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ZEBRAFISH AS A MODEL FOR PARKINSON'S DISEASE-INDUCED COGNITIVE DYSFUNCTION: A PHARMACOLOGICAL, BIOCHEMICAL AND BEHAVIORAL APPROACH

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ABSTRACT

The human brain is responsible for performing numerous complextasks that are essential for the survival and leading a healthylife. Cognitive functions like learning and memory, complexation, executive function, language, motor and social cognition are important characteristics of healthy brain. This is achieved with coordinated functioning of billions of neurons. Cognitive dysfunction is a disturbance in one or more of these six cognitive domains. Parkinson's disease (PD), Alzheimer's disease (AD) are the most common progressive neurodegenerative disease which affects cognitive functions of an individual. Rats and Mice are the most commonly used animal species for modeling cognitive dysfunctional though multiple models and tests are required to improve validity. This is a financial, logistical and cost constraint for scientific community. RecentlyZebrafish has emerged as a model organism for a variety of diseases. Zebrafish could be the answer to this challenge as it has many advantages overrodents.Important one is their high breeding rate which makes high-throughput screening more feasible and thus increases cost-effectiveness.This review's objective is to provide a brief discussion on the superiority of zebrafish over rodentsas a model organism for cognitive disorders especially the Parkinson's disease.

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INTRODUCTION:

Introduction to Zebrafish:

A zebrafish happens to be a tiny fish featuring striped pattern on its body that is typically under five centimeters long. Due to their low maintenance requirements, they are frequently maintained as aquarium creatures. George Streisinger suggested zebrafish for biological study in the year 1981. Zebrafish were amongst the animals that he considered because they have a perfect set of traits compared to other organisms. They are very active throughout daytime and sleep at nighttime. They are omnivorous. Insects and zooplankton are its natural food sources[1].

Zebrafish as a model animal:

The usage of zebrafish as a model species advanced in the course of the 1960s, and once the zebrafish genome was first sequenced, its utilization skyrocketed (post-1996)[1]. They gained popularity as a model species for human illnesses considering their genetic structure is extremely close to humans' [2]. The majority of their current applications revolve around the fields of genetics, developmental biology, neuroscience, and the field of molecular biology[1]. Zebrafish serve as a useful animal model due to their tiny size, quick generation, and simple, affordable care[3]. Another advantageous characteristic of zebrafish larva is the fact that they are translucent, making it easy to see how their tissues and organs are growing[4]. Their growth happens quickly. One-week-old larvae have proven to be capable of catching tiny prey, hiding off predators, and stabilizing their position in turbulent waters[5].

Zebrafish female and male:

Female zebrafish are distinguished by their bluish-white striped patterns and larger bellies. Male zebrafish have straight, slender bodies along with more golden fins than females.

Figure 1: Male and female zebrafish.

A summary of the available animal models regarding cognitive impairment:

It is abundantly evident from many reviews that living creatures have proven to be a helpful resource for simulating disorders in four out of six areas of cognition, despite the fact that it continues to be difficult to simulate a malfunction in the linguistic and perceptual motor areas of cognition.

Although non-human primates, cats, and dogs have also been utilized as models for cognitive impairment, rodents remain certainly the most popular species of animals[6]. Despite widespread disagreement on the efficacy of rodent simulations in contrast with non-human primate simulations, most research facilities throughout the globe prefer using rats since using non-human primates is financially, logistically, and ethically problematic.Yet, the reliability of outcomes from rodent models being applied to human clinical investigations has been criticized[7,8]. Using multiple approaches and replicating the research in various settings are two potential ways to increase the predictability of rodent models[7].

Zebrafish as the new animal model for investigating impaired cognition:

The conflict between legitimacy and applicability might be resolved by substituting zebrafish (Danio rerio) for rodents as the model organismwhich, as a result of their rapid rate of reproduction, are growing in popularity[9], making rapid screening possible as a result.This is due to the oviparous zebrafish's year-round, constant breeding and their brief, 3–5 months generation periods[10]. Zebrafish that are matured ex utero in the springtime also develop swiftly; they can see, swim unrestrictedly, and eat throughout the span of 72 hours.Zebrafish larvae can thrive in about 50 µl of solution throughout the initial stages of growth,allowing the rapid screening of chemicals on microtiter plates to find those that have a specific intended impact[11]. Another technique involving zebrafish is to simply dissolve the substances being examined directly into the tank water, removing the requirement for invasive therapies like injections.Zebrafish are believed to absorb chemicals dispersed in the tank's water both through their digestive tract as well as, in the instance of young zebrafish, through their skin.Such absorption methods are likely in young zebrafish who begin ingesting on their 3rd day after birth and do not begin to acquire scales as long as they've reached a few weeks of age.Nevertheless, there is a 75% homology between their genes and human genes.

This makes it quite easy to determine those human orthologues that exist in zebrafish.Zebrafish additionally possess tight junctions that are extremely permeable towards macromolecules for their blood-brain barriers, implying that zebrafish will respond strongly when exposed to substances. Zebrafish brains are anatomically comparable to the brains of humans since they both consist of a peripheral nerve system having motor, sensory, as well as autonomic elements and a forebrain, midbrain, and hindbrain that are well delineated. Additionally, zebrafish display 'higher' behaviours that incorporate brain processes like memory, programmed responses, including social behaviour. Zebrafish are a desirable model species for studies on cognitive impairment because of all these factors.

The progress towards developing a zebrafish model of cognitive dysfunction:

Zebrafish were discovered to exhibit a reduction in cognitive impairment with age, comparable to what is seen in various different animal models, including rats.Sadly, due to the zebrafish's undeveloped brain network during the larval phase, the majority of zebrafish cognitive impairment models—with the exception of those that include unrestricted or reflexive behaviors—may require to be performed in adults.

Complex attention:

Since there isn't a universally approved zebrafish attention test covering the cognitive area of complex attention, learning exercises using validated zebrafish behaviour are employed for inferring attention. The 'virtual object identification test', for example, had been designed as an exercise to assess complicated attentionin addition to the "3-choice serial reaction time task" for adult zebrafish.

Executive function:

Reversal learning tests were employed to show how the executive function-related behavioural adaptability involving adult zebrafish in the cognitive area of executive function. But it needs to be noticed that operating memory as well as feedback/error corrections are also a part of executive function[12]. Accordingly, considering the proposed definition for the range that executive function encompasses, zebrafish models intended to evaluate the cognitive areas of complex attention along with retention and recall may additionally be utilised for assessing executive function.

Learning and memory:

The three-choice discriminating tests, conditioned place preference, predator evasion, plus maze, T-maze, and three-compartment zebrafish maze are some of the behavioural activities employed to evaluate the acquisition and memory cognition areafor adult zebrafish. By administering substances like antiepileptic medications to adult zebrafish, the pharmacological approach to creating cognitive impairment have previously been employed to develop zebrafish exhibiting learning and memory deficiencies.

A summary of various assessments for cognitive impairment created for zebrafish:

Three-choice discrimination:

Purpose:

Assesses each of the five attentional domains described by Bushnell (orienting, expectation, stimulus distinction, sustained focus, as well as concurrent processing) within an animal once the domains have been expanded to incorporate extra stimuli that might serve as diversions.Demands zebrafish that they be capable of self-orientation along with the ability to predict the outcome of an activity.

Procedure:

A zebrafish is taught to navigate itself employing nourishment as a way to reinforce through an initial chamber towards one of its three chambers.

White light is used for illuminating the proper chamber that holds the nourishment, while the erroneous compartments that are vacant are left in darkness.

Critical assessment:

For selecting the right chamber, the zebrafish has to conquer its fondness for shadowy areas, which shows how it is learning instead of relying only on instincts.

The T-maze is improved with the incorporation of an extra chamber since it lowers the probability rate from fifty percent to thirty-three percent.

Three-choice serial reaction time:

Purpose:

Assesses a zebrafish's capacity for learning when faced with a behavioural challenge.

Showcases the zebrafish's ability to absorb information and remember.

Procedure:

Employing nourishment as encouragement, a zebrafish is initially taught to swim towards the response opening of the tank once it gets lighted by an external light.

After that, the conditioned zebrafish is put inside a container containing 3 response openings and provided a brief window of time to stick its nose into the illuminated opening to obtain food reinforcement.

Critical assessment:

As a result of structural variations among the two species, it remains unknown how well this assessment correlates with its rodent analogue.

It is also unclear if the stimulus light is visible from all areas of the test tank and thus afailure of the zebrafish to see the stimulus light could be incorrectly perceived as a deficitin learning and memory.

The amount of time that the zebrafish is given to eat the food reinforcement should also be carefully chosen to allow sufficient time for the zebrafish to eat the food.

Three-compartment zebrafish maze:

Purpose:

Evaluates spatial discrimination learning in zebrafish and also demonstrates avoidancediscrimination learning in zebrafish.

Procedure:

A zebrafish is first placed in the middle of a three-chambered tank, with partitions at eitherend of the central chamber.
After a minute, the partitions are lifted, and the zebrafish is allowed to swim into either theleft or right chambers.

If the zebrafish swims into the chamber designated as the 'wrong' side, the partition ispushed until it is within 1 cm from the end of the tank, in order to continue the zebrafishand 'punish' it.
After 10 seconds, the zebrafish is allowed to return to the central chamber, and the protocolcan be repeated as needed.

Critical assessment:

Zebrafish can reliably learn and remember the response contingencies when this behavioraltask is repeated multiple times.

Plus maze:

Purpose:

Evaluates a zebrafish's ability to learn and remember the association between a single visualcue and a food reward (simple associative learning), as well as the location of the foodreward (spatial learning).

Procedure:

The plus maze is placed on a rotating circular platform, and a zebrafish is first transferredinto the middle of the plus maze (four-armed radial maze), which is bounded by start boxto prevent the zebrafish from entering the arms before the start of the experiment.

The zebrafish then undergoes habituation (food provided in all arms) and shaping trials(food provided only in certain arms next to a visual cue, such as a red plastic card).

The initial training step involves the use of paired and unpaired groups whereby the food isprovided together with or independently of the visual cue.

Zebrafish learning can then be tested by providing only the visual cue with the food beinginaccessible (associative learning) or by providing the food in a fixed location relative toexternal visual cues such as room equipment, without any visual cues in the maze itself(spatial learning).

Parameters such as the time spent in the target arm and the number of entries into the targetarm versus other arms are quantified to assess zebrafish learning and memory.

Critical assessment:

The four distinct spatial locations provided by the plus maze is again an improvement overthe T-maze.
The plus maze provides stimuli in a predictable way and is thus unsuitable for measuringsustained attention.
It is also possible that the spatial learning task is accomplished by the zebrafish via nonspatialstrategies such as by remembering a single salient cue next to the target location.

Zebrafish larvae visual/acoustic stimuli habituation:

Purpose:

Evaluates zebrafish larvae nonassociative learning as the test requires a degree of memory storage and retrieval.
Zebrafish larvae show long-term habituation to visual stimuli and short-term habituation to acoustic stimuli, as demonstrated by a decline in the rate of characteristic movements in response to repeated exposure to visual or acoustic stimuli.

Procedure:

Visual stimulation is provided by equilibrating a zebrafish larva to a uniformly lit testingchamber and then abruptly extinguishing the light, which triggers a unique turningbehavior termed the O-bend:

The training procedure is then repeated at desired intervals in either a massed or spacedfashion, before returning the zebrafish to a holding tank.

The visual stimulation can then be repeated after a desired amount of time to determinehow much of the habituation training remains.

Acoustic stimulation is provided by exposing a zebrafish larva to a series of acoustic stimuliwith varying intensities and intervals between each stimulus, to trigger a characteristickinematic startle response termed 'short-latency C-start':

After a short resting period of several minutes, the protocol is repeated to determine thedegree of habituation.

Critical assessment:

Being able to use larval zebrafish allows for high-throughput screening methods.

The characteristic responses are relatively simple and may not be suitable for testing highercognitive functions.

Memory retention time also appears to be relatively short, ranging from minutes in short-termhabituation to hours in long-term habituation. Thus, zebrafish larvae may not be suitablefor testing learning and memory over longer periods of time.

Conclusions and future directions:

Zebrafish as a model organism in PD:

The zebrafish, Danio rerio, a vertebrate model organism for genetics that has emerged in the past decade or so, is a relatively uncharted system for modeling Parkinson's disease (PD).

It has become a widely used model system for the study of development and gene function. They are relatively smallfish (3–4 cm long as an adult) that can be easily managed in large numbers in specializedfacilities. Zebrafish have a short generation time (3 months) and breed prodigiously (hundreds of offspring per female per week). Embryos develop externally, can be readily manipulated genetically and are transparent. Touch and behavioral responses such as movement patterns can bemonitored; and cardiovascular, anti-angiogenic and anti-cancer drugs elicit compatibleresponses in zebrafish embryos to those in mammalian systems[13]. Zebrafish mutationsphenocopy many human disorders and the genome sequence of zebrafish is near completion[14].

Induction of Parkinson's disease in zebrafish:

Some of the toxins known to induce DA cell loss in other animal models have now also beentested in adult zebrafish[15]. Four toxic substances are commonly used to produceexperimental parkinsonism, they are MPTP, 6-hydroxydopamine, rotenone, paraquat.Systemic injection of MPTP or 6-hydroxydopamine did not alter the number of DA neurons,but DA and noradrenaline concentrations in brain tissue were significantly decreased withouta concomitant change of tyrosine hydroxylase (TH) or caspase 3 protein levels. Theswimming velocity and total distance moved decreased after exposure to both neurotoxins.Given the ease of delivering chemical compounds to zebrafish (by simply adding them to thetank water, which enables access to the CNS), the potential PD-inducing effects of MPTP, itsmetabolite MPP, and the pesticides including rotenone and paraquat, have been evaluated inboth larval and adult zebrafish[15–18]. Motor behavior (e.g. Swimming) can also bealtered by administration of the pesticide's rotenone and paraquat in both larval and adultzebrafish[16]. Adult fish were exposed to rotenone and paraquat via immersion (pesticidesdiluted in tank water) and exposed to MPTP and MPP+ via intraperitoneal injections. In adultzebrafish, only the highest single dose of MPTP resulted in a measurable effect on locomotoractivity, and no effect was seen with rotenone or paraquat at sublethal doses.This so-calleddrug-induced Parkinson is usually developing within 1 month of the initiation of theoffending medicationin approximately 60% patients and in approximately 90% within 3months. Theconditions are generally reversible, once the medications are removed, suggesting that thedrugs produce an interference with neuronal function rather than killing the neurons.Antipsychotics fluphenazine and haloperidol also impair locomotor activity in larvalzebrafish.Behavioral parameters in zebrafish for testing catalepsy:Catalepsy is the major symptom of Parkinson's disease. It can be induced in zebrafish usingstandardized dose of haloperidol (9 µg) by giving direct exposure to fishes. During inductionof catalepsy, fish will start showing aberrant swimming patterns like upside down, arrow likeswimming, circular swimming, and finally state of complete catalepsy can be achieved.

Induction of Catalepsy in zebrafish in haloperidol solution:

(a) Fish justintroduced in haloperidol solution.

(b) Aberrant swimming patterns shown by fish after sometime.

(c) State of complete catalepsy. Examination tank will be filled with fresh aerated tank water. It consisted of a 5-L tank (30 ×15 × 10 cm, length × height × width) with number of vertical lines drawn on one of the facesof tank at the spacing of 5 cm and with one horizontal line which divides the water filledportion of the tank into two equal halves. Vertical lines on tank are used to calculate thespeed of fish by measuring the time taken by fish to travel from first vertical line to last andhorizontal line gave idea about the time spent in the upper and lower half of the tank by thefish.

Examination tank used for behavioral evaluations:

Following behavioral parameters in zebrafish will be evaluated:

1. Latency to travel from one fixed point to another: In this time taken by the fish to travelfrom first vertical line to last was calculated. This gives an idea about the speed of fish underexamination.
2. Complete cataleptic time: Time for which the fish did not move at all, i.e., the time forwhich fish remained completely cataleptic.
3. Time spent near the bottom of the tank: Time spent below the horizontal line drawn ontank will be measured here. This will give an idea about the anxious behavior of the fishunder study.

1) Latency to travel from one point to another: Catalepsy diminishes the speed of fishes dueto rigidity of muscular movements. After induction of catalepsy, rigidity of fins will beobserved due to which there will be difficulty in swimming experienced by the fishes. Thus,they took longer to travel from one particular point of tank to another. Keeping this intoconsideration, time taken by fish to travel from first vertical line of examination tank to lastline will be measured at different time interval.

2) Complete cataleptic time: Time for which the fish will be in completely immovable statewill be used as index of locomotor activity in fishes.

3) Time spent near the bottom of the tank: It is a well-known fact that zebrafish are surfacefish, i.e., they swim near the surface of the water. When they are transferred to a newenvironment (tank) they initially spend more time near the bottom of the tank and after sometime they come toward surface, this is attributed towards their exploratory and most often dueto their anxiety. Thus, the time spent near the bottom of the tank gives idea about the extentof anxiety of fish. It will be calculated by measuring the time spent by the fish below thehorizontal line drawn on the examination tank[6].

CONCLUSION

Considering the evidence mentioned previously, it could be inferred that zebrafish not exclusively make excellent models for a variety of human illnesses and fundamental physiological functions in mammals, additionally, they could potentially possess the potential to serve as a model organism for various pharmaceutical treatments. Yet another intriguing approach is to use embryos from zebrafish in high-throughput screening to find novel therapeutically potent chemicals, especially once automated readings are made accessible.

In conclusion, zebrafish have the potential to replace rodents as the most widely used model of cognitive dysfunction, mainly due to their higher cost-effectiveness, and may have already begun doing so as several zebrafish models of cognitive dysfunction have already been developed. Future areas of research into the zebrafish model of cognitive dysfunction could focus on producing new models of cognitive dysfunction to complement the existing models, as well as to expand the scope of research into cognitive dysfunction that is possible using zebrafish. Already existing models should also be further examined to validate them in hopes that in the near future, certain tasks will become widely accepted tests of cognitive dysfunction in zebrafish. Another potential area of research is the development and characterization of mutant zebrafish strains to produce genetic models of cognitive dysfunction in zebrafish.

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CONFLICT OF INTEREST

The creators announce that there are no irreconcilable circumstances with respect to the production of this paper.

ABBREVIATIONS

PD : Parkinson’s disease.
DA : Dopaminergic cells groups.
TH : Tyrosine hydroxylase.
MPTP : 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.
MPP+ : 1-Methyl-4-phenylpyridinium.
CNS : Central nervous system.

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