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Advancing Neuroscience through Zebrafish: Challenges, Innovations, and Future Directions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Genetic similarity of zebrafish and humans, their transparency, and rapid development has propelled them to be one of the most preferred models for research in neuroscience. Within this article, there is a brief discussion on zebrafish in the field of neuroscience with the considerations of their neuroanatomy, advantages, recent achievements, drawbacks, and the possible routes for future exploration. Zebrafish are a rare model for neuronal development and or translating/creating neurological disorders. Neuroscientists that study zebrafish have occasionally faced problems with behavioral complexity and lack of established methods. Forward-looking actions include

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optimization of behavioral assays, adoption of CRISPR/Cas9 for genotype modification, and unification of drug discovery and testing of toxicological effects. Through zebrafish neuroscience research, we are heading towards great heights as the potential role in bridging the knowledge on the brain and neurological disorders is very promising. The successful adoption of new technologies and collaborative methods will be the attending factors to reach a goal of zebrafish model fully exploiting the potential for the neuroscience research.

Keywords: Zebrafish; neuroscience; model organism; genetic manipulation; neurodevelopment; behavioral assays; drug discovery.

1. INTRODUCTION

1.1 Zebrafish as a Model Organism in Neuroscience Research

This organism is unique, in that it has a very mammalian kind of brain and structure of arrangements, as well as the function, a model for research. Nevertheless, both the way of functioning and the building blocks and method of constructing those structures like the hippocampus, diencephalon, tectum, tegmentum, and cerebellum are shared by the zebrafish and the mammals [1]. The research points out the fact that zebrafish development and neuro-disorders have a common element, and this idea should be emphasized [2].

Zebrafish model is among the most preferred animal models for research, as its number of advantages simply outweighs the others. These, rather, have a short life cycle, especially during the embryonic period of development, less maintenance costs, and multiple methods for genetic engineering, as well as polygenic diseases [3]. In a similar way, at least one orthologue of a human gene is present in about 70% of the repertory in zebrafish [4]. However, zebrafish have a lot of similar genes in common with human beings, and this is why it is challenging to study zebrafish's activities [5].

Zebrafish is an interesting model that undergoes external fertilization and the formation of embryos, thereby making it simple for researchers to observe the development and place the embryos for phenonic screening, manipulation, and observation using chemicals or mutagens [6]. As an important note, zebrafish may give average 200–400 embryos following mating, while rodent females may yield 10–12 embryos [7]. Zebrafish as a species is readily adaptable to multi-conditional experiments and high-throughput screening [8].

1.2 Importance of Understanding the Challenges and Future Directions in this Field

The understanding of the blockages encountered as well as different ways to go in the aspect of zebrafish neuroscience research is highly critical. It should be pointed out that there are disadvantages although they are not hindering of zebrafish models despite their benefits. Zebrafish have a significantly increased behavioral complexity that creates a huge barrier which requires developing newly complex assays and analytical methods to achieve any reliable evaluation of neurological function [9]. Besides that, it is important to recognise interspecies differences in brain microstructures and functions as these variations should be taken into account before simulating complex neurological diseases in zebrafish are viewed as relevant to humans. Integrating knowledge from these fields, i.e. computational biology, genetics, neurology, and some other fields, is the main phase to deal with these problems [5].

Stable standardized protocols should be designed for zebrafish experiments as well that would permit the experiments in the field again perfectly acheiveable with high precision. Multiple neuroscience experiments performed on zebrafish require method development and this is simply because the lack of techniques is leading to unsound findings and is hampering the field's progress [10]. Research can be made more robust and strict by introducing generalized protocols of the experiments, which would increase the number of good papers on zebrafish neuroscience research [11].

Both the direction of neuroscience research on zebrafish can be quite full of hope in future for many people. The advent of highly efficient gene editing devices, which include genome engineering tools such as CRISPR/Cas9, has triggered a new epoch in the zebrafish studies since it is now possible to create exact modifications of the zebrafish genome [12]. The advent of high technology now made it possible to discover the mechanism of gene function and obtain the models of human diseases from zebrafish. In addition, advanced imaging techniques of the brain can be combined with gene manipulation strategies that will tell much about the brain circuits and behaviors of zebrafish. Moreover, we can stumble upon new brain science discoveries in this way [13].

2. NEUROANATOMY AND NEURODEVELOPMENT OF ZEBRAFISH

As opposed to other models of the nervous system, the zebrafish seems to be the most preferred by neuroscientists for its individual anatomical and developmental features that resemble those of mammals. Understanding the brain structure of zebrafish facilitates the isolation of different neurobiological and behavioral elements that are crucial to the study [14].

2.1 Zebrafish Brain Structure

The central nervous system of a great number of zebrafish species, having the system Cypriniforms, is perfectly structured since it is much simpler than the central nervous system of mammals [15]. The regions of the adult zebrafish brain that one could watch from this perspective are the forebrain, midbrain, hindbrain, and spinal cord. The structure is comprised of sub-regions performing different functions and networks, which set the stage for their operation as such [16].

The amygdala and hippocampus are the mid-brain structures that belong to the limbic system and function as emotion and memory-related components. The subdivisions of the diencephalon, thalamus, and hypothalamus translate sensory information and control brain hormones [1].

The optic tectum which is a vital spot for acquiring visual information is located in the midbrain. Zebrafish optic tectums that are highly developed visually are a highly searched suggestion whether for scientists or researchers, and this sheds some light on how such visual process mechanisms work [17].

Towards the posterior end of the zebrafish brain lies the hindbrain, which encompasses two key structures: the brain stem, which contains the cerebellum and medulla. The cerebellum is a very important part of the structure as it helps to coordinate all the motor functions. In contrast, the medulla oblongata, which is part of the hindbrain, is what controls the autonomic processes on the body level that regulate vital functions. Straight from the hindbrain is the spinal cord, which is the major point of communication that enables the transfer of both the up and down sensory information from the brain to the rest of the organism's body [18].

2.2 Neurodevelopment of Zebrafish

Zebrafish is one of the compound model organisms very common in neuroscience studies due to the ease in which they can develop an embryo within two days and thus their embryo will be visible to the eye which enables one the ability to observe neuronal growth and development in a short time [19]. The early stages of neural development in the zebrafish embryo take place as early as several hours after fertilization, indicating that these species develop fast at onset [20].

The neurodevelopment, in zebrafish, is exhibited in the early stages of the neural tube formation which further contributes to the origin of brain and spinal cord [21]. As the neural tube in zebrafish embryos undergoes its cell proliferation, migration and differentiation into neuronal and glial cell, it becomes the mature zebrafish brain comprised of a wide range of different types of cells [22].

2.3 Comparison with Mammalian Brains

Researchers need to be cognizant of whether the findings from zebrafish neuroscience experiments are relevant to humans, or if there are differences in the neuroanatomy of the zebrafish and mammalian brains [15].

Different than mammals and their having relatively simple brain anatomy with less brain parts and simple communications system. Regardless, zebrafish and mammals possess almost the same basic compartments, such as back, mid, and lower brains, namely forebrain, midbrain, and hindbrain [14]. For example, it may contain information on how a particular zebrafish has a small shape, but its telencephalon is of the same size as the cerebral cortex in mammalian.

The optic tectum in zebrafish is, in a similar way as the mammalian superior colliculus, responsible for the marketing of visual information [17].

Mammalian brains and zebrafish brains are very different in size as the former has a substantial neocortex while the latter is devoid of this brain part. Mammals' intensely evolved neocortex is functionally in-charge of intelligence that includes the advanced processes of language, decision-making, and complex sensory processing. Finally a Zebrafish has no neocortex but it does have the areas that are homologous together even if rather primitively, the functions are comparable [23].

Regardless of this diversity, it turns out that the brain of zebrafish and mammals fundamentally resemble each other a lot in the bottom of the molecular and genealogical research. Zebrafish also has many genes which affect development, function and neurological disorders of their brain together with mammals. This is possible because of their genetic similarities and hence, scientists can utilize zebrafish to mimic human brain illness and by catapulting default disease mechanisms and relatable cure targets [24].

3. ADVANTAGES OF USING ZEBRAFISH IN NEUROSCIENCE RESEARCH

3.1 Genetic Similarity to Humans

Human species and zebrafish have highly evolved away, but they surprisingly resemble each other in terms of their genetic similarities [25]. Zebrafish orthologs for nearly and seventy percent of human genes indicate that these genes belong to the common ancestral origin of human and other species and this common origin maintains their similar mode of operation in other species as well. Due to their phylogenetic closeness to humans, which sometimes acquires the level of genome linkage with genes responsible for neurology, zebrafish are considered as a perfect model for studying the genetics of neurodevelopment and neurological disorders [26].

Gene-precise modifications to zebrafish that are viewed as one of its unique features that helps it stand out among other experimental animals in neuroscience research [27]. zebrafish have provided us with information relating the functions of certain genes that are responsible for neurodevelopment and behavior [26]. Exploring zebrafish, for instance, has updated scientists with genes that regulate the development of a brain [14]. Moreover it is reported that zebrafish have been attracted in work on genetic basis of neurodevelopmental diseases including epilepsy and autism spectrum disorders (ASD) through which new candidate gene and pathways have been found for these conditions [28,29].

3.2 Optical Transparency and Imaging Techniques

Zebrafish have an edge among other model organisms used in neurological studies, because of three special qualities of them. First, it is the fact that they are optically transparent in the early stages of embryo development. Since zebrafish embryo's early stages they're translucent, allowing researchers to seem the whole neural system in live, undamaged specimens. By being open to all researchers, the crossing of neurons and buildup of the architecture can be observed in real-time, which is especially good in research about neurodevelopment [30].

To ensure that the capabilities of clear zebrafish embryos is exploited fully, a large number of advances in imaging techniques have been established. Such as light-sheet microscopy in which a narrow sheet of light is used to illuminate the sample so that it won't get worn down(photobleach) and will do no harm(phototoxicity) thus, enabling images to be taken in the low-resolution areas within the sample. Major progresses in the areas of neurodevelopment dynamics, such as the circuit formation and neurons' migration, have been made with this particular method [31].

A significant technique in fluorescence discovered by intensity scanning is confocal microscopy in zebrafish brain studies. Contrast and clarity of the specimen images are guaranteed by an imaging technique called confocal microscopy which uses only the light that the pinhole has allowed the unused beams of light to be removed. This approach allows to observe a development of cells and their connections that could play a role in the circuit formation and follow its functioning [32].

To keep taking advantage of zebrafish imaging methods, scientist have developed a lot of genetic and lighthouse- tools now available together with known imaging methods. For example, calcium probe genes activated ensure researchers monitor brain activities in real time and hence paint a clear picture on how the circuits function. To make the changes in networks of neurons and behaviors of zebrafish the technique is called optogenetics, genetical engineering of apparatus and control by that being used by light [33].

3.3 Rapid Development and High Fecundity

Zebrafish as model organism shed light on neurodevelopment research and clone a few generations of fish to evaluate numerous treatments in neuroscience experiments due to their abbreviated life cycle and high fecundity [8].

The zebrafish embryos developed rapidly; and in a period of 2-3 days, major morphogenetic processes occurred after fertilization [20]. As they embryos of the zebrafish are transparent, neural tube formation, neural crest migration, and neural circuit growth can be watched in the process directly. The fast result of these studies provides a good chance to look at them closely and change gene expression or brain activity by using them to learn more about the influence of such factors on the course of neurological development or disease [32].

A zebrafish is indeed very fertile. For this to happen, there should be a suitable environment for the female. Researchers are able to perform high-throughput genetic and medication screens through random generation of multiple embryos due to high fecundity [8], which is conducive to selection of that gene combination that has the most beneficial effect for the offspring. Through detection of genes or substances that influence neurodevelopment or cognitive functionality, these types of tests provide information on the nature of the disease and thus stimulate new approaches to treatment [26].

The fish have been effective to become as the best experimental system in investigating the developmental neuro disorders like seizures and ASD due to its high fertility and quick development [28,29]. Younger people really have an advantage in that they can use these aspects to conduct the investigations which can be impossible or difficult in other model organisms, therefore facilitating neuroscience scientific development [34].

4. CHALLENGES IN ZEBRAFISH NEUROSCIENCE RESEARCH

4.1 Behavioral Complexity and Modeling of Neurological Disorders

Zebrafish have an eclectic array of behaviors that one such do in the field of neurological diseases study. Such responses as reactions to sensory perception, learning and remembering, social interactions, and walking are where brain activity is detected. Zebrafish behavior is naturally more complex and diverse [2]. Thus, standard laboratory setups, which often involve the use of tanks and a distracting background, can make the task of capturing and analyzing these behaviors particularly challenging [35].

Creating behavioral tests that can evaluate, in a subtle way, complex actions is the main problem in a zebrafish neuroscience research, where many intricate procedures are required. To illustrate, assessments that successfully measure learning process and memory function like tests must be developed for researchers to correctly assess the cognition abilities in zebrafish animals. The assays, which are developed for accurate measurement of social or repetitive behaviors in zebrafish, are also needed for creating the models of such behaviors which are the distress symptoms of varied mental diseases [35].

Despite the challenges brought by the peculiar behavior of zebrafish, scientist have worked conscientiously to devise various behavioral tests and analytical methods to match the different kinds of zebrafish behaviors. For instance, the shoaling assay will help to access the social interactions and the size of the school while the novel tank test is usually known for assessing zebrafish fear like behaviour. In addition to this, techniques having been designed in order to clarify these organisms brain mechanisms involving memory and learning [36].

The last but not the least cerebrality of neurological disease involving zebrafish is that nothing from the behavioral model can be directly linked to the features in humans. Tiny zebrafish are very much capable to display human illness-like behavior, but at the same time, the brain circuits and mechanisms underlying such behavior may be different across animal species. Thus this means that we should have a very deep knowledge of the neurobiology of both fishes and fetuses and a thorough validation of the model will bring up the constraints [37].

Zebrafish have invested a lot into the understanding of neurodevelopmental and neurological disorders despite these disadvantages simply because of their genetic proximity to humans. Through the genetical tractability, transparency and intricate behaviors of lower organisms, scientists can create new models which enable them to get great insight on the underlying mechanisms of those illnesses and suggest possible therapeutic strategies [27].

4.2 Lack of Standardized Protocols for Certain Experiments

The following limitation of zebrafish neuroscience research is no such regulation procedure in the certain cases. To ensure the reproduction of the research outcome and effects and make comparing different studies easier, standardization is crucially important. However, the creation of standard protocols may be challenging because so many different research Zebrafish investigations are available, as well as because a wide variety of experimental approaches are being used [35].

The need arises for the same screening protocols to be implemented in the drug trails. Due to the ability of zebrafish to produce a large number of offspring and develop quickly into an adult, they are often used in drug discovery research as they allow for a large scale screening- the preliminary examination of chemicals [8]. On the opposite side, dissimilar end results could happen due to an alteration of the experimental setting, like the duration and amount of drug exposure. Developing uniform screening methods for zebrafish drug testing is an essential step on the path to ensuring screening outcomes correct and facilitating the process of finding probable treatments for neurological issues [38].

4.3 Ethical Considerations and Regulatory Issues in Zebrafish Neuroscience Research

Being rapid, clear and genetically similar as humans to zebrafish, they have become a famous model organism which is widely applied in neurological studies [8]. The use of humanize animals in research gives rise to the most consequential ethical and legal issues that require decision and solutions.

The animals well-being is the main ethical issue when we speak about zebrafish using for research. Zebrafish are likely to be a creature with small-scale sensory circuits as they have a simple neural system however; there is ample proof that they can experience pain and discomfort. For the purpose of prevention of any potential suffering from occurred, scientists must ensure that P. leerii species are held and cultured following ethical standards [39].

Another ethical concern is the employing of genetically organism zebrafish. Because of the development of technologies like CRISPR/Cas9, researchers can now fine-tune their edits to create the zebrafish with the level of precision that can be used to build disease models. Though the point is establishing genetic modification as the method for improving agricultural production, some moral issues appear about the welfare of animals, environmental effect and unpredictable consequences [40].

Extra standardization is expected when doing zebrafish research and treatment. There is an added step for ethics. Plenty of governmental organizations take part in the regulation of research using animals like zebrafish to make sure that there is no unethical research and it is done as painless and humanly as possible. Before carrying out any zebrafish experiments, investigators get to seek and gain approval from some ethics committees [41].

Basically we face the need of ethical and legal problems in each of zebrafishes brain researching. Similarly with making sure that not only are their studies done ethically but also in accordance with the law, researchers can also answer to some of the brain and neurological conditions through the use of this well thought approach [41].

5. RECENT ADVANCES IN ZEBRAFISH NEUROSCIENCE RESEARCH

5.1 Innovative Techniques and Tools in Zebrafish Neuroscience Research

Zebrafish have come to serve as a very valuable generic organism for neuroscience research due to the invention of some new methods of observations and instruments that make the zebrafish brain and behavior to be investigated in detail and accurately.

The finding of optogenetics along with that, is as well one of the wonder of all the zebrafish neuro science research. In optogenetics the modified group of neurons that are formed genetically to be activated by light is manipulated in a manner that permits the monitoring of light in order to determine the level of activity. Human trial with the utilization of this technology, helps in the control of brain waves at a sub microscopic level to study how neuronal circuits distil different functions in the motor cortex of the brain. Optogenetics has enabled us to track the output of zebrafish action patterns, including the processes of decision making, movement coordination and social interaction. Through this mapping, we have learned how certain behaviors as have depended on specific neurological processes [42].

Calcium imaging is the primary way to study zebrafish behavior in the context of both neuroscience research as well as the field of post - genomic medicine. It is now possible for scientists to pinpoint exact single neurons or the bunches of neurons in zebrafish brain becasue of calcium imaging. Scientists may track brain activity in real time with the use of the fluorescent level of the calcium indicator as it varies with the changes of the calcium levels.Using this method, researchers have mapped the neural circuits that underlie zebrafish sensory processing and decision-making as well as examined patterns of brain activity during behavior [43].

In addition to these procedures, the development of imaging technology has provided us with entirely new abilities to see the zebrafish brain which were never available to us. For instance, light-sheet microscopy provides an excellent resolution imaging of large tissue volumes cutting down on the photobleaching or phototoxicity. The excitation-contraction coupled processes like neurite outgrowth and synaptogenesis have been held in investigation which has also been part of the neural networks in zebrafish brains using this technique [31].

5.2 Key Findings in Neurobiology and Behavior in Zebrafish

The evaluation of neural networks underlying behaviors in zebrafish is just one of the fields of research that has added to the scientific understanding of these fish [44]. The brain's elaborate circuitry, which provides zebrafish with the capacity for movement, social interactions, and dodging predators, is managed by complex brain circuits [14]. The field of optogenetics and calcium imaging produces the results that innervate which specific neuronal circuits in the zebrafish brain are responsible for these behaviors [42,43].

Pinpointing conserved molecular screen halting neurodevelopment is another of the most significant achievements in the neurobiology of zebrafish. One of the main factors that makes zebrafish an excellent model for studying development of the human nervous system is that there are many human genes that are involved in neurodevelopment which are also present in this fish and research carried on them has revealed vital information about the functions of these genes [45]. Due to this road map, which can be altered to have developmental abnormalities, information on the genetics of neurodevelopmental disorders could be discovered [26].

The findings based on zebrafish behaviors uncovered hidden connections on the neurological and genetic grounds of the social behavior. The formation of schools by zebrafish, who are apparently gregarious swimmers, face group swimming in synchronized movements. Studies have disclosed that zebrafish genes involved in their sociable behaviors share the same genes with humankind, thus, transfiguring social behavior as a trait of evolution [2].

Consequently, zebrafish have provided a considerable contribution to research done to understand behavior and neurobiology leading to the gathering of fundamental facts about the structure and process of the vertebrate’s nervous system. via harnessing special understanding from the zebrafish model, scientists come into a point where they can further define basic brain activities like those which bear translatability to the general health and sickness of human beings [46].

6. FUTURE DIRECTIONS AND POTENTIAL APPLICATIONS

6.1 Enhancing Behavioral Assays and Modeling Neurological Diseases

However, the key approaches to zebrafish neuroscience research will probably be enhancing the accuracy of behavioral tests and creating neurological diseases that mimic human disorders. These initiatives should also be able to provide a clearer and more detailed information about the zebrafish behavioral research as to be more sensitive and relevant to neural disorders by providing a focused and precise research [5].

Forming new assays that are closer to the phenomenon of a healthcare problem or behavior in humans is a certain way of making these trials better. Currently, scientists are trying to improve those assays within zebrafish research with ever more precision in order to make a coincidence closer to ASD social disorders. To measure these behaviors with higher precision, one may need to develop new assay with the capability of detecting and analyzing specific factors of social behaviors eg social mating or social acceptance and use complex data processing techniques and automatic monitoring system [47].

The employment of strategies that contribute to improving the current behavioral assays and of those that boost behavioral assay repeatability and sensitivity is also an accomplished strategy. To minimize variation in studies, such an approach may comprise changing the experimental environment including brightness and enriched surroundings. In order to have more comprehensive reviews about zebrafish behavior, scientists are as well suggesting the use of newer behavioral outcomes for instance, decision-making and cognitive paradigms.

Scientists are attempting to develop novel zebrafish models of neurological disorders, which are more similar to the genetic and behavioral characteristics of the human illnesses [48]. This may include techniques like CRISPR/Cas9 genome editing, for example, to create genes linked to some neurological diseases. These models can be deployed thereafter in disease mechanism studies and treatment efficacy evaluations in an environment that is more physiologically appropriate [49].

Summing up, perfecting behavioral tests and zebrafish modeling of brain diseases represent indispensable factors for acquiring more sophisticated insights into the brain and designing cutting-edge therapies for neurological diseases. Scientists can enhance the precision and significance of behavioral studies in zebrafish by means of boosting the accuracy and translational value of those studies. This will in the long run be good for the patients with neurological disorders [27].

6.2 Incorporating Advanced Technologies Like CRISPR/Cas9 for Genetic Manipulation

Zebrafish genetic modification has reached a new level of acquisition in recent years due to the rise of CRISPR/Cas9 technology, which allows scientists to modify genes with extraordinary accuracy and speed. The employment of CRISPR/Cas9 as well as other innovative genetic techniques in zebrafish brain research is the gateway for probing the genetics of neurodevelopmental and neurological disorders [12].

One of the main goals of CRISPR/Cas9 in zebrafish neuroscience research is to design and carry out genetic modifications to investigate the role of particular genes or pathways, which play a key role when it comes to brain development and disease. Scientists can tell how genes are functioning in neurodevelopment or the illnesses of the neurological system through using CRISPR/Cas9 to introduce targeted mutations, or by introducing, or deleting specific genes, as well as modifying gene expression levels [50].

Similarly, accurate and effective attempts to establish zebrafish models for neurological disorders in humans can be made only with the help of CRISPR/Cas9 technology instead of traditional methods. Through the possible construction of models that mimic the essentials of human diseases this research can be done by simply inserting mutations related to disease into the genetic makeup of zebrafish. This can give us useful information about the underlying biochemical processes and elluciate possible targets for treatment [50].

6.3 Potential for Drug Discovery and Toxicology Studies

Zebrafish owing to their ability to develop rapidly, its optical transparency, known genetic tractability have been proven to be a good model organism for genetic research on chemical toxicity and drug discovery [48]. Zebrafish can serve as a great model for the finding of multiple drug candidates which may have therapeutic effects with the help of this feature. Safety profile of the drugs or environmental pollutants can also be easily determined with the help of this species [51].

Zebrafish have been placed at the top of the phenomenon of accelerated development as well as high fecundity which give scientists an opportunity to treat big doses of material and therefore have vast number of embryos for screening [8]. The zebrafish embryos are transparent and then this gives us the ability to see the effect of medications on the development in a more precise way in real time as compared to other methods [52]. Zebrafish furthermore provide an almost unparalleled means for studying abnormalities in the developmental process, as well as for evaluating the potential toxicity of different substances by the time that they enter the cell [47].

By using zebrafish models of neurological illnesses, namely epilepsy, Parkinson's disease, and Alzheimer's disease, and their associated phenotypes, our scientists can search for chemicals that can modify these traits and utilize these models for research. These models allow scientists to save money and time by only testing the compounds identified by the models for further testing in other types of nonmammalian models [53,54].

In addition, zebrafish become more important tool for toxicologists in order to assess medicinal drug safety and other environmental toxins. On the other hand, this species is used to discover drugs. Zebrafish embryos are very susceptible to chemical exposure; therefore, its use is also a valid modeling of an unfavorable chemical environment during embryogenesis process. Furthermore, zebrafish are used in behavioral investigations to estimate neurotoxicity of substances, which help upgrading the health safety assessments of similar products intended for human use [55].

Zebrafish are an easy and inexpensive model system that makes it possible to select the most promising chemical compounds which can be used in medicine and assess the safety of chemicals control [51]. They bear the hope for pharmaceuticals as well as for the prediction of the side effects. Scientists are more able to identify new treatments for neurodegenerative illnesses earlier, and that there is a deep understanding of mechanisms on how environmental contaminants affect the health of humans, which is made possible by the presence of the zebrafish model [56].

7. CONCLUSION

Zebrafish have become a significant model organism among neuroscientists, firstly, due to their unique features that are useful in understanding the processes that are responsible for the behavior and work of brain. Zebrafish’s diverse behaviors and many genetic and neurobiological similarities to human, despite of the fact that most of them are small, simple model organisms, have made Zebrafish a good model for research in neurology and neurodevelopment. However, there are some challenges to consider with zebrafish neuroscience research, and overcoming the hurdles would not only delve deeper into the branch, but would allow using the fish to its full potential as well [57].

The complexity of learning and analysis of the behavior in zebrafish which is addressed through advancement in testing techniques, stands as one of the chief barriers in neuroscience research using zebrafish [38]. As a result, the development of behavioral assays with a greater representation of neurological disorders and their corresponding human activities will be critically important for raising the translational value. The researchers could come up with new assays and set standard methods for behavioral assays with zebrafish by collaboration and pooling their knowledge and expertise [34].

Insufficiency of standard operating procedures (SOPs) for some studies is also an issue participants might confront. Consequently, these researchers might not obtain the same results and therefore make comparison of the studies more challenging. Repeatbility of zebrafish research can be improved significantly only by the adoption of detailed standard operating procedures normally tested prior to the experiment [58]. The drug screen and behavioural tests are the most applicable among genetic screening, histopathology, and molecular analysis. Providing recommendations for scientists, funders, regulatory bodies as well as sponsored the teamwork is important to establish the best practices and guidance for zebrafish research [27].

By no means these obstacles are insurmountable; thus, the field of zebrafish neuroscientific researches should still have great potential for extending our knowledge about the brain and developing new therapies for neurological illnesses. In the near future, the spotlight is going to fall on high-tech methods of genome editing, such as CRISPR/Cas9 and experiments involving zebrafish for drug testing and toxicological studies. Apart from that, the broadening of the region and the transformation of research results into processes of treatment will require permanent study of the zebrafish model as well as constant communication between researchers [50].

The neuroscience research on zebrafish provides a lot of opportunities to enrich our knowledge of the brain and of neurological disorders as well [37]. Scientists have indeed been very productive in embarking on the zebrafish model to address some of the critical health issues by applying cutting edge technologies and operating with a common goal. However, whether this potential would be realised and the direction of the future research is dependent on further studies and the international cooperation [59].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bally-Cuif L, Vernier P. Organization and physiology of the zebrafish nervous system. In: Fish Physiology. Elsevier. 2010;29:25-80.
   Accessed April 5, 2024.
   Available:https://www.sciencedirect.com/science/article/pii/S154650981002902X
2. Mrinalini R, Tamilanban T, Kumar VN, Manasa K. Zebrafish–The Neurobehavioural Model in Trend. Neuroscience. 2023;520:95-118.
3. Soares J, Coimbra AM, Reis-Henriques MA, et al. Disruption of zebrafish (*Danio rerio*) embryonic development after full life-cycle parental exposure to low levels of ethinylestradiol. Aquat Toxicol. 2009;95(4):330-338.
4. Davis EE, Frangakis S, Katsanis N. Interpreting human genetic variation with in vivo zebrafish assays. Biochim Biophys Acta BBA-Mol Basis Dis. 2014;1842(10):1960-1970.
5. Kari G, Rodeck U, Dicker AP. Zebrafish: An emerging model system for human disease and drug discovery. Clin Pharmacol Ther. 2007;82(1):70-80.
   DOI:10.1038/sj.clpt.6100223
6. Baxi D. Zebrafish: A versatile animal model to study tumorigenesis process and effective preclinical drug screening for human cancer research. In: Pathak S, Banerjee A, Bisgin A, eds. Handbook of Animal Models and Its Uses in Cancer Research. Springer Nature Singapore; 2023:1039-1049.
   DOI:10.1007/978-981-19-3824-5_53
7. Nasiadka A, Clark MD. Zebrafish breeding in the laboratory environment. ILAR J. 2012;53(2):161-168.
8. Lessman CA. The developing zebrafish (*Danio rerio*): A vertebrate model for high-throughput screening of chemical libraries. Birth Defects Res Part C Embryo Today Rev. 2011;93(3):268-280.
   DOI:10.1002/bdrc.20212
9. Moretz JA, Martins EP, Robison BD. Behavioral syndromes and the evolution of correlated behavior in zebrafish. Behav Ecol. 2007;18(3):556-562.
10. Bruneel B, Witten PE. Power and challenges of using zebrafish as a model for skeletal tissue imaging. Connect Tissue Res. 2015;56(2):161-173.
    DOI:10.3109/03008207.2015.1013193
11. Detrich III HW, Westerfield M, Zon LI. Overview of the zebrafish system. Methods Cell Biol. 1998;59:3-10.
12. Liu K, Petree C, Requena T, Varshney P, Varshney GK. Expanding the CRISPR toolbox in zebrafish for studying development and disease. Front Cell Dev Biol. 2019;7:13.
13. Wyatt C, Bartoszek EM, Yaksi E. Methods for studying the zebrafish brain: Past, present and future. Eur J Neurosci. 2015;42(2):1746-1763.
    DOI:10.1111/ejn.12932
14. Wulliman MF, Rupp B, Reichert H. Neuroanatomy of the zebrafish brain: A topological atlas. Birkhäuser; 2012.
    Accessed April 5, 2024.
    Available:https://books.google.com/books?hl=en&lr=&id=yMrzBwAAQBAJ&oi=fnd&pg=PA1&dq=Neuroanatomy+and+Neurodevelopment+of+Zebrafish&ots=y5uLPEiDyA&sig=4S8JPFJzx0LvspoYAcEGpGpfUwg
15. Panula P, Chen YC, Priyadarshini M, et al. The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. Neurobiol Dis. 2010;40(1): 46-57.

16. Chong M, Drapeau P. Interaction between hindbrain and spinal networks during the development of locomotion in zebrafish. Dev Neurobiol. 2007;67(7):933-947.
DOI:10.1002/dneu.20398
17. Sajovic P, Levinthal C. Visual cells of zebrafish optic tectum: mapping with small spots. Neuroscience. 1982;7(10):2407-2426.
18. Bally-Cuif L, Vernier P. Organization and physiology of the zebrafish nervous system. In: Fish Physiology. Vol 29. Elsevier; 2010:25-80.
Accessed April 5, 2024.
Available:https://www.sciencedirect.com/science/article/pii/S154650981002902X
19. Saint-Amant L, Drapeau P. Time course of the development of motor behaviors in the zebrafish embryo. J Neurobiol. 1998;37(4):622-632.
DOI:10.1002/(SICI)1097-4695(199812)37:4<622::AID-NEU10>3.0.CO;2-S
20. Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. Dev Dyn. 1995;203(3):253-310.
DOI:10.1002/aja.1002030302
21. Lewis KE, Eisen JS. From cells to circuits: development of the zebrafish spinal cord. Prog Neurobiol. 2003;69(6):419-449.
22. Lyons DA, Talbot WS. Glial cell development and function in zebrafish. Cold Spring Harb Perspect Biol. 2015; 7(2):a020586.
23. Kaas JH. Evolution of the neocortex. Curr Biol. 2006;16(21):R910-R914.
24. Guo S. Linking genes to brain, behavior and neurological diseases: What can we learn from zebrafish? Genes Brain Behav. 2004;3(2):63-74. doi:10.1046/j.1601-183X.2003.00053.x
25. MacRae CA, Peterson RT. Zebrafish as tools for drug discovery. Nat Rev Drug Discov. 2015;14(10):721-731.
26. Sakai C, Ijaz S, Hoffman EJ. Zebrafish models of neurodevelopmental disorders: past, present, and future. Front Mol Neurosci. 2018;11:294.
27. Burgess HA, Burton EA. A critical review of zebrafish neurological disease models- 1. The Premise: Neuroanatomical, Cellular and Genetic Homology and Experimental Tractability. Oxf Open Neurosci. 2023;2:kvac018.
28. Kundap UP, Kumari Y, Othman I, Shaikh M. Zebrafish as a model for epilepsy-induced cognitive dysfunction: a pharmacological, biochemical and behavioral approach. Front Pharmacol. 2017;8:249388.
29. Tayanloo-Beik A, Hamidpour SK, Abedi M, et al. Zebrafish modeling of autism spectrum disorders, current status and future prospective. Front Psychiatry. 2022;13:911770.
DOI:10.3389/fpsyt.2022.911770
30. Lawson ND, Weinstein BM. In vivo imaging of embryonic vascular development using transgenic zebrafish. Dev Biol. 2002;248(2):307-318.
31. Keller PJ, Schmidt AD, Wittbrodt J, Stelzer EHK. Reconstruction of Zebrafish Early Embryonic Development by Scanned Light Sheet Microscopy. Science. 2008; 322(5904):1065-1069.
DOI:10.1126/science.1162493
32. Graeden E, Sive H. Live imaging of the zebrafish embryonic brain by confocal microscopy. J Vis Exp JoVE. 2009;(26).
Accessed April 8, 2024.
Available:https://www.academia.edu/download/71165747/601c7409c98a8cfa0de6d57fe295cfc56e73.pdf
33. Martin NR, Plavicki JS. Advancing zebrafish as a model for studying developmental neurotoxicology. J Neurosci Res. 2020;98(6):981-983.
DOI:10.1002/jnr.24621
34. Gerlai R. Reproducibility and replicability in zebrafish behavioral neuroscience research. Pharmacol Biochem Behav. 2019;178:30-38.
35. Stevens CH, Reed BT, Hawkins P. Enrichment for laboratory zebrafish—A review of the evidence and the challenges. Animals. 2021;11(3):698.
36. Lovett-Barron M. Learning-dependent neuronal activity across the larval zebrafish brain. Curr Opin Neurobiol. 2021;67: 42-49.
37. Shams S, Rihel J, Ortiz JG, Gerlai R. The zebrafish as a promising tool for modeling human brain disorders: A review based upon an IBNS Symposium. Neurosci Biobehav Rev. 2018;85:176-190.

38. Stewart AM, Braubach O, Spitsbergen J, Gerlai R, Kalueff AV. Zebrafish models for translational neuroscience research: from tank to bedside. Trends Neurosci. 2014;37(5):264-278.
39. Ji P, Wang Y, Peron T, Li C, Nagler J, Du J. Structure and function in artificial, zebrafish and human neural networks. Phys Life Rev; 2023.
Accessed April 8, 2024.
Available:https://www.sciencedirect.com/science/article/pii/S157106452300043X
40. de Abreu MS, Giacomini AC, Echevarria DJ, Kalueff AV. Legal aspects of zebrafish neuropharmacology and neurotoxicology research. Regul Toxicol Pharmacol. 2019;101:65-70.
41. Canedo A, Saiki P, Santos AL, et al. Zebrafish *(Danio rerio*) meets bioethics: the 10Rs ethical principles in research. Ciênc Anim Bras. 2022;23:e-70884.
42. Del Bene F, Wyart C. Optogenetics: A new enlightenment age for zebrafish neurobiology. Dev Neurobiol. 2012;72(3):404-414.
DOI:10.1002/dneu.20914
43. Kettunen P. Calcium Imaging in the Zebrafish. In: Islam MdS, ed. Calcium Signaling. Vol 740. Advances in Experimental Medicine and Biology. Springer Netherlands; 2012;1039-1071.
DOI:10.1007/978-94-007-2888-2_48
44. Girdhar K, Gruebele M, Chemla YR. The behavioral space of zebrafish locomotion and its neural network analog. PloS One. 2015;10(7):e0128668.
45. Colson V, Cousture M, Damasceno D, et al. Maternal temperature exposure impairs emotional and cognitive responses and triggers dysregulation of neurodevelopment genes in fish. PeerJ. 2019;7:e6338.
46. Levin ED, Cerutti DT. Behavioral neuroscience of zebrafish; 2011.
Accessed April 8, 2024.
Available:https://europepmc.org/article/nbk/nbk5216
47. Garcia GR, Noyes PD, Tanguay RL. Advancements in zebrafish applications for 21st century toxicology. Pharmacol Ther. 2016;161:11-21.
48. Saluja D, Jhanji R, Kaushal S, et al. Importance of zebrafish as an efficient research model for the screening of novel therapeutics in neurological disorders. CNS Neurol Disord-Drug Targets Former Curr Drug Targets-CNS Neurol Disord. 2021;20(2):145-157.
49. Hruscha A, Krawitz P, Rechenberg A, et al. Efficient CRISPR/Cas9 genome editing with low off-target effects in zebrafish. Development. 2013;140(24):4982-4987.
50. Liu J, Zhou Y, Qi X, et al. CRISPR/Cas9 in zebrafish: an efficient combination for human genetic diseases modeling. Hum Genet. 2017;136(1):1-12.
DOI:10.1007/s00439-016-1739-6
51. Kanungo J, Cuevas E, F Ali S, Paule MG. Zebrafish model in drug safety assessment. Curr Pharm Des. 2014; 20(34):5416-5429.
52. Yang L, Ho NY, Alshut R, et al. Zebrafish embryos as models for embryotoxic and teratological effects of chemicals. Reprod Toxicol. 2009;28(2):245-253.
53. Doyle JM, Croll RP. A critical review of zebrafish models of Parkinson’s disease. Front Pharmacol. 2022;13:835827.
54. Saleem S, Kannan RR. Zebrafish: an emerging real-time model system to study Alzheimer’s disease and neurospecific drug discovery. Cell Death Discov. 2018;4(1):45.
55. Sipes NS, Padilla S, Knudsen TB. Zebrafish—As an integrative model for twenty-first century toxicity testing. Birth Defects Res Part C Embryo Today Rev. 2011;93(3):256-267.
DOI:10.1002/bdrc.20214
56. Fitzgerald JA, Könemann S, Krümpelmann L, Župančič A, Vom Berg C. Approaches to Test the Neurotoxicity of Environmental Contaminants in the Zebrafish Model: From Behavior to Molecular Mechanisms. Environ Toxicol Chem. 2021;40(4):989-1006.
DOI:10.1002/etc.4951
57. Choi TY, Choi TI, Lee YR, Choe SK, Kim CH. Zebrafish as an animal model for biomedical research. Exp Mol Med. 2021;53(3):310-317.
58. Baker MR, Goodman AC, Santo JB, Wong RY. Repeatability and reliability of exploratory behavior in proactive and reactive zebrafish, Danio rerio. Sci Rep. 2018;8(1):12114.

59. Rosello M. Cutting Edge Genome Editing Technologies in Zebrafish: From Efficient and Precise Genetic Modifications to Disease Modeling and Genetic Tool Engineering. PhD Thesis. Sorbonne Université; 2021.

Accessed April 8, 2024.

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