of the honeybee's total content. With the first neurones emerging 7.5 hours after fertilisation, the brain of an embryonic zebrafish contains more complex neural connections than those of an adult .

Future perceptive

The zebrafish model has emerged as a pivotal organism in neurology research due to its unique advantages in studying the molecular and cellular mechanisms underlying neurological disorders. With its genetic tractability, transparent embryos, and rapid development, zebrafish allow real-time observation of neural processes, offering insights into both basic neuroscience and disease pathophysiology. The future perspective of zebrafish in neurology lies primarily in its application for understanding complex neurological diseases, including neurodegenerative disorders (e.g., Alzheimer's, Parkinson's), autism spectrum disorders, and epilepsy. Advances in CRISPR/Cas9 technology and gene-editing tools have further enhanced the utility of zebrafish, enabling

the creation of precise models that replicate human genetic mutations associated with these conditions. Additionally, the ability to monitor neuronal activity and behavior *in vivo* using high-resolution imaging technologies positions zebrafish as an ideal platform for drug discovery and therapeutic interventions. Moreover, the zebrafish central nervous system exhibits notable regenerative capacities, making it an attractive model for studying neurodegeneration and potential therapies for neurodegenerative diseases. As our understanding of the genetic and environmental factors influencing neurodevelopment expands, zebrafish will likely play a central role in unravelling the complex molecular pathways involved in synaptic plasticity, neuroinflammation, and neurodegeneration. In conclusion, zebrafish will continue to be a powerful model in the advancement of neurology research, particularly as precision medicine and regenerative strategies gain traction in clinical settings. Its utility in drug screening, disease modelling, and understanding neurodevelopmental processes holds significant promise for advancing neurological therapies.

CONCLUSION

The zebrafish, or *Danio rerio*, is an important vertebrate model for neuroscientific studies because of its cheap maintenance requirements, transparent development, and high degree of genetic similarity to humans. Behavioral experiments in zebrafish mimic human neurological disorders, and the fish can reproduce quickly, making them an ideal model for high-throughput screening. This is in contrast to classic mammalian models. In particular, they help researchers understand anxiety, Alzheimer's disease, Parkinson's disease, stroke, and epilepsy. Modeling brain disorders, understanding disease pathways, and accelerating treatment development are all possible using zebrafish, despite limits such as anatomical divergence and variances in drug metabolism. Because of their growing translational relevance and ethical feasibility, they are becoming an indispensable tool in neuropharmacology.

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