LEARNING AND MEMORY IN ZEBRAFISH LARVAE

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A project report submitted in partial fulfilment of the requirements for the award of Bachelor of Engineering (Honours) Biomedical Engineering

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

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ABSTRACT

Understanding the mechanisms of learning and memory can be challenging due to the intricacy of the brain circuits and the constrained capability to observe neuronal activity *in vivo*. Zebrafish *(Danio Rerio)* is an emerging animal model which solves these complications due to their high behavioral repertoire and manageable brain. Over the years, different types of learning and memory tests have been devised but they were mostly catered for rodents, and limited behavioral paradigms were designed for zebrafish. In this project, a new test was developed to study the learning and memory in zebrafish larvae. Unlike the previous tests which associates cues with rewards or shocks, this test incorporates a colored T maze with light stimulus. The choice of color for T maze is based on the natural color preference of zebrafish larvae with blue as the most preferred color and yellow as the least preferred color. Light stimulus (red laser beam) was used to train the larvae to swim towards the correct pool. Zebrafish larvae treated with MK-801 (schizophrenic model) and morphine (drug abuse model) were found to take a significantly longer time to learn to swim to the correct (yellow) arm compared to the control, with the morphine-treated larvae spent the longest time, indicating different levels of cognitive deficit. This test opens a new avenue for investigating the effects of pharmacological manipulations of learning and memory in larval zebrafish, and provides an association of cognitive deficits between schizophrenia and drug abuse.

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LIST OF SYMBOLS / ABBREVIATIONS

M	Molecular Concentration, Mol
dpf	Days Post Fertilization
3D	3-Dimentional
E3	Embryo Medium
CR	Conditional Response
CS	Conditional Stimulus
US	Unconditional Stimulus
RV	relief valve
AD	Alzheimer ’s Disease
S	Schizophrenia
VTA	Ventral Tegmental Area
CNS	Central Nervous System
VOR	Vestibule-Ocular Reflex
VLNOR	Visual Lateralization Novel Objects Recognition
LES	Left Eye System
RES	Right Eye System
SAB	Spontaneous Alternation Behavior
ASR	Acoustic Startle Response
PCP	Phencyclidine
DMSO	Dimethyl Sulfoxide

CHAPTER 1

INTRODUCTION

1.1 General Introduction

This research aims to develop a new methodology which is able to study the impact of associative learning and memory for zebrafish larvae for a schizophrenic model and drug abuse model. Schizophrenia is recognized as a serious threat to humanity. About 1% of the worldwide population is diagnosed with this disease. Yearly, around 1.5 million people were diagnosed with this terrible disease. It affects people throughout their lifespan, especially during their adulthood. On the other hand, although it is also relatively rare among children or elder people, it is still possible. For male, the probability of getting diagnosed with schizophrenia increased in the teen years, reaching the top of vulnerability around age of 18 to 25 years old. Meanwhile, the vulnerability of female is twice as much as man, which is around the age of 25 to 30 years (Mentalhelp.net, 2019).

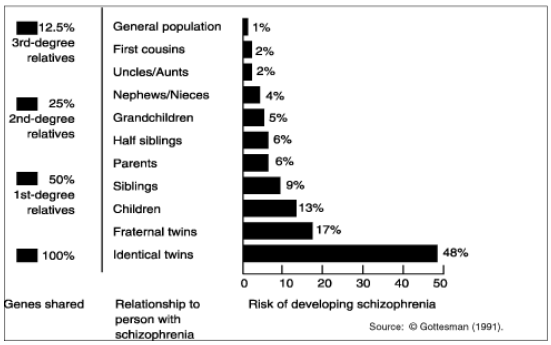

Figure 1.1: Risks of getting schizophrenia (Schizophrenia.com, 2019)

In developed countries, schizophrenia is one of the top 10 causes of disability. The risk factor of schizophrenia is illustrated in Figure 1.1. The chances for a person to be diagnosed with schizophrenia are of seven to nine percent if the family has a history of schizophrenia. Besides that, if one of the parents has schizophrenia, the child has approximately ten to fifteen percent of

chances of getting this disease. Increase of affected family members will multiply the probability of getting schizophrenia (Schizophrenia.com, 2019).

It is rather difficult to detect schizophrenia. The abuse of drugs, such as morphine, sometimes may cause a person to behave rather similar to a schizophrenic patient. Besides, most of those who were diagnosed do not believe they have this disease. Patient tends to be lack of awareness that they are ill and this in turn greatly complicates treatment. There is no exact cure for this illness, but there are several ways to treat and manage it such as using antipsychotic medications and going through psychotherapy. Schizophrenia is a type of psychotic disorder. Psychosis remains as one of the main symptoms of schizophrenia. It is a condition in which the functionality of the brain is disrupted which causes a strange state of consciousness. Schizophrenia has two main divisions of symptoms, namely positive and negative symptoms.

Schizophrenia and addiction, or disorder related to substance use, often co-occur. Schizophrenic people often engage in substance abuse as a way to self-medicate or ease feelings of anxiety and depression (Addiction Centre, 2018).

Morphine is a class of drugs known as opioid (narcotic) analgesics. Opiates are poppy-derived drugs or substances. Examples of opiates include codeine and morphine. Opiates are extremely addictive narcotics which are able to produce a robust high and a sense of well-being. Psychosis is included in one of the signs of abuse of certain opiates. The conditions or symptoms of patient are rather similar to schizophrenia, such as hallucinations and also delusions (Dual Diagnosis, 2020).

Morphine is used to treat slight to extreme pain. The short-acting formulations of morphine are taken as needed to relieve pain. Though it facilitates many people, this remedy may on occasion causes addiction. For instance, morphine can able to induce drowsiness and difficulty in defaecation, and, relying on the dosage, it is also able to compromise breathing. Taking a high dose may also motive extreme respiratory depression, loss of conscious or even death. In fact, prolonged usage of morphine may cause to physical dependency, which leads to addiction (Sinha, 2019).

Opioid addiction is a lifelong (chronic) illness which leads to significant social, health and economic issues. Opioid dependence is categorized as a robust, obsessive urge to consume opioid medications even if it is medically unnecessary. Persons who have become addicted can prioritize the use of these drugs in their life, often affecting their profession (Genetics Home Reference, 2020).

According to Nusslein Volhard and Dahm (2002), zebrafish, a species known as Danio Rerio, is a type of teleost fish in the class of ray-finned fishes. There are different stages of embryonic growth of zebrafish. Period of embryogenesis is divided into seven main periods Kimmel et al. (1995). The names and stages of the developmental process are based on their respective morphological structures identified from stereo microscope. Based on Singleman and Holtzman (2014), learning of biological concepts such as genetics, development, and behaviour of zebrafish is able to help researches to study of the human genome better. The stages of zebrafish from larval to adulthood have been examined extensively. Learning and memory of zebrafish larvae can be tested based on several types of experiment which tackle different types of learning areas. The genome of zebrafish is also very similar compared to the human genome.

In the study of Eyjolfsson et al. (2019), glutamate-induced neurotoxicity displays a significant part in neurological and psychiatric illnesses. Hence, much consideration has been given to the possible neuroprotective part of glutamate receptor antagonists, particularly those performing on the N-methyl-d-aspartate (NMDA) subtype. However, these mixtures have also neurotoxic and psychotogenic possessions. MK801, a non-competitive NMDA receptor antagonist, is able to produce schizophrenia-like changes in performance and brain metabolism in animals.

1.2 Problem Statement

Previous studies on learning and memory were mostly performed on rodents, and therefore there are limited behavioural paradigms that were designed for testing learning and memory in zebrafish. Zebrafish has been identified as an essential vertebrate model in the research on human neuropsychological

disorder such as schizophrenia because the genome of zebrafish is highly similar compared to the human's genome. Continuous research on schizophrenia is relatively important in finding the cause and cure of this illness. NMDA MK-801 is recognized as the most common type of drug administered in animal models in order to model this type of disease and there are various types of learning and memory tests that have been performed on zebrafish larvae such as motor adaptation learning and memory tests and classical conditioning learning and memory tests. However, they are rather complicated and requires longer data processing time.

On the other side of the view, there has been speculations stating that schizophrenia and drug addiction often co-relate to each other because they tend to have very similar side effects such as psychosis. Although both schizophrenia and morphine use has led to cognitive deficits, the extent of impairment remains unknown. There are limited resources that compare the associative learning and memory of zebrafish larvae that are subjected to schizophrenia drug and morphine drug.

1.3 Aims and objectives

This research aims to develop a new methodology that is able to identify the impact of associative learning and memory behavior of zebrafish larvae treated with MK-801 and morphine. Besides that, this research also aims to study the impact of acute drug exposure, morphine, on the learning and memory behavior of zebrafish larvae. The results obtained from morphine-treated larvae will then be used to compare with MK-801-treated larvae to study the association between acute morphine drug exposure and schizophrenia.The deficits of learning and memory in schizophrenia model and drug abuse model is to be studied if they both have the same extent

The specific objectives of the project include the following:

1. Develop a new methodology to investigate the impact of associative learning and memory behavior of zebrafish larvae.

2. Compare the associative learning and memory of zebrafish larvae between drug abuse model and schizophrenic model.

1.4 Scope and limitation of the project

Whole-syndrome modelling of schizophrenia in animals may experience limitation and often fails because of the heterogeneous nature and complexity of schizophrenia. However, developing models according to a specific symptom of schizophrenia, gives much advantage and success rate as the results are stable across the species boundary. Results from the animal model may be translated to results in patient with schizophrenia to validate the effect in order for the animal model to be useful in developing treatments. This research aims to introduce a simple yet effective methodology which uses the natural color preference of zebrafish larvae to study the learning and memory behavior of schizophrenic model by administrating compounds into zebrafish larvae that can induce psychosis in human, specifically MK-801 antagonist. Moreover, drug abuse model will also be mimicked using morphine in order to study the effect on associative learning and memory of drug abuse model and schizophrenia model.

The experiment was conducted on a T maze. Zebrafish larvae of age 10dpf to 12dpf were treated with MK-801/morphine and allowed to swim on its own preference. A 3D printed T maze with its left arm and pool painted with blue color while the right arm and pool painted in yellow will be used. By the fish's natural preference, it will be attracted to swim towards the blue area instead of yellow area. During the test, a laser pointer will be used to beam red laser at the blue pool for 5 seconds for every 30 seconds of interval. If the fish fails to learn to stay away from the blue pool and enters it, it will be placed back to the starting arm. This step is repeated until the fish learns to swim to the yellow arm. The time needed for each fish to learn to swim to yellow pool is observed manually and recorded. This type of learning and memory is known as operant conditioning. Learning and memory capability of zebrafish can be identified by observing the time needed to train the fish to swim towards the yellow pool. However, there is some limitation of the study. For instance, the zebrafish is randomly being assigned to tests without considering

their state of health. Fishes that are impaired since they hatched were unable to be detected.

1.5 Contribution of study

Models of animals (in this case zebrafish) can be a stable platform for researches to conduct experiment to find ways to treat or to reverse the adverse effect of the schizophrenia. This study aims to develop a simple yet effective paradigm based on the natural color preference of zebrafish larvae and light stimulus to study the impact MK-801 on associative learning and memory behavior of zebrafish larvae in mimicking schizophrenia. Findings from this study are able to develop a new testing method which saves times, energy and improved efficiency. Moreover, this study also contributes to the study on the effect of associative learning and memory for zebrafish larvae exposed with acute drug (morphine). There will be a clearer view on the comparison of associative learning and memory for a drug abuse model and also a schizophrenia model.

1.6 Outline of Report

Chapter 2 of this report outlines the literature review on selection of suitable animal to model schizophrenia and also the reason behind the selection. Besides that, discussions on current pharmacologic models that are designated for schizophrenia are also included. In addition, the morphology and tests of morphine on zebrafish will be discussed. Types of learning and memory in zebrafish larvae are analyzed along with different methods to access them are also compared.

Chapter 3 discuss about the methodology developed to assess associative learning and memory of zebrafish larvae treated with MK-801 and morphine. Different conceptual design has been proposed and analyzed to compare the efficiency of each.

Chapter 4 shows the result obtained from the experiment conducted in this study. The effect of learning and memory for all the groups tested were discussed and compared.

Chapter 5 includes the conclusion obtained from this study and proposes future recommendation or work that is able to further improve the work performed in this study to achieve better and more accurate results.

CHAPTER 2

LITERATURE REVIEW

2.1 Animal Model

2.1.1 Zebrafishas as an emerging animal model

Zebrafish, a species known as *Danio Rerio*, is a type of teleost fish under the category of ray-finned fishes, which is also known as Actinopterygii (Nusslein-Volhard and Dahm, 2002). The genome of zebrafish is 1.7 GB in size, more than half of a human's genome. When zebrafish hatched, they are considered as larvae, when they reached the age of 45 days post fertilization (dpf), they are considered as juvenile and when they are 90 dpf or beyond, they are considered as adult zebrafish (Singleman and Holtzman, 2014).

Zebrafish has been used extensively in modelling different types of neuronal diseases due to the high similarity between its genome compared to the human genome. In the study of Howe et al. (2013), the nucleotide sequence of zebrafish shows 70% homology to the human genes. 84% of the human genes are proven to be linked with the zebrafish counterparts. According to Howe et al. (2013) zebrafish is also often involved in investigation of brain disorders such as hopelessness, autism, insanities and also misuse of drugs. Zebrafish has high sensitivity towards neurotropic drugs which includes antipsychotic drugs, mood stabilizers and anxiolytics.

The brain morphology of the zebrafish and mammal models which includes the general macro-organization and cellular morphology is surprisingly similar (Panula et al., 2006). Studies of neuronal network in zebrafish are able to help understand the human brain functioning better. Zebrafish has all crucial neuro mediator systems which are similar to the human brain such as neurotransmitter receptors, transporters and enzymes, which makes it perfect for modelling neurophenotypic and associated disorders.

Table 2.1: Zebrafish in modelling neurophenotypic and Associated Disorders

Disorders	Observable Changes	Reference
Neurodegeneration	Decrease in locomotion	(Lopes da Fonseca et al., 2013)
Cognitive behavior	Long-term and short-term memory in learning tasks	(Yu et al., 2006)
Eating disorders	Reduced or increased eating	(Nguyen et al., 2013)
Reward-related behavior	Fondness towards stimuli such as nutrients and mistreated substances that is rewarding	(Stewart et al., 2011)
Psychotic Disorders (Schizophrenia)	Impaired cognitive processes	(Kokel and Peterson, 2008)

2.1.2 Comparison between different animal models

In depth research in psychotic behavior of schizophrenia may involves various animal models as a tool. Modelling schizophrenia may range from full extend of symptoms which are found in schizophrenia or specific modelling which focus on the efficiency of antipsychotic drugs. According to Feifel and Shilling (2013), modelling schizophrenia may be a great challenge since the characteristic of the illness includes acoustic delusion and delusional beliefs which might be difficult to be modelled in animal. Feasibility of animal to be modelled for schizophrenia generally depends on how the animals are able to prompt quantifiable behavioral deviations that are in line with features of schizophrenia. Animals that has been used for modelling of schizophrenia includes rats, monkey, and zebrafish. The advantage and disadvantage of animal model is summarized in Table 2.2.

Table 2.2: Advantage and disadvantage of different animal models

Animal Model	Advantage	Disadvantage	Reference
Zebrafish	• Rapid growth rate • Optical clarity • Genetic malleability • Low cost • Easy maintenance • Share 70% genome sequence identity with humans	• Short life span	(Saleem and Kannan, 2018)
Monkey	• Share 86% genome sequence identity with humans • Longer life span	• Expensive • Requires intensive regulation to maintain highest ethical standards	(Rogers and Hixson,1997); (Simen, DiLeone and Arnsten, 2019)
Rats	• Fully characterized genetic aspects • Share 64% genome identity with humans	• Short life span • Slow growth rate	(Nature,2004)

According to Table 2.2, the animal that has the highest genome similarity to a human genome is monkey. However, monkey requires intensive care to make sure that they are in good health condition to perform tests. Hence the cost of living for monkey is the highest among all the three animals listed. Rats also have fully characterized genetic aspects which help researches to understand neurochemical and neuroanatomic pathways of the brain better. However, rats have a short life span and slow growth rate. Recently, there has been a shift in using zebrafish larvae instead of rodents. Zebrafish, on the other hand, despite having a short life span have a very rapid growth rate with minimum maintenance required. Besides that, it also has a relatively high genome similarity with human, which is about 70%.

By comparing the animals listed in Table 2.2, zebrafish has the most advantage. In the study of Saleem and Kannan (2018), female zebrafish also lays about more than 200 eggs per week and this allows larger samples of specimens to be obtained for research work. The neurochemical and neuroanatomic pathways of the brain of zebrafish exhibit a very profound resemblance as compared to the human brain. The social behavioral pattern, emotional pattern and also the physiological pattern is relatively similar to the human. The nervous system of the zebrafish is also considered simple and not complex, which is very suitable to stimulate the pathology of illness such as Alzheimer's disease (AD).

According to Leonard (2019), the neuronal network of zebrafish has already fully developed at a very young age. Hence, different types of learning and memory tests can be performed on zebrafish larvae. Zebrafish in larval stage are more preferable compared to adult stage. This is because they need lesser time of maintenance and do not need to take up a large space. The externally developing embryo of zebrafish is also very clear which allows direct visualization of the fish's internal organ during the period of its growth.

Meanwhile, according to Saleem and Kannan (2018), it is much easier to maintain them in the laboratory as compared to other mammals such as rat and monkey due to the simplicity of their natural habitat. In conjunction with that, high throughput screening of the neuroactive compounds can also be easily performed because the size of the larvae is relatively small. Hence,

zebrafish poses great model systems as compared to other rodents as they allow in vivo analysis without interrupting the physiological milieu of the disease. In short, zebrafish is the most suitable animal amongst the models.

2.2 Types of learning and memory in larval zebrafish

Zebrafish larvae adopt seven types of learning and memory at different stages of their growth. According to Roberts et al. (2011), habituation learning is shown when zebrafish shows a startle response, which is the C-start by stimulus such as auditory, visual, or tactile stimulus. This response exhibit a related escape behavior during an abrupt stimulus. Most of the test performed to assess learning and memory behavior of zebrafish larvae is based on habituation learning.

For instance, acoustic startle response was performed on zebrafish to test the habituation learning. In this study, zebrafish larvae had shown reduced response towards continuous stimulus. Upon a series of acoustic stimuli, researches have demonstrated that zebrafish larvae at 7dpf exhibit reduction of startle response, showing signs of habituation, a form of learning and memory. In contrast, administration of chemicals such as Rolipram, (PDE4 inhibitor), donepezil (acetylcholinesterase inhibitor), and Memantine (NMDA receptor antagonist) in zebrafish larvae shows no reduction of acoustic startle response, which leads to decreased habituation (Best et al., 2007).

Spontaneous alternation behavior (SAB) test, is another form of habituation learning which defines that in consecutive tuning, the teleost fish tends to alternate their turn direction (Bögli and Huang, 2016). In this research, zebrafish larvae at 6dpf have already possess this type of behavior. This occurrence of SAB in larval zebrafish shows signs of memory in their early stage of growth.

In the research of Andersson, Ek and Olsson (2015), habituation learning by using visual stimuli can be investigated by using visual lateralization novel objects recognition (VLNOR) method. VLNOR test shows preserved visual bias of left-eye system (LES) of teleost fish in assessing novelty. 8dpf zebrafish shows clear preference by using LES in the first few minutes for assessing novel objects which then gradually switched to RES. In

order to mimic schizophrenia, MK-801 is used to treat the fish in order to block non-competitive NMDA receptor to test cognitive deficits. Results have shown that individuals treated with the drug failed to recognize the object.

Dishabituation is the second form of learning in zebrafish larvae. In recent research of Wolman and Granato (2012), dishabituation of C-start has been proven successful. A zebrafish larva that is habituated in C-start in response to acoustic stimuli is successfully dishabituated by exerting tactile stimulus to the head of the larva.

Classical conditioning describes the learning capability of associationof a neural stimulus with a strengthening stimulus. There are only two successful classical conditioning learning tests on zebrafish from the larval to juvenile stage. In the study of Aizenberg and Schuman(2011), a touch on the body of zebrafish larva at the side is able to train the larva to associate the light that is moving. Motor adaptation is another type of learning in which zebrafish larvae learns motor adaptation to the environment. As stated by Mo et al. (2010), calibration of Vestibule-ocular reflex (VOR) is one of the types of motor learning. VOR, a type of motor learning plays a part in generating the compensatory eye movement that opposes direction from head movement.

According to Lee et al. (2010), in the case of operant conditioning, a shuttle box is used as training procedure to prove avoidance conditioning in larvae zebrafish. Zebrafish larvae were successfully trained to stay away from the sideways of a shuttle box that is illuminated by using a red light.

In the study of Aizenberg and Schuman (2011), a classical conditioning model is formed whereby zebrafish larvae learned to associate a light stimulus, which is also known as a conditioned stimulus (CS) with a tactile stimulus, which is known as unconditioned stimulus (US). Paired CS and US shows increase neurons in cerebellum being activated as a response towards conditioning response during trial 7. This confirms that the fish is able to adapt and learn better under paired CS and US.

As said by Engeszer et al. (2007), social learning includes learning a series of social behavior, for example preference of shoaling. According to Park et al. (2016), if the point of an investigation is to deliver quick, high asymptote learning, it may be beneficial to use red colored hints. Zebrafish

show natural preferences for blue color, followed by reds, greens and finally yellow. This data is helpful in picking future shading based learning and memory ideal models. Zebrafish is also able to detect light by the age of 3.5dpf and shows mobility at onset of 5dpf.

Table 2.3: Types of Learning and Memory in Zebrafish Larvae

Types of learning and memory	Days Post Fertilization	Description	Reference
Habituation (Touch Stimuli)	2	Respond towards a series of repeated presentation of stimulus with fixed intensity	(Roberts et al., 2011).
Habituation (Auditory Stimuli)	5		
Dishabituation	5	Respond towards introduction of sensitization that diminishes the habituation of zebrafish	(Wolman and Granato, 2012)
Sensitization	5	Response enhancement towards exposure of an arousing stimulus, usually painful or noxious	(Wolman and Granato, 2012)
Classical Conditioning	5	Learning ability to associate a neural stimulus with a reinforcing stimulus	(Aizenberg and Schuman, 2011)

Motor Adaptation	5	Motor adaptation towards environment such as stabilize moving image during head turn by Vestibule-ocular reflex (VOR) system	(Mo et al., 2010).
Social Learning	7	Learning series of social behavior such as swimming in group	(Engeszer et al., 2007).
Operant Conditioning	12	Reaction towards strengthening stimulus which changes the upcoming likelihood of the animal's reaction	(Lee et al., 2010)

Among all the types of learning and memory discussed, only classical conditioning and operant conditioning are associative. The major difference between these two is that classical conditioning prompt involuntary response from animal model while operant conditioning prompts voluntary response from animal model. Besides that, the response of animal model in classical conditioning comes after stimulus while operant conditioning comes before.

This research incorporates the natural colour preference of zebrafish in a T maze. According to Park et al. (2016), blue is the most preferred color while yellow is the least preferred color.For this new methodology, zebrafish larva that swims towards the blue is applied with stimulus (threat), hence for their next attempt, they should learn and memorize the existence of threat and swim towards yellow arm voluntarily. The type of learning and memory implemented is operant conditioning, it matches the aim of the study, which is to trigger the animal model to learn and memorize and behaves according to the stimulus applied. This methodology is different from the previous learning and memory tests because it is the first methodology that uses combination of the natural colour preference and light stimulus to train the zebrafish to learn and memorize.

2.3 Schizophrenia and Opioid Misuse

The comorbidity of schizophrenia and drug abuse in recent years has attracted more and more attention with multiple potential links that are under discussion, including neurobiological aspects, side effects of drugs and psycho-social considerations. Specific neurobiological mechanisms and disorders tend to be commonly implicated in different psychiatric conditions and addiction (Brady K. and Sinha R., 2005).

One of the main symptoms of schizophrenia is cognitive deficits. Schizophrenic patients tend to lose their ability to learn and memorize. However, high intake of drug such as opioid also leads to cognitive deficit. Schizophrenia and addiction, or disorder related to substance use, often co-occur. Schizophrenic people often engage in substance abuse as a way to self-medicate or ease feelings of anxiety and depression (AddictionCenter, 2018).

2.3.1 Schizophrenia - Origin of Madness

Schizophrenia is a type of chronic and severe mental disorder that highly affects a person's daily living in terms of the method or pathway a person reasons, expresses feelings, perform, recognizes realism, and associate with others. As compared to other major illness such as Alzheimer's disease, schizophrenia is rather uncommon. Patients suffering from this illness face problems such as not doing well in the public, at labor, at institute, or even relationships. This is mainly because they might feel frightened and withdrawn, and seems to have lost touch with reality world.

Positive symptoms of schizophrenia are symptoms that present in schizophrenia patients in which other non-schizophrenia patient will not experience. Negative symptoms are symptoms which include lack of some characteristics that are often found in schizophrenia patient that are "healthier". The word negative is used to describe a feeling of lost or a sense of disappearance from patient's experience.

One of the core features of schizophrenia is cognitive dysfunction. This type of deficits ranges from moderate to severe in different aspects which includes working memory, attention, executive functions and also verbal

learning and memory. These deficits are stable throughout the course of illness in patients and indicate the onset of psychosis (Bowie and Harvey, 2006).

The exact cause of schizophrenia still remains unknown. However, there are several theories behind this psychotic disease. According to Knight (1982), genetic abnormality may be one of the factors that cause schizophrenia.

People who have a family history of psychiatric diseases will surge the probability of having schizophrenia, for about ten percent. Antipsychotic drugs block the dopamine receptors and this perception has prompted the speculation that over action of dopaminergic pathways is the reason for the psychotic symptoms. Dopamine-receptor-invigorating autoantibodies are hypothesized to cause the dopaminergic hyperactivity, in this way representing the irregularity.

However, in the research of Olney, Newcomer and Farber (1999), hypofunction of the NMDA glutamate receptor gives a more promising concept in causing schizophrenia. Drugs that were used to block NMDA glutamate receptors, such as phencyclidine (PCP) and MK-801all triggers acute psychotic reactions. Hence, disorder prompted by tricNMDA antagonist can be recognized as a model in which the mechanism of the disease can be used to explain symptoms of schizophrenia.

2.3.2 Comparison on pharmacologic models

A common approach to develop animal models that mimic schizophrenia is to apply pharmacologic treatment. There are several drugs that are able to represent one or more of the signs of schizophrenia. Different pharmacologic models of schizophrenia are developed by considering both the effect of patient treated with drug and also supporting evidences from animal models.

Table 2.4: Pharmacologic models of schizophrenia

Drug	Description	Reference
Dopaminergic (Eg: amphetamine, cocaine)	• Patients with schizophrenia has abnormal dopaminergic function • Overactive mesolimbic dopaminergic transmission leads to	(Davis et al, 1991) ; (Kapur et al, 2005)

	positive delusional symptoms • Dopamine system that is underactive in frontal cortex leads to negative symptom of schizophrenia	
Glutamatergic (Eg : Ketamine, phencyclidine (PCP), MK-801)	• Usage of NMDA receptor antagonists induces locomotor, behavioral, cognitive changes that is similar to schizophrenia • Ketamine worsen the positive symptoms and also the negative symptoms of patients with forerunning schizophrenia symptoms • Acute doses of ketamine lead to impairment in cognition, especially memory and paranoid delusions • Chronic PCP leans to greater auditory rather than visual hallucinations • MK-801 is associated with side effects that includes cognitive disruption and psychotic-spectrum reaction • Chronic PCP, Ketamine, and MK-801 shows persistent schizophrenia-like symptoms	(Coan, Saywood and Collingridge, 1987); (Lahti et al, 1995); (Stone et al, 2012) ; (Jentsch and Roth, 1999); (Jones et al, 2011)
Serotonergic (Eg: Serotonin)	• Serotonin (5-HT) system induces psychotic symptom which includes anxiety, visual hallucination, anxiety and illusion, which are similar to first psychotic episode of	(Vollenweider and Geyer, 2001)

	schizophrenia	

According to Pratt et al (2012), although dopaminergic psychostimulants is able to mimic and induce schizophrenia, the cognition and also negative symptoms of schizophrenia is not precisely mimicked. In contrary, in the research of Morrison et al (2009), NMDA receptor antagonist, which is under glutamatergic psychostimulants is able to produce a more complete model of schizophrenia by taking consideration into the features of positive and negative intellectual signs. Based on Roth et al (2004), serotonergic models have been proven to develop cognitive or negative symptoms in patients with schizophrenia but it is still lack of effectiveness against positive symptoms. By considering all of the choices above in Table 2.4, NMDA receptor antagonist is the best preferred choice.

NMDA receptor antagonist functions to inhibit the action of NMDA receptor. Examples of antagonists include ketamine, phencyclidine, methoxamine and dizocilpine (MK-801) (Coyle, 2012). These antagonist is able to replicate the entire range of psychotic, cognitive, negative and even physiologic features of schizophrenia. Consistent with Tricklebank et al (1989), among all three types of antagonists, MK-801 is the strongest, followed by PCP, and lastly Ketamine. Hence, MK-801 NMDA antagonist is preferably used in study of schizophrenia.

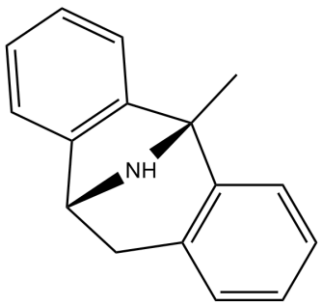

Figure 2.1: Molecular Structure of MK-801(Rung et al., 2005).

MK-801 is one of the non-competitive antagonists of NMDA receptor, which is a glutamate receptor (Rung et al., 2005). Models treated with MK-801 are able to mimic positive and negative symptoms of schizophrenia by single injection.

As stated by Sison and Gerlai (2011), amongst the concentration of 0, 2, 20 and 100 μM concentration of MK-801 applied on zebrafish larvae, 20 μM is the most suitable dosage used in treating the zebrafish larvae in order to perform tests that analyse the learning and memory because this dosage does not lead to major performance shortages. Hence, MK-801 of 20 μM is the optimum drug dosage for zebrafish larvae to perform neurological tests.

2.4 Morphine

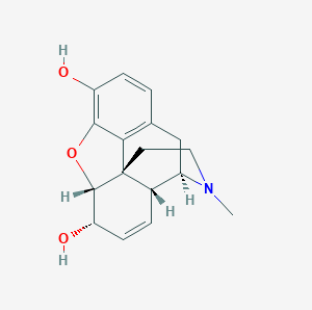

Figure 2.2: Molecular Structure of Morphine (Pubchem.ncbi.nlm.nih.gov, 2020)

The class of morphine that is used in this experiment is morphine sulphate. Morphine sulphate has a molecular formula of 383.4 g/mol with a molecular structure of $C_{17}H_{21}NO_7S$. Morphine is an opiate alkaloid that is synthetically produced and isolated from papaversomniferum plants. Morphine adheres to and stimulates certain opiate receptors such as delta, mu, and kappa which each participate in regulatory various brain tasks (Pubchem.ncbi.nlm.nih.gov, 2020). This agent has extensive impact in CNS and gastrointestinal systems, including analgesics, anxiolysis, and smooth muscle contractions of the gastrointestinal system Morphine is a highly potent opioid analgesic psychoactive drug, a morphinane alkaloid. The morphine acts on the central nerve directly to alleviate pain, but has high addiction potentials with rapidly developing tolerance and physical and psychological dependence. Morphine binding in the opioid receptors blocks nociceptive signal transmission, enables the transmission of pain modulating neurons in the reverse cord and inhibits

the signaling of sensory dorsal horneal projection cells from primary afferent nociceptors(Pubchem.ncbi.nlm.nih.gov, 2020).

Table 2.5: Experiments on Effect of Morphine to Zebrafish

Type of Test	Description / Findings	Citation
Response to nociception	The results suggest that larvae respond similarly to adult zebrafish and other vertebrates to a noxious challenge and that the effect on the nociception can be improved through the use of analgesics.	(Lopez-Luna et al., 2017)
Conditioned place preference paradigm	Zebrafish show strong behaviors of preferences caused by natural reward foods and morphine addictive drugs.	(Lau et al., 2006)
Habituation	Acute dosage of morphine (2.0 mg/L × 15 min) exposure to adult zebrafish shows no effect on habituation.	(Wong et al., 2010)
Gene expression	With chronic exposure, morphine causes changes, as in humans and rodents, in the expression of the genes in zebrafish embryos.	(Herrero-Turrión et al., 2014)

Based on Table 2.5, there had been many studies done on zebrafish by using morphine. According to Lau et al. (2006), it has been proven that zebrafish exhibit an extreme place preference towards morphine after being exposed to it, suggesting addiction. It is found that when the system water is mixed with morphine, quantifiablealtitudes of drug can be noticedwithin zebrafish's brain and this induces dynamic conditioning place preference, suggesting quite rapid metabolism. This shows successful drug modelling in zebrafish.

According to the study of Lopez-Luna et al. (2017), zebrafish larvae as early as 5dpf already exhibit responds towards morphine, similar of those adult zebrafish. This has allowed the direct replacement of larval zebrafish to a protected adult fish with a non-protected form in pain- and nociception-related

research. Morphine may not necessarily have effect on all the aspects of behavior of a zebrafish. For instance, in the study of Wong et al. (2010), acute morphine does not have an effect on the habituation of zebrafish larvae.

The comparison between the effect of learning and memory of zebrafish larvae on acute exposure of morphine (drug abuse model) and also schizophrenic model has not been thoroughly studied and verified yet. The result obtained from this study is able to provide a clearer view on the impact and association of learning and memory between drug abusive model and schizophrenia model whether their learning capability is up to the same extent.

CHAPTER 3

METHODOLOGY

3.1 Animal Maintenance and Breeding

All procedures performed in this study were complied with Universiti Tunku Abdul Rahman Research Ethic and Code of Conduct. Embryos of either sex were obtained from natural spawning of AB wild-type adult zebrafish and kept in a beaker containing E3 embryo medium (5mM NaCl, 0.17mM KCl, 0.33mM CaCl2, 0.33mM MgSO4). The embryos were kept at 28°C and maintained at 14 hour light/10 hour dark cycle (Hinz et al., 2013). The larvae were transferred to dechlorinated water maintained at pH of 6.8 to 7.5 upon hatching. Dechlorinated water was prepared by leaving a beaker of tap water overnight before use. In order to maintain good water quality, ten percent of water in the beaker was changed each day. The zebrafish larvae were fed twice or trice daily. They were fed with paramecium starting 5 days post fertilization (dpf) and with brine shrimps when they reached 9 dpf.

To culture paramecium, 900 ml of tap water and two red beans were added into a 1-litre empty bottle. The bottle was covered and autoclaved. After the red bean solution has cooled down, half a tea spoon of yeast was added and swirled. 100 ml of paramecium master stock was subsequently added. The entire bottle was maintained at room temperature with the cap loosen. It was allowed to sit for two days under light. After two days, the presence of paramecium can be confirmed by checking a drop of solution under the microscope.

3.2 Pharmacology

3.2.1 Stock Preparation

3.2.1.1 MK-801

MK-801 was purchased from Sigma-Aldrich (St. Louis,MO). It was dissolved in dimethyl sulfoxide (DMSO) to make stock solution. The amount (mass) of MK-801 powder needed to dissolve in DMSO in order to achieve the desired concentration can be calculated using Equation 3.1.

$$\text{Mass (g)} = \text{Concentration (mol/L)} \times \text{Volume (L)} \times \text{Molecular Weight (g/mol)} \quad (3.1)$$

The stock solution was prepared at 20mM. It was diluted to 20 μM before as this concentration was found to be the most optimum for treating zebrafish larvae (Sison and Gerlai, 2011). Equation 3.2 shows the dilution equation.

$$M1\ (\text{mol/L}) \times V1\ (\text{L}) = M2\ (\text{mol/L}) \times V2\ (\text{L}) \quad (3.2)$$

Where,

M1 =initial concentration in molarity

V1 = volume of concentrated solution

M2 = final or desired concentration in molarity

V2 = volume of diluted solution

Once the stock solution was prepared, it was tightly sealed and stored at -20°C. It was thawed to room temperature before use. The drug can be used up to one month from the day of preparation.

3.2.1.2 Morphine

Morphine sulphate of 10mg/ml was first diluted in distilled water to prepare the stock solution at 2.6 μM. It was diluted to a concentration of 0.8μM for the use of this experiment. This is the most optimum dosage used to treat zebrafish larvae to achive optimum response (Bretaud et al., 2007).

3.2.2 Treatment

Zebrafish larvae were divided into four groups, DMSO, MK-801, morphine and control, each consists of 30 larvae. Zebrafish larvae under control group was immersed in dechlorinated water, under DMSO group was immersed in 0.1% DMSO, under morphine was immersed in morphine with concentration of 0.8μM, while under treated group was immersed in MK-801 with a concentration of 20 μM. Each larva was placed individually inside the well of 96-well microtiter plates. They were treated with the drugs three hours prior to the experiment.

3.3 T maze

3.3.1 Conceptual Design 1

Conceptual Design 1 aims to assess the Spontaneous Alteration Behaviour (SAB) of zebrafish larvae at 12dpf. The maze design was adopted from Bögli and Huang (2016). It was 3D printed in white to ease the observation of the location of the zebrafish larvae.

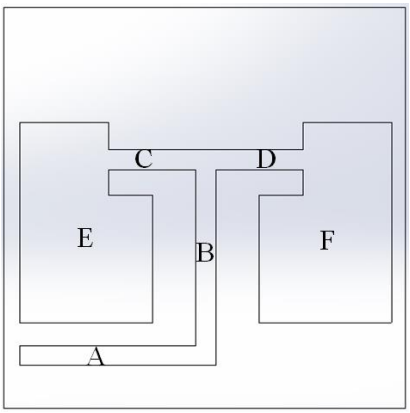

Figure 3.1: Schematic diagram of T maze drawn using *Solidworks*

Figure 3.2: Photograph of the 3D-printed T maze (top view)

The maze consists of four arms and two pools. The starting arm (A) and the main arm (B) are 1.97 inch in length, while the left and the right arms (C/D) are 0.98 inch in length. Each arm has a width of 0.2 inch, a depth of 0.39 inch, and interlocking intersections of 0.98inch^2. There are two goal arms

that lead to two separate pools (E/F)with a size of 76.77inch^2. Dechlorinated water was used to fill the maze until it reached a height of 0.2 inch.

SAB defines the tendency of teleost fish to alternate their direction in consecutive turns. Each choice that they make depends on the previous one. This behaviour requires no prior training or reinforcement. For example, after the zebrafish made a left turn from starting arm A to arm B, they would have a higher preference to make a right turn to arm D. Punishment is given to the larvae that made a right turn by pushing them out from arm D so that they would avoid taking the same route in the next attempt.

A preliminary test has been performed using this method. However, the results obtained were not satisfying. After the zebrafish larvae made a left turn from arm A to arm B, the number of larvae which made a right turn from arm B to arm D was almost equal to the larvae which made a left turn from arm B to arm C. The zebrafish larvae did not produce convincing result that they possess the SAB strongly. Hence this design was dropped.

3.3.2 Conceptual Design 2

After reconsidering all the natural behaviour of zebrafish larvae, the maze from conceptual design 1 was modified to incorporate colour preference of zebrafish larvae.

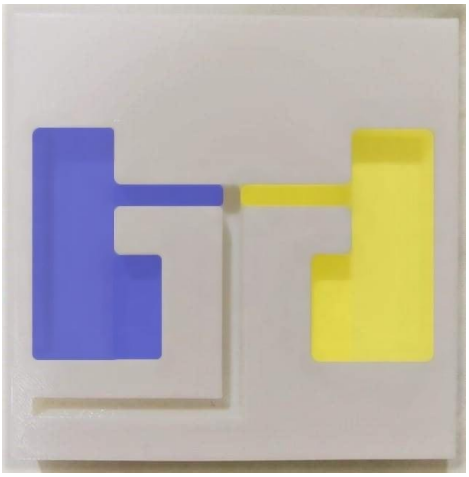

Figure 3.3: Modified T maze from Conceptual Design 1

A T maze with the exact same dimension as in Conceptual Design 1 was modified by painting the left arm and pool blue (most preferred colour)

while the right arm and pool yellow (least preferred colour). The center of the maze is remained in white. It acts as a starting point whereby the fish was placed at the beginning of the test. This design allows the zebrafish larvae to view the two choices of colour when they are oriented at the center of the maze. The colour selection for the maze is based on the innate color preference of the zebrafish larvae – blue is the most preferred colour while yellow is the least preferred colour. The ideology of this design includes the natural colour preference of zebrafish larvae and also a light stimulus. The main idea is to train the fish to swim towards the least preferred zone (yellow). A light stimulus (red laser pointer) was beamed at the blue pool. This light stimulus 'threatens' the larvae, triggering them to learn and memorize to swim away from the blue pool.

3.4 Behavioral Test

Zebrafish is most active during day time, and less active during night time (López et al, 2006). Hence the experiment was conducted during day time from 11am to 4pm.

Colour Preference Test

Before proceeding to learning and memory test, the natural colour preference of zebrafish larvae was first validated to prove that they have a natural tendency to swim towards blue coloured arm instead of the yellow coloured arm. 81 zebrafish larvae of 10dpf to 12dpf were divided into three groups with each consisting 27 larvae, and were tested consecutively. Firstly, a group of zebrafish larvae were placed at the starting arm A of the T maze. The pathway between arm A and arm B was blocked by a cylindrical tube for fifteen minutes to allow the larvae to familiarize themselves with the surrounding of the maze. After 15 minutes, the obstacle was removed and the larvae were allowed to swim freely. After another 15 minutes, the number of larvae in the yellow-coloured and the blue-coloured pools was recorded.

Learning and Memory Test

First of all, the larva was placed at the starting arm A of the T maze. The pathway between arm A and arm B was blocked by a cylindrical tube for fifteen minutes to allow the larvae to familiarize with the surrounding of the maze. After 15 minutes, the obstacle was removed and the larva was allowed to swim freely. During the test, a telescopic laser pointer with wavelength of 650nm was used to beam red laser at the blue pool for 5 seconds for 30 seconds of interval. If the fish fails to learn to stay away from the blue pool, it was placed back to the starting arm A. This step is repeated until the fish learns to recognize and remember the existence of threat (red laser as stimulus) at blue pool and learns to swim to the yellow pool. The time needed for each fish to learn to swim to yellow pool was recorded.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Colour Preference Test

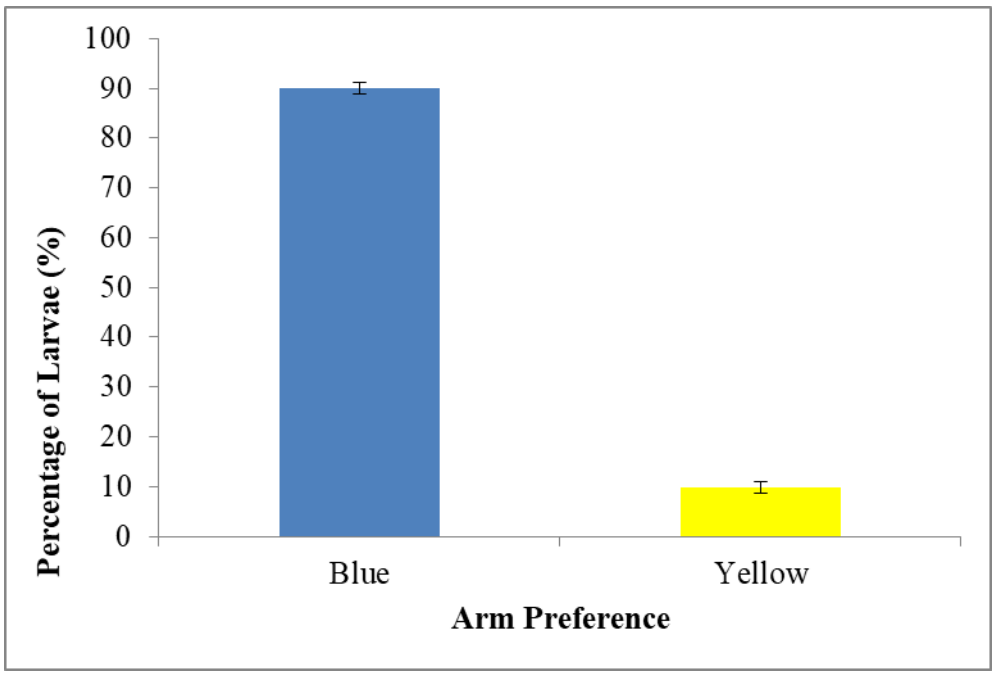

Figure 4.1: Bar chart showing the percentageof larvaeaccording colour preference

Colour Preference Test reveals that among 81 zebrafish larvae tested, the number of zebrafish larvae that enters blue arm are 90%±2.5%, while the number larvae which enter the yellow arm are 10%±2.5%, showing distinct colour preference for both colour groups.

4.2 Learning and Memory Test

Figure 4.2 illustrates the average amount of time required for learning and memory each group. The mean time for the morphine group, to larvae to learn and memorize, is the highest (588.83±164.937seconds), followed by MK-801(413.10±149.179 seconds), DMSO (230.87±114.711 seconds), and lastly, control group (223.37±120.142 seconds).

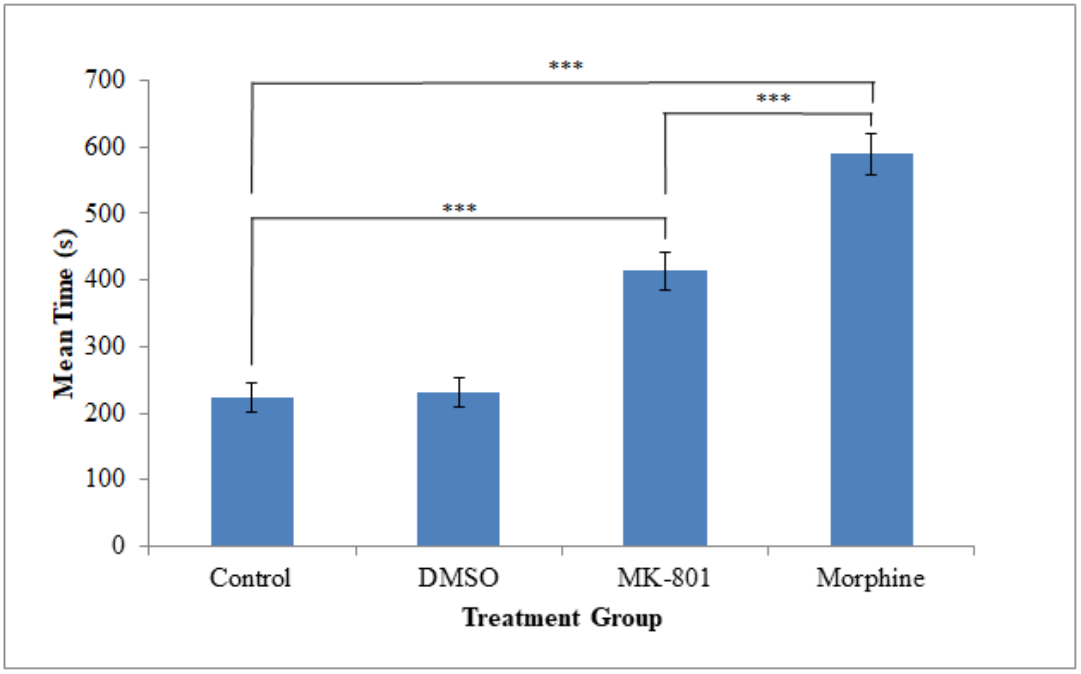

Figure 4.2: Mean time needed to learn and memorize for each group

A one-way analysis of variance ANOVA was conducted to compare the effect of associative learning and memory for control, DMSO, MK-801 and morphine treatments group. There was a significant difference of associative learning and memory capability between the groups [F (3,116) =46.963, p=0.001]. Post hoc comparisons using the Tukey HSD test indicated that the mean time needed for learning and memory for zebrafish larvae is significantly higher for morphine group (M=588.83, SD = 164.937, p <0.001) and MK-801 group (M=413.10, SD = 149.179, p <0.001) compared to control group (M=223.37, SD = 120.142). In addition, test results also revealed that morphine group and MK-801 group are significantly different to each other as well (p <0.001). However, the mean time needed for learning and memory for zebrafish larvae is not significantly different between control and DMSO group (M = 230.87, SD = 114.711, p= 0.997).

4.3 Summary

Results obtained from the colour preference test showed that zebrafish larvae exhibits higher favourability towards blue colour instead of yellow colour, as the number of larvae that stays in blue pool was found to be higher than yellow pool. This result is in agreement with the findings of a recent study which claims that zebrafish larvae have a clear preference of entering blue zones and to avoid yellow zones (Park et al., 2016). Zebrafish larvae have a very distinct colour preference and colour discrimination during larval stage as early as 5dpf (Park et al., 2016). Recent studies have found that the location of the colour also plays an important role as presentation of colour on the walls of the tank triggers an approach response while presentation of colour on the bottom might induce an aversion (Peeters BW et al., 2016). However, this study shows that this effect does not inhibit the urge of zebrafish larvae to enter the blue goal arm as their natural preference. Upon confirming the natural preferred colour of zebrafish larvae, this specific preference of zebrafish larvae was incorporated in the learning and memory test of zebrafish larvae.

Zebrafish larvae were observed to be able to recognize and memorize the existence of threat (red laser beam) at blue pool, and swim towards the yellow pool. This type of learning adopted is known as operant conditioning learning method, whereby the larvae show avoidance conditioning via a stimulus. The successful usage of red laser in training zebrafish larvae was observed in the study of Lee et al (2010), which pairs red laser (stimulus) with mild shock to train larvae to stay put from the sideways of a shuttle box illuminated using red light. In this study, red laser (stimulus) was paired with the natural color preference of larvae for training. Experimental test results from this newly developed methodology affirmed the effectiveness of using red laser beam as stimulus as the larvae were successfully trained to enter yellow (least preferred color) pool.

This methodology had revealed that MK-801 has imposed a significantly adverse impact on the learning and memory of zebrafish larvae. The results obtained from this study is consistent with the findings of

Andersson, Ek and Olsson (2015), which showed impaired learning and memory of zebrafish larvae in the visual lateralization novel objects recognition (VLNOR) test on 8dpf treated with MK-801. Zebrafish larvae shows preserved visual bias of left-eye system (LES) in assessing novelty and they have shown to have use longer period of LES to access novelty after treated with MK-801, suggesting slower familiarization or recognition of novelty. Impairment of learning and memory in zebrafish larvae administered with MK-801 was unlikely due to disruption of perception or motor function, but due to decrease in the glutamatergic neuro-transmission by NMDA receptor antagonists (Xu X. et al., 2004).

Zebrafish exhibited aggressive and erratic swimming behaviour when first transferred to the T maze, mimicking the anxiety-like symptoms. Restlessness with recurrent short hyperactivity bouts were similarly observed in previous studies involving acute administration of morphine (Stewart A. et al., 2011). Opioid antagonists such as morphine manage to trigger anxiety in zebrafish larvae usually by inhibiting the functioning opioid ligands that are “anti-anxiety” (Stewart A. et al., 2011). Remarkably, zebrafish treated with morphine also spent the longest time to learn to enter the yellow pool, suggesting greater cognitive deficits for morphine group compared to the MK801-treated group.

The behavioral paradigm developed in this study enables one to assess associative learning and memory in zebrafish larvae with relative ease. The findings from the learning and memory test also provide a clearer view on comparison between a drug abuse model and a schizophrenia model, which was not reported earlier.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In a nutshell, this newly developed methodology which incorporates the natural colour preference of zebrafish larvae were able to successfully assess the associative type of learning and memory of zebrafish larvae treated with MK-801. In addition, comparison between drug abusive model and schizophrenic model has been made and test results has revealed that zebrafish larvae treated with morphine (drug abusive model) shows greater adverse impact on associative learning and memory as compared with MK-801 (schizophrenic model). All in all, this study is able to develop a new methodology which applies the natural colour preference of zebrafish larvae and a light stimulus on a T maze to study the associative learning and memory of zebrafish larvae. This research is also able to provide a clearer view on the comparison between associative learning and memory for a schizophrenic model and drug abuse model.

5.2 Recommendations for future work

Future investigation may be done to further characterize the learning and memory response by environmental aspects such as stressors (e.g., crowding effect), predator exposure, different genetics, or gender based test experiment. Chronic morphine exposure on zebrafish larvae could also be performed to identify its impact on learning and memory of zebrafish larvae.

APPENDIX

Appendix A: Statistical Test Results

Descriptive Data for Each Group

Time needed								
					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Control	30	223.37	120.142	21.935	178.50	268.23	23	480
DMSO	30	230.87	114.711	20.943	188.03	273.70	53	530
MK801	30	413.10	149.179	27.236	357.40	468.80	120	630
Morphine	30	588.83	164.937	30.113	527.25	650.42	263	898
Total	120	364.04	203.919	18.615	327.18	400.90	23	898

Anova

Time needed					
	Sum of Squares	Degree of Freedom	Mean Square	F	Sig.
Between Groups	2713891.492	3	904630.497	46.963	0.000
Within Groups	2234485.300	116	19262.804		
Total	4948376.792	119			

Post Hoc

Dependent Variable: Time needed						
Tukey HSD						
					95% Confidence Interval	
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Control	DMSO	-7.500	35.836	.997	-100.91	85.91
	MK801	-189.733*	35.836	.000	-283.14	-96.32
	Morphine	-365.467*	35.836	.000	-458.88	-272.06
DMSO	Control	7.500	35.836	.997	-85.91	100.91

	MK801	-182.233*	35.836	.000	-275.64	-88.82
	Morphine	-357.967*	35.836	.000	-451.38	-264.56
MK801	Control	189.733*	35.836	.000	96.32	283.14
	DMSO	182.233*	35.836	.000	88.82	275.64
	Morphine	-175.733*	35.836	.000	-269.14	-82.32
Morphine	Control	365.467*	35.836	.000	272.06	458.88
	DMSO	357.967*	35.836	.000	264.56	451.38
	MK801	175.733*	35.836	.000	82.32	269.14
*. The mean difference is significant at the 0.05 level.						

APPENDIX B: Specifi cation for Telescopic Laser Pointer

Specifications:
Product Name: 5 in 1 Telescopic Laser Pointer
Optical power: 5MW
The laser wavelength: 650nm
Powered by: 3pcs x AG3 button cell batteries(Included)
Material: Aluminum
Color: Silver
Pen length: app.15cm/5.91in
The maximum tensile size: 45cm/17.72in

REFERENCES

Andersson, M.Å., Ek, F. and Olsson, R., 2015. Using visual lateralization to model learning and memory in zebrafish larvae. *Scientific reports*, *5*, p.8667.

AddictionCenter. (2018). *Schizophrenia and Addiction - Addiction Center*. [online] Available at: https://www.addictioncenter.com/addiction/schizophrenia/ [Accessed 12 Feb. 2020].

Aizenberg, M. and Schuman, E., 2011. Cerebellar-Dependent Learning in Larval Zebrafish. *Journal of Neuroscience*, 31(24), pp.8708-8712.

Best, J., Berghmans, S., Hunt, J., Clarke, S., Fleming, A., Goldsmith, P. and Roach, A., 2007. Non-Associative Learning in Larval Zebrafish. *Neuropsychopharmacology*, 33(5), pp.1206-1215.

Bögli, S. and Huang, M., 2016. Spontaneous alternation behavior in larval zebrafish. *The Journal of Experimental Biology*, 220(2), pp.171-173.

Bowie, C. and Harvey, P., 2006. Cognitive deficits and functional outcome in schizophrenia. *Neuropsychiatric Disease and Treatment*, 2(4), pp.531-536.

Brady K. , Sinha R. Co-occuring mental and substance use disorders: the neurobiological effects of chronic stress. *Am J Psychiatry*. 2005;162:1483–1493

Bretaud, S., Li, Q., Lockwood, B.L., Kobayashi, K., Lin, E. and Guo, S., 2007. A choice behavior for morphine reveals experience-dependent drug preference and underlying neural substrates in developing larval zebrafish. *Neuroscience*, *146*(3), pp.1109-1116.

Coan, E., Saywood, W. and Collingridge, G., 1987. MK-801 blocks NMDA receptor-mediated synaptic transmission and long term potentiation in rat hippocampal slices. *Neuroscience Letters*, 80(1), pp.111-114.

Coyle, J., 2012. NMDA Receptor and Schizophrenia: A Brief History. *Schizophrenia Bulletin*, 38(5), pp.920-926.

Davis, K.L., Kahn, R.S., Ko, G. and Davidson, M., 1991. Dopamine in schizophrenia: a review and reconceptualization. *The American journal of psychiatry*.

Dual Diagnosis. (2020). *Schizophrenia and Opiate Use | Dual Diagnosis*. [online] Available at: https://dualdiagnosis.org/mental-disorders-caused-addiction/schizophrenia-and-opiate-use/ [Accessed 12 Feb. 2020].

Engeszer, R., Da Barbiano, L., Ryan, M. and Parichy, D., 2007. Timing and plasticity of shoaling behaviour in the zebrafish, Danio rerio. *Animal Behaviour*, 74(5), pp.1269-1275.

Eyjolfsson, E., Brenner, E., Kondziella, D. and Sonnewald, U., 2019. *Repeated injection of MK801: An animal model of schizophrenia?*.

Fader, S., 2019. *What Is Dopamine And How Does It Affect The Brain And The Body? | Betterhelp*. [online] Betterhelp.com. Available at: https://www.betterhelp.com/advice/medication/what-does-dopamine-do-in-the-brain-and-what-is-its-function/ [Accessed 27 Jul. 2019].

Feifel, D. and Shilling, P., 2013. Modeling Schizophrenia in Animals. *Animal Models for the Study of Human Disease*, pp.727-755.

Genetics Home Reference. (2020). *Opioid addiction*. [online] Available at: https://ghr.nlm.nih.gov/condition/opioid-addiction [Accessed 12 Feb. 2020].

Grundt, P., Jane Husband, S., Luedtke, R., Taylor, M. and Newman, A., 2007. Analogues of the dopamine D2 receptor antagonist L741,626: Binding, function, and SAR. *Bioorganic & Medicinal Chemistry Letters*, 17(3), pp.745-749.

Herrero-Turrión, M., Rodríguez-Martín, I., López-Bellido, R. and Rodríguez, R. (2014). Whole-genome expression profile in zebrafish embryos after chronic exposure to morphine: identification of new genes associated with neuronal function and mu opioid receptor expression. *BMC Genomics*, 15(1), p.874.

Hinz, F., Aizenberg, M., Tushev, G. and Schuman, E., 2013. Protein Synthesis-Dependent Associative Long-Term Memory in Larval Zebrafish. *The Journal of Neuroscience*, 33(39), pp.15382-15387.

Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L. and McLaren, S., 2013. The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*(7446), pp.498-503.

Jentsch, J.D. and Roth, R.H., 1999. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology*, *20*(3), pp.201-225.

Jones, C.A., Watson, D.J.G. and Fone, K.C.F., 2011. Animal models of schizophrenia. *British journal of pharmacology*, *164*(4), pp.1162-1194.

Kamboj, S.K., Tookman, A., Jones, L. and Curran, H.V., 2005. The effects of immediate-release morphine on cognitive functioning in patients receiving chronic opioid therapy in palliative care. *Pain*, *117*(3), pp.388-395.

Kapur, S., Mizrahi, R. and Li, M., 2005. From dopamine to salience to psychosis—linking biology, pharmacology and phenomenology of psychosis. *Schizophrenia research*, *79*(1), pp.59-68.

Kimmel, C., Ballard, W., Kimmel, S., Ullmann, B. and Schilling, T., 1995. Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203(3), pp.253-310.

Knight, J., 1982. DOPAMINE-RECEPTOR-STIMULATING AUTOANTIBODIES: A POSSIBLE CAUSE OF SCHIZOPHRENIA. *The Lancet*, 320(8307), pp.1073-1076.

Kokel, D. and Peterson, R., 2008. Chemobehavioural phenomics and behaviour-based psychiatric drug discovery in the zebrafish. *Briefings in Functional Genomics and Proteomics*, 7(6), pp.483-490.

Lahti, A.C., Koffel, B., LaPorte, D. and Tamminga, C.A., 1995. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology*, *13*(1), pp.9-19.

Lau, B., Bretaud, S., Huang, Y., Lin, E. and Guo, S. (2006). Dissociation of food and opiate preference by a genetic mutation in zebrafish. *Genes, Brain and Behavior*, 5(7), pp.497-505.

Lee, A., Mathuru, A., Teh, C., Kibat, C., Korzh, V., Penney, T. and Jesuthasan, S., 2010. The Habenula Prevents Helpless Behavior in Larval Zebrafish. *Current Biology*, 20(24), pp.2211-2216.

Leonard, I., 2019. Zebrafish: A New Model for Human Disease. [online] Available at: https://genome.cshlp.org/content/9/2/99.full [Accessed 21 Jul. 2019].

Li, P., L. Snyder, G. and E. Vanover, K., 2016. Dopamine Targeting Drugs for the Treatment of Schizophrenia: Past, Present and Future. *Current Topics in Medicinal Chemistry*, 16(29), pp.3385-3403.

Lopes da Fonseca, T., Correia, A., Hasselaar, W., van der Linde, H., Willemsen, R. and Outeiro, T., 2013. The zebrafish homologue of Parkinson's disease ATP13A2 is essential for embryonic survival. *Brain Research Bulletin*, 90, pp.118-126.

Lopez-Luna, J., Al-Jubouri, Q., Al-Nuaimy, W. and Sneddon, L. (2017). Reduction in activity by noxious chemical stimulation is ameliorated by immersion in analgesic drugs in zebrafish. *The Journal of Experimental Biology*, 220(8), pp.1451-1458.

López-Olmeda, J., Madrid, J. and Sánchez-Vázquez, F., 2006. Light and Temperature Cycles as Zeitgebers of Zebrafish (Danio rerio) Circadian Activity Rhythms. *Chronobiology International*, 23(3), pp.537-550.

Mental Health America., 2019. *Schizophrenia*. [online] Available at: https://www.mentalhealthamerica.net/conditions/schizophrenia [Accessed 16 Jul. 2019].

Mentalhelp.net., 2019. *Schizophrenia Symptoms, Patterns and Statistics and Patterns*. [online] Available at: https://www.mentalhelp.net/schizophrenia/statistics/ [Accessed 16 Jul. 2019].

Moawad, H., 2017. *What Is a Dopamine Antagonist? | Livestrong.com*. [online] LIVESTRONG.COM. Available at:

https://www.livestrong.com/article/232329-what-is-a-dopamine-antagonist/ [Accessed 27 Jul. 2019].

Morrison, P.D., Zois, V., McKeown, D.A., Lee, T.D., Holt, D.W., Powell, J.F., Kapur, S. and Murray, R.M., 2009. The acute effects of synthetic intravenous Δ 9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychological medicine*, *39*(10), pp.1607-1616.

Mo, W., Chen, F., Nechiporuk, A. and Nicolson, T., 2010. Quantification of vestibular-induced eye movements in zebrafish larvae. *BMC Neuroscience*, 11(1), p.110.

Ng MC, Hsu CP, Wu YJ, Wu SY, Yang YL and Lu KT. Effect of MK-801-induced impairment of inhibitory avoidance learning in zebrafish via inactivation of extracellular signal-regulated kinase (ERK) in telencephalon. Fish physiology and biochemistry 2012; 38(4): 1099-1106.

Nguyen, M., Yang, E., Neelkantan, N., Mikhaylova, A., Arnold, R., Poudel, M., Stewart, A. and Kalueff, A., 2013. Developing 'integrative' zebrafish models of behavioral and metabolic disorders. *Behavioural Brain Research*, 256, pp.172-187.

Noldus.com., 2019. *Zebrafish research: behavioral differences between wild-type strains*. [online] Available at: https://www.noldus.com/blog/zebrafish-research-behavioral-differences-between-wild-type-strains [Accessed 16 Jul. 2019].

Nusslein-Volhard, C. and Dahm, R. eds., 2002. *Zebrafish*. Oxford University Press.

Olney, J., Newcomer, J. and Farber, N., 1999. NMDA receptor hypofunction model of schizophrenia. *Journal of Psychiatric Research*, 33(6), pp.523-533.

Panula, P., Sallinen, V., Sundvik, M., Kolehmainen, J., Torkko, V., Tiittula, A., Moshnyakov, M. and Podlasz, P., 2006. Modulatory Neurotransmitter Systems and Behavior: Towards Zebrafish Models of Neurodegenerative Diseases. *Zebrafish*, 3(2), pp.235-247.

Panula, P., Sallinen, V., Sundvik, M., Kolehmainen, J., Torkko, V., Tiittula, A., Moshnyakov, M. and Podlasz, P., 2006. Modulatory Neurotransmitter Systems and Behavior: Towards Zebrafish Models of Neurodegenerative Diseases. *Zebrafish*, 3(2), pp.235-247.

Park, J., Ryu, J., Choi, T., Bae, Y., Lee, S., Kang, H. and Kim, C. (2016). Innate Color Preference of Zebrafish and Its Use in Behavioral Analyses. *Molecules and Cells*, 39(10), pp.750-755.

Peeters BW, Moeskops M and Veenvliet AR. Color preference in Danio rerio: effects of age and anxiolytic treatments. Zebrafish 2016; 13(4): 330-334.

Pratt, J., Winchester, C., Dawson, N. and Morris, B., 2012. Advancing schizophrenia drug discovery: optimizing rodent models to bridge the translational gap. *Nature reviews Drug discovery*, *11*(7), p.560.

Psychology Today., 2019. *What is dopamine?*. [online] Available at: https://www.psychologytoday.com/us/blog/mouse-man/200904/what-is-dopamine [Accessed 27 Jul. 2019].

Pubchem.ncbi.nlm.nih.gov. (2020). *Morphine*. [online] Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Morphine#section=Impurities [Accessed 12 Feb. 2020].

Rat Genome Sequencing Project Consortium, 2004. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature*, *428*(6982), p.493.

Roberts, A., Bill, B. and Glanzman, D., 2013. Learning and memory in zebrafish larvae. *Frontiers in Neural Circuits*, 7.

Roberts, A., Reichl, J., Song, M., Dearinger, A., Moridzadeh, N., Lu, E., Pearce, K., Esdin, J. and Glanzman, D., 2011. Habituation of the C-Start Response in Larval Zebrafish Exhibits Several Distinct Phases and Sensitivity to NMDA Receptor Rogers, J. and Hixson, J.E., 1997. Baboons as an animal model for genetic studies of common human disease. *The American Journal of Human Genetics*, *61*(3), pp.489-493.

Roth, B.L., Hanizavareh, S.M. and Blum, A.E., 2004. Serotonin receptors represent highly favorable molecular targets for cognitive enhancement in schizophrenia and other disorders. *Psychopharmacology*, *174*(1), pp.17-24.

Rung, J., Carlsson, A., Rydén Markinhuhta, K. and Carlsson, M., 2005. (+)-MK-801 induced social withdrawal in rats; a model for negative symptoms of schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 29(5), pp.827-832.

Saleem, S. and Kannan, R., 2018. Zebrafish: an emerging real-time model system to study Alzheimer's disease and neurospecific drug discovery. *Cell Death Discovery*, 4(1).

Sawa, A., 2009. Genetic animal models for schizophrenia: advantages and limitations of genetic manipulation in drosophila, zebrafish, rodents, and primates. *Progress in Brain Research*, pp.3-6.

Schizophrenia.com., 2019. *Schizophrenia Facts and Statistics*. [online] Available at: http://www.schizophrenia.com/szfacts.htm [Accessed 16 Jul. 2019].

Singleman, C. and Holtzman, N., 2014. Growth and Maturation in the Zebrafish, Danio Rerio: A Staging Tool for Teaching and Research. *Zebrafish*, 11(4), pp.396-406.

Sinha, S. (2019). *Morphine: Uses, Dosage, Side Effects, Warnings - Drugs.com*. [online] Drugs.com. Available at: https://www.drugs.com/morphine.html [Accessed 12 Feb. 2020].

Simen, A., DiLeone, R. and Arnsten, A., 2019. *Primate models of schizophrenia: future possibilities*.

Sison, M. and Gerlai, R., 2011. Behavioral performance altering effects of MK-801 in zebrafish (Danio rerio). *Behavioural Brain Research*, 220(2), pp.331-337..

Stewart, A., Wong, K., Cachat, J., Gaikwad, S., Kyzar, E., Wu, N., Hart, P., Piet, V., Utterback, E., Elegante, M., Tien, D. and Kalueff, A., 2011. Zebrafish models to study drug abuse-related phenotypes. *Reviews in the Neurosciences*, 22(1).

Stewart, A., Wu, N., Cachat, J., Hart, P., Gaikwad, S., Wong, K., Utterback, E., Gilder, T., Kyzar, E., Newman, A. and Carlos, D., 2011. Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(6), pp.1421-1431.

Stone, J.M., Dietrich, C., Edden, R., Mehta, M.A., De Simoni, S., Reed, L.J., Krystal, J.H., Nutt, D. and Barker, G.J., 2012. Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. *Molecular psychiatry*, *17*(7), p.664.

Timothy J. Legg, C., 2019. *Schizophrenia: Symptoms, causes, and treatments*. [online] Medical News Today. Available at: https://www.medicalnewstoday.com/articles/36942.php [Accessed 27 Jul. 2019].

Tricklebank, M., Singh, L., Oles, R., Preston, C. and Iversen, S., 1989. The behavioural effects of MK-801: a comparison with antagonists acting non-competitively and competitively at the NMDA receptor. *European Journal of Pharmacology*, 167(1), pp.127-135.

Vollenweider, F.X. and Geyer, M.A., 2001. A systems model of altered consciousness: integrating natural and drug-induced psychoses. *Brain research bulletin*, *56*(5), pp.495-507.

Wolman, M. and Granato, M., 2012. Behavioral genetics in larval zebrafish: Learning from the young. *Developmental Neurobiology*, 72(3), pp.366-372.

Wong, K., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Roy, S., Goodspeed, J., Suciu, C., Tan, J., Grimes, C., Chung, A., Rosenberg, M., Gaikwad, S., Denmark, A., Jackson, A., Kadri, F., Chung, K., Stewart, A., Gilder, T., Beeson, E., Zapolsky, I., Wu, N., Cachat, J. and Kalueff, A. (2010). Analyzing habituation responses to novelty in zebrafish (Danio rerio). *Behavioural Brain Research*, 208(2), pp.450-457

Xu X, Scott-Scheiern T, Kempker L and Simons K. Active avoidance conditioning in zebrafish (Danio rerio). Neurobiology of learning and memory 2004; 87(1): 72-77.

Yu, L., Tucci, V., Kishi, S. and Zhdanova, I., 2006. Cognitive Aging in Zebrafish. *PLoS ONE*, 1(1), p.e14.

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