Effects of chronic exposure to selenium on learning and memory in zebrafish (*Danio rerio*)

A Thesis Submitted to the College of
Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
For the Degree of Doctor of Philosophy
In the Department of Biology
University of Saskatchewan
Saskatoon

By

Mohammad Naderi

Permission to use

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

Disclaimer

Reference in this thesis to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes. Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Department of Biology
112 Science Place
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5E2
Canada

Or

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9
Canada

Acknowledgements

I would first like to thank Dr. Som Niyogi and Dr. Doug Chivers, my supervisors, for their constant support and encouragement throughout my doctoral program. I believe that I am a better researcher and writer because of their mentorship. My sincere thanks also go to my advisory committee: Drs. Maud Ferrari, David Janz, and Christy Morrissey for their kind efforts and proper guidance from the beginning to the end of my research work. I acknowledge the financial support from Natural Sciences and Engineering Research Council (NSERC), and the Department of Biology, University of Saskatchewan.

I would also like to extend my gratitude to the faculty, and staff of the Department of Biology. I am especially thankful to Joan Virgil, Marlynn Mierau, Gousheng Liu, Diedre Wasyliw, and Halyna Heisler for their tireless efforts in enabling great science in 'our' department.

I am thankful to my fellow graduate students of the Department of Biology, especially Arash Salahinejad, Anoosha Attaran, and Dr. Ankur Jamwal for all the assistance with my experiments. I also thank the members of Niyogi and Chivers lab Dr. Adam Crane, Dr. Dale Jefferson, Dr. Denis Meuthen, Kamran Shekh, Brandon Demuth, and Kevin Bairos-Novak for your assistance during my Ph.D.

It would have been impossible to complete this research without the support of my friends. Thank you to Faham, Farzaneh, Shahrzad, Kazem, and Maryam for your constant love, support, and encouragement. I am especially thankful to my friend Mohammad Shabani for his help and support throughout my Ph.D. studies. I would like to wish my heartfelt thanks to my cousin Fardin who supported me in every possible way to see the completion of this work.

Last but not the least, I would like to express my very profound gratitude to my parents and to my sisters for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. They are the most important people in my world and I dedicate this thesis to them.

Abstract

I investigated the direct and transgenerational effects of chronic dietary exposure to selenium (Se) on learning and memory in zebrafish (*Danio rerio*), with a particular focus on alterations in dopaminergic neurotransmission. Zebrafish possess a conserved dopaminergic system with receptors homologous to mammals. However, the role of dopamine (DA) receptors in learning and memory in zebrafish was not clear. Therefore, my first two studies (Chapters 2 and 3) were focused on the role of DA receptors in learning and memory in zebrafish. To gain insights into the role of DA receptors in learning and memory in zebrafish, two different learning paradigms were employed: a latent learning task (unreinforced learning) and an associative learning task (reward-related learning). Pharmacological manipulations of DA receptors in the zebrafish brain showed that D2 receptors play a prominent role in the acquisition and consolidation of latent learning in zebrafish. While exposure to a D2 receptor family agonist impaired the acquisition and consolidation of latent learning, antagonism of D2 receptors improved both phases of latent learning in zebrafish (Chapter 2). In the associative learning task (Chapter 3), however, D1 receptors showed a more important role in the regulation of acquisition and consolidation of learning. In this study, exposure to both D1 receptor agonist and antagonist improved acquisition and consolidation of associative learning performance in adult zebrafish. However, manipulation of D2 receptors mainly affected retrieval of associative learning. After determining the role of DA receptors in two different forms of learning, the effects of chronic dietary exposure to Se (as selenomethionine; SeMet) on latent learning and associative learning performance in adult zebrafish were investigated in Chapters 4 and 5 of this thesis, respectively. Dietary exposure to high concentrations of Se (32.5 and 57.5 µg Se/g dry weight) impaired latent learning performance in zebrafish (Chapter 4). The impaired learning was associated with the induction of oxidative stress and alterations in the mRNA expression of genes involved in DA synthesis and re-uptake in the zebrafish brain. The results of this study showed that dietary Se exposure decreased the mRNA abundance of the D1 receptor, while it increased the mRNA expression of D2 receptor subtypes in the zebrafish brain. In addition, the exposure to high concentrations of dietary Se (27.4 and 63.4 µg Se/g dry weight) also impaired associative learning performance in adult zebrafish (Chapter 5). Similar to observations in Chapter 4, Se exposure induced oxidative stress in the zebrafish brain. Moreover, an increase in DA levels of the brain was recorded, which was in line with the up-regulation of genes involved in the DA synthesis and re-uptake. However, unlike the previous

study, Se exposure led to up-regulation of both D1 and D2 receptors. Following this experiment, SeMet treated female fish were bred with untreated male fish to generate the F1-generation required to investigate the transgenerational effects of dietary Se on learning and memory in zebrafish (Chapter 6). To this end, embryos were raised up to the age of six months in clean water and fed on a normal diet, and learning performance of fish was tested in the latent learning task. Maternal exposure to dietary Se was found to impair latent learning in zebrafish offspring as well. This behavioural impairment was associated with an elevated level of DA in zebrafish brain along with an increase in the mRNA expression of genes involved in the synthesis, storage, re-uptake, and degradation of DA. In addition, the mRNA abundance of different DA receptors also showed a significant increase in the zebrafish brain. This hyperfunction of the dopaminergic system resulted in the induction of oxidative stress in the zebrafish brain. Collectively, the results of my research suggest that DA neurotransmission plays a fundamental role in learning and memory in zebrafish. However, this neurotransmitter system is highly sensitive to oxidative insult. Overall, the research presented in this thesis suggests that both chronic and maternal exposure to dietary Se leads to learning impairment in zebrafish via induction of oxidative stress and dysfunction of the dopaminergic system.

Dedication

To my parents and my sisters

Table of Contents

List of tables

List of figures

List of abbreviations

%	Percent
~	Approximately
<	Less than
>	Greater than
±	Denotes error of a statistic
µg	Microgram
µl	Microliter
µM	Micromolar
•OH	Hydroxyl radical
AADC	Aromatic amino acid decarboxylase
AC	Adenylyl cyclase
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
APO	Apomorphine
BDNF	Brain-derived neurotrophic factor
℃	Degree Celsius
Ca^{2+}	Calcium cation
cAMP	Cyclic adenosine monophosphate

CAT	Catalase enzyme
CCME	Canadian Council of Ministers of the Environment
C-FOS	Cellular proto-oncogene FOS
C-JUN	Cellular proto-oncogene JUN
cm	Centimetre
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
CREB	cAMP response element-binding protein
CS	Conditioned stimulus
Cu/Zn-SOD	Copper/zinc superoxide dismutase
Cyp2D	Cytochrome P450 2D
D1	D1 receptor family
D2	D2 receptor family
DA	Dopamine
DAT	Dopamine transporter
DBH	Dopamine-β-hydroxylase
DNA	Deoxyribonucleic acid
DOLT-4	Dogfish liver-certified reference material
DOPAC	3, 4-dihydroxyphenylacetic acid
DOPAL	3, 4-dihydroxyphenylacetaldehyde
DOPET	3, 4-dihydroxyphenylethanol
dpf	Days post-fertilization
DRD1	Dopamine receptor D1

DRD2	Dopamine receptor D2
DRD3	Dopamine receptor D3
DRD4	Dopamine receptor D4
DRD5	Dopamine receptor D5
dw	Dry weight
ECCC/HC	Environment and Climate Change Canada and Health Canada
EDTA	Ethylenediaminetetraacetic acid
EFSA	European Food Safety Authority
EGR-1	Early growth response protein 1
ERK	Extracellular signal-regulated kinase
ETIC	Eticlopride hydrochloride
Fe^{2+}	Ferrous iron
g	Gram
g	Gravitational force
GABA	Gamma-aminobutyric acid
GPX	Glutathione peroxidase
GPX1a	Glutathione peroxidase 1a
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
hpf	Hours post-fertilization
hrs	Hours
IAEs	Incorrect arm entries

IEGs	Immediate early genes
kg	Kilogram
l	Liter
$_{L}$-DOPA	3, 4-dihydroxyphenylalanine
LPO	Lipid peroxidation
LTD	Long-term depression
LTP	Long-term potentiation
M	Molar
MAO	Monoamine oxidase
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
mg	Milligram
min	Minute
ml	Milliliter
Mn-SOD	Manganese superoxide dismutase
mRNA	Messenger RNA
N	Normal
n	Sample size
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
NEUROD 1	Neuronal differentiation 1
nm	Nanometer
NMDA	N-methyl-D-aspartic acid

NPAS4	Neuronal PAS domain protein 4a
$O2^{\bullet-}$	Superoxide anion
OH^{-}	Hydroxide
OPT	o-Phthalaldehyde
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFC	Prefrontal cortex
pg	Picogram
pH	Potential of hydrogen
PKA	Protein kinase A
PKC	Protein kinase C
PTN	Posterior tuberal nucleus
PVO	Paraventricular organ
QUIN	Quinpirole hydrochloride
RDA	Recommended dietary allowance
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
s	Second
SCH	SCH-23390 hydrochloride
Se	Selenium
Se(0)	Elemental selenium
Se^{2-}	Selenide

SECIS	Selenocysteine insertion sequence
SEM	Standard error of the mean
SeMet	Selenomethionine
SeO_3^{2-}	Selenite
SeO_4^{2-}	Selenate
SKF	SKF-38393 hydrochloride
SOD	Superoxide dismutase enzyme
S-S	disulfide linkages
TAEs	Target arm entries
TH	Tyrosine hydroxylase
TPp	Periventricular nucleus of the posterior tuberculum
tRNA	Transfer ribonucleic acid
TTA	Time in target arm
TYR	Tyrosinase
UGA	Opal suppressor
US	Unconditioned stimulus
USEPA	United States Environmental Protection Agency
v/v	Volume/volume
VMAT2	Vesicular monoamine transporter 2
vs.	Versus

Chapter 1: General introduction

1.1. Introduction

Learning and memory are basic functions of the brain in all animals that enable individuals to respond plastically to their changing environments. In fact, instead of genetic processes which may take decades or even hundreds of years to produce organisms adaptable to their environmental conditions, learning and memory offer an optimal adaptation to environmental challenges within a fairly short time frame (Gerlai, 2016). These cognitive processes are mediated by the rapid forming and remodelling of synaptic connections in specific brain regions – a process known as synaptic plasticity (Abbott and Nelson, 2000). Neurotransmitters – the brain chemicals that mediate communication between neurons – play a central role in the modulation of synaptic plasticity and memory formation. Various psychiatric disorders and neurodegenerative diseases have some roots in the dysfunction of neurotransmitter systems (Pokorski, 2014). A growing body of evidence suggests that only a small portion of neurological diseases have a strict genetic etiology, while the gene × environment interaction provides a plausible explanation for the majority of cases (Johnson and Atchison, 2009; Tsuang et al., 2004). There is conclusive evidence that environmental (chronic) exposure to metals can interfere with normal functions of several neurotransmitter systems and contribute to neuropsychiatric and neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, schizophrenia, and attention deficit-hyperactivity disorder (Modgil et al., 2014; Pinto et al., 2017). A majority of the existing research has focused on the detrimental effects of non-essential trace metals. However, the exposure to non-essential metals represents only a fraction of the total environmental contaminants (Karri et al., 2016). In the past two decades, there has been an increasing concern about the role of essential trace elements (mainly their deficiency) in the pathogenesis of several brain disorders (Chen and Berry, 2003; Hegde et al., 2004). However, the knowledge about neurobehavioral effects of essential trace elements and their possible mechanisms of neurotoxicity is still scarce. In this regard, utilizing the relevant animal models is the only means by which we can predict the possible harmful effects of suspicious chemicals on cognitive functions including learning and memory (Levin and Buccafusco, 2006).

1.2. Learning and memory

1.2.1. Basic processes of learning and memory

Learning and memory are two closely related but separate functions of the brain, which produce adaptive changes in organisms' behaviour. Learning can be conceptualized as the process of acquiring information which can lead to changes in behavioural responses to environmental stimuli (Sweatt, 2009). It is of note that learning *per se* is not a response to an environmental stimulus, but rather is an alternation in that response which roots from an environmental stimulus. On the other hand, memory can be defined as the storage of the information acquired during learning, which can be subjected to later retrieval (Sweatt, 2009). Memory formation is a complex process which can be segmented into three basic stages: (1) the "acquisition" which refers to the encoding of information into a neuronal memory trace, (2) the "consolidation" of the fresh memory trace for long-term storage, and (3) "recall" of the learnt material. All of these stages are prone to distortions and may result in false memories (Straube, 2012).

Memory is typically classified into short-term and long-term memory (Sato, 2017). The short-term memory refers to the ability to briefly store small amounts of information and to recall those details immediately. Items stored in short-term memory can be retained by rehearsal, but are easily erased by shifting attention to other things. The short-term memory system is divided into three basic components: sensory memory, short-term storage, and working memory, each with different functions (Sato, 2017; Sweatt, 2009). The sensory memory as the first component of the short-term memory system temporarily stores the information received from the senses. Once placed in short-term storage, information can then either be transferred into long-term storage or it can be decayed and eliminated. The information can also be held and actively processed in short-term storage. This process is called working memory (Sweatt, 2009). Long-term memory represents a more permanent storage that can retain information over lengthy periods of time and is subdivided into non-declarative and declarative memory. The non-declarative memory – also known as implicit memory – refers to the unconscious storage of information that can be subjected to either conscious or unconscious recall. This type of memory includes procedural memory, associative conditioning (e.g., classical conditioning and operant conditioning), non-associative memory, etc. The declarative memory, on the other hand – alternatively referred to as explicit memory – implies the conscious acquisition of facts or events, and refers to those memories that can be consciously recalled. The declarative memory can be broadly categorized into the semantic memory and the

episodic memory, but it also includes spatial learning. The semantic memory corresponds to the general knowledge of concepts, words and objects, their meaning, their history, and relationship to each other, whereas episodic memory stores and retrieves information about autobiographical events such as times, places, and associated emotions (Baddeley, 2004; Sato, 2017; Squire, 1992). Moreover, spatial learning is a process through which animals acquire information about their surrounding environment. This helps them to facilitate navigation through space and recall the location of motivationally relevant stimuli (Byrne, 2010).

1.2.2. Associative learning and latent learning

Associative learning is the ability of living organisms to perceive a link between two or more stimuli or events. This is a vital component of adaptive behaviour across the animal kingdom as it allows anticipation of an event on the basis of another (Seel, 2011). In fact, during this process, an animal learns to associate a neutral conditioned stimulus (CS) with a biologically relevant event with either appetitive (e.g., food, water, etc.) or aversive (e.g. electric shock) properties known as the unconditioned stimulus (US) (Seel, 2011; Sweatt, 2009). This phenomenon was first described by the Russian physiologist Ivan Pavlov (1927). He found that after pairing the ringing bell with a neutral stimulus such as the meat powder on several occasions, the ringing bell would come to elicit the same response as the meat powder (i.e. salivation) even in the absence of meat powder (Pavlov, 1927). Pavlov's finding is now known as classical conditioning or classical associative learning. It is of note that in classical conditioning both CS and US lead to identical behavioural outputs. This is not the case for all forms of associative conditioning. Therefore, classical conditioning is considered as a subset of the broader category, associative conditioning (Sweatt, 2009). Operant conditioning (also called instrumental conditioning) is another main experimental procedure for the study of associative learning, where a behaviour is controlled by its consequences (Seel, 2011). However, classical associative learning is the learning procedure used in my research and henceforth referred to as “associative learning”. The results of Pavlov's study also established stimulus-response association theories which were dominant theories of learning until the mid-nineteenth and early twentieth century (Jensen, 2006; Seel, 2011). The stimulus-response theorists believed that learning occurs only when behaviours become associated with a reward/reinforcement (Jensen, 2006). However, the initial demonstrations of spatial latent learning refuted this basic assertion. Blodgett (1929), as well as Tolman and Honzik (1930), demonstrated that reinforcement is not necessary for learning to take place. They showed that food-deprived rats

that were given several trials of free exploration of a maze could learn it better than rats that had not explored the maze. Their performance was also equal to rats that were trained with the food reward. They believed that during the exploration phase rats were able to incidentally learn a spatial map of the environment and could recall and use this spatial map when a relevant reinforcer was introduced into the maze. Since the acquisition of this information lies hidden in the brain and does not immediately affect behaviour, it has been called latent learning. Therefore, latent learning is defined as the incidental and unreinforced acquisition of information that is not apparent at the time of learning, but can be recalled and utilized later when suitable motivation and circumstances appear (Blodgett, 1929; Tolman and Honzik, 1930).

Associative learning and latent learning are important parts of animal cognition. Animals forage and survive in challenging environments by learning about the events surrounding them and adjusting their behavioural responses accordingly. Associative learning enables animals to use environmental cues to predict and adapt to their environmental needs. Based on this form of learning, animals can navigate, find suitable food, avoid toxic food, escape from predators, and enhance the effectiveness of their sexual behaviours (Adkins–Regan and MacKillop, 2003; Ferrari et al., 2010; Roberts and Pearce, 1999; Skelhorn et al., 2016). Latent learning also plays a crucial role in the spatial navigation of animals within their environment. In fact, the incidental learning about the landmarks/stimuli of the environment allows animals to demonstrate efficient, goal-directed, and error-less navigation to the reward, which can subsequently affect foraging ability and anti-predatory behaviours (Brown et al., 2008).

It is now clear that different types of memory are modulated by different anatomical structures in the brain. In mammals, the amygdala, cerebellum, and hippocampus are the brain regions implicated in different forms of associative conditioning including classical associative learning (Sweatt, 2009). Moreover, the hippocampus, fimbria-fornix, amygdala, and entorhinal cortex are brain regions known to be involved in latent learning (Gaskin and White, 2007; Stouffer and Klein, 2013; Stouffer and White, 2006). Interestingly, impaired associative learning and latent learning are two of the main cognitive deficits manifested in patients with neurological disorders, including schizophrenia, attention deficit-hyperactivity disorder, Alzheimer's disease, and Parkinson's disease (Diwadkar et al., 2008; Hadj-Bouziane et al., 2013; Hemsley, 1996; Ouchi et al., 2013; Quenon et al., 2015).

1.2.3. The neurological basis of learning and memory

It is widely accepted that memories are encoded in the brain as the activity-dependent modification of the strength or efficacy of synaptic transmission between neurons, a process known as synaptic plasticity (Bermúdez-Rattoni, 2007). This idea was first suggested over 120 years ago by Santiago Ramón y Cajal, who proposed that information storage results from changes in synaptic strength between neurons that are active. It was further advanced in the late 1940s by Donald Hebb, who postulated that when two interconnected neurons fire at the same time, the synaptic connection between them becomes stronger and persists for a long time afterward (Hebb, 1949; y Cajal, 1894). This form of synaptic strengthening has come to be called Hebbian synaptic plasticity. The first experimental support for the Hebbian type modification, provided by Bliss and Lomo (1973) who demonstrated that repetitive activation of excitatory synapses in the hippocampus led to the persistent changes in synaptic strength that could last for hours or even several days (Bliss and Lømo, 1973). This phenomenon is commonly referred to as long-term potentiation (LTP). An opposite phenomenon, long-term depression (LTD), is another form of activity-dependent synaptic plasticity that reduces the efficacy of neuronal synapses. It is widely believed that LTP and LTD are fundamental cellular mechanisms underlying learning and memory (Bermúdez-Rattoni, 2007). Indeed, once a group of neurons is activated by a high-frequency stimulation (e.g. memorizing words) post-synaptic cells persistently respond to it with a greater intensity which results in storage of "memory" for that event at those synapses. It means that LTP occurred. When memories are recalled the same synaptic input is reactivated in order to recreate the memory of that event. At the same time, LTD could be induced in the surrounding fibers to reduce the synaptic activity, thereby increasing the signal-to-noise ratio, or LTD could be induced in the same fibers to return formerly potentiated responses back to the baseline to perhaps erase a memory (Bermúdez-Rattoni, 2007; Lynch, 2004). These processes (LTP and LTD) typically occurs in the hippocampus, but can also be detected in other brain areas including the amygdala, striatum, and nucleus accumbens (Anwyl, 2006; Neves et al., 2008).

Many molecular mechanisms have been implicated in the regulation of LTP and LTD. However, there is a general consensus that the pre-synaptic release of glutamate and subsequent activation of post-synaptic glutamate receptors are the essential mechanisms involved in these processes (Citri and Malenka, 2008). Following pre-synaptic activity and post-synaptic depolarization, calcium (Ca^{2+}) ions flow into the post-synaptic cell through N-methyl-D-aspartic acid (NMDA)-type

glutamate receptors. This process is followed by a rapid insertion of additional alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors into the post-synaptic membrane, leading to the strengthening of excitatory glutamatergic synapses (Kessels and Malinow, 2009; Nicoll and Roche, 2013). This process, which leads to the acquisition of new information is also known as early LTP. In a second phase lasting a few hours after the initial encoding period (known as late LTP or consolidation) the elevation of intracellular Ca^{2+} can further influence the stabilization of LTP through activation of a number of kinases including calcium-calmodulin kinase II, extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK), cyclic adenosine monophosphate-dependent protein kinase A (PKA), and protein kinase C (PKC) (Bermúdez-Rattoni, 2007; Citri and Malenka, 2008). Activation of these kinases can lead to phosphorylation of transcription factors, such as cAMP response element-binding protein (CREB). Subsequently, CREB triggers the expression of immediate early genes (IEGs) such as C-FOS, C-JUN, early growth response protein 1 (EGR-1), and brain-derived neurotrophic factor (BDNF). IEGs are a family of rapidly and transiently inducible genes that are transcriptionally activated in response to a wide variety of stimuli. In the brain, IEGs are rapidly induced as the earliest genomic response to synaptic activity (Clayton, 2000). These genes encode both transcriptional regulators and direct effector molecules, such as signalling enzymes, structural proteins, and growth factors that participate in synaptic plasticity and long-term memory formation (Guzowski, 2002; Minatohara et al., 2016). Although the molecular mechanisms underlying the acquisition and consolidation processes have been extensively studied, memory retrieval has attracted less attention because it was believed to be only a passive readout of stored plasticity in the synapses induced by acquisition and consolidation of information. Studies now show that the rapid activation of metabotropic glutamate receptors and AMPA receptors in several brain regions is necessary for memory retrieval. Several downstream protein kinases such as PKC and MPAK have been shown to participate in memory retrieval (Szapiro et al., 2002).

LTD, contrary to LTP, is characterized by a decrease in synaptic efficacy and induced by repetitive low-frequency stimulation. Like LTP, LTD also depends upon an NMDAR-dependent increase in post-synaptic Ca^{2+} influx. However, the induction of LTD requires a modest increase in Ca^{2+} levels leading to preferential activation of protein phosphatases such as calcineurin, which results in the removal or endocytosis of AMPA receptors from post-synaptic cells and reduction of synaptic efficacy (Citri and Malenka, 2008).

1.3. Dopaminergic system

1.3.1. The dopaminergic system in higher vertebrates

Dopamine (3, 4-dihydroxyphenylethylamine; DA) is a catecholamine[1]neurotransmitter. It was first synthesized by Barger and Ewens (1910) by decarboxylation of L-DOPA (3, 4-dihydroxyphenylalanine). The name "dopamine" was first suggested by Dale (1952) because it was an amine produced from the precursor L-DOPA. Before the 1950s, DA was assumed to be merely a precursor in the synthesis of norepinephrine and epinephrine or considered to be an intermediate in the tyrosine degradation (Blaschko, 1939; Blaschko, 1942; Holtz, 1939). However, in 1957, a Swedish pharmacologist Arvid Carlsson found that DA was a neurotransmitter in the human brain, not an intermediary in the synthesis of norepinephrine and epinephrine (Carlsson et al., 1957). This discovery, in combination with his later works on DA, rendered Arvid Carlsson the Nobel Prize for Physiology and Medicine in 2000.

In mammals, dopaminergic neurons are located predominantly in the ventral mesencephalon (midbrain) within the substantia nigra and the ventral tegmental area gives rise to three main dopaminergic pathways (Beaulieu and Gainetdinov, 2011; Missale et al., 1998). 1) The nigrostriatal pathway originates in the substantia nigra and projects to dorsal striatum. This pathway is associated with movement in a system called the "basal ganglia motor loop". Moreover, it is known to play an important role in learning. 2) The mesolimbic pathway originates from the ventral tegmental area and projects to the nucleus accumbens, amygdala, and hippocampus. This pathway is known to be involved in emotional processing, reward, motivation, feeding, and sleep. 3) The mesocortical pathway which transmits DA from the ventral tegmental area to cortical structures, where it is involved in cognitive functions (such as learning and memory) and affective regulation. In addition, there is also a fourth dopaminergic pathway known as the tuberoinfundibular pathway which projects DA from the hypothalamus to the pituitary gland, where it influences the secretion of certain hormones, specifically prolactin (Beaulieu and Gainetdinov, 2011; Missale et al., 1998). Besides its role in a variety of brain functions, DA is also involved in a wide range of physiological processes. In the periphery, DA is implicated in the regulation of olfaction, retinal processes, hormonal regulation, cardiovascular functions, immune system, and renal functions, among other functions (Armando et al., 2011; Beaulieu and Gainetdinov, 2011; Missale et al., 1998). DA

[1] A catecholamine is a monoamine, an organic compound that has a catechol (benzene with two hydroxyl side groups at carbons 1 and 2) and a side-chain amine.

innervation and receptor expression appear early during nervous system development and alter brain structure and connectivity with long-lasting anatomical and behavioural effects through adulthood. For example, DA via activation of DA receptors regulates proliferation and differentiation of neuronal progenitors during the brain development (Belinsky et al., 2013; Spencer et al., 1998). Dopaminergic innervation during the embryonic period controls neuronal migration of glutamatergic and GABAergic[1]neurons which are critically involved in excitation-inhibition balance in cortical structures (Money and Stanwood, 2013). Moreover, DA plays a fundamental role in synaptogenesis in the central nervous system (CNS) (Kozorovitskiy et al., 2015; Spencer et al., 1998). Considering its role in a plethora of critical functions, it is not surprising that multiple neurodegenerative and neurodevelopmental disorders have been related to the dysfunction of the dopaminergic system. The most recognized DA-related disorders are Parkinson's disease, attention deficit-hyperactivity disorder, and schizophrenia. The first two brain disorders result from a decrease in DA functioning (i.e. hypofunctional DA states). However, schizophrenia originates from DA hyperactivity in the brain (Iversen and Iversen, 2007; Money and Stanwood, 2013).

DA is synthesized within the brain from the amino acid tyrosine. In the first step, tyrosine is converted into L-DOPA by the enzyme tyrosine hydroxylase (TH). L-DOPA is further decarboxylated to DA by aromatic amino acid decarboxylase (AADC, also known as DOPA decarboxylase). This two-step process is the main DA biosynthesis pathway in catecholaminergic neurons (Fig. 1.1) (Meiser et al., 2013). However, DA can also be produced by the activity of tyrosinase. Like TH, this enzyme catalyzes the hydroxylation of tyrosine to L-DOPA which can lead to the production of either DA or dopaquinone[2]. A third DA-producing pathway was also shown to exist in rats. In this pathway, tyrosine is decarboxylated by AADC to yield tyramine and then hydroxylated by Cyp2D enzymes belonging to the cytochrome-P450 family to produce DA (Fig. 1.1) (Delcambre et al., 2016; Meiser et al., 2013).

[1] GABAergic neurons generate gamma aminobutyric acid (GABA), the main inhibitory neurotransmitters in the central nervous system.

[2] Dopaquinone, also known as o-dopaquinone, is a metabolite of L-DOPA and a precursor of melanin, which is also directly derived from tyrosine by tyrosinase. It is well known that dopaquinone is a highly reactive intermediate which can induce a wide range of chemical and/or conformational changes within protein and peptides.

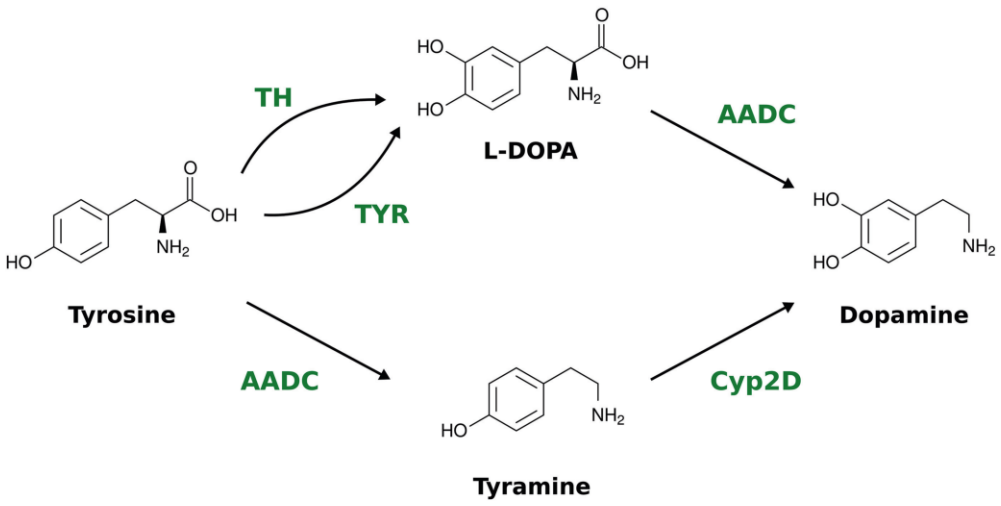

Figure 1.1. DA synthesis pathways. DA can be synthesized via three different pathways. The major pathway for DA biosynthesis consists of the hydroxylation of tyrosine by TH followed by AADC-catalyzed decarboxylation. Under TH deficient conditions, TYR can produce L-DOPA in order to ensure sufficient DA production. In a third pathway, tyrosine is first decarboxylated to form tyramine, which is subsequently hydroxylated by the P450 enzyme Cyp2D. Abbreviations: TH, tyrosine hydroxylase; AADC, aromatic amino acid decarboxylase; TYR, tyrosinase; Cyp2D, Cytochrome P450 2D (Figure adapted from Delcambre et al., 2016).

The synthesized DA is stored into synaptic vesicles by the vesicular monoamine transporter 2 (VMAT2) and released into the synaptic cleft following neuronal firing. After release, DA can travel across the synaptic cleft to interact with the post-synaptic DA receptors or regulatory pre-synaptic DA receptors (known as autoreceptors). The activity of the released DA is mainly terminated by re-uptake into pre-synaptic neurons. The re-uptake of DA from the synaptic cleft is mediated by the DA transporter (DAT) localized in the plasma membrane. DA re-uptake by DAT can be followed again by sequestration into the synaptic vesicles (Delcambre et al., 2016; Meiser et al., 2013). However, intra-neuronal DA that is not stored or leaked from synaptic vesicles is enzymatically degraded by monoamine oxidase (MAO) (Fig. 1.2). Oxidative deamination of DA by MAO leads to the production of hydrogen peroxide (H_2O_2) and the reactive 3, 4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL can be inactivated by either reduction to the corresponding alcohol 3, 4-dihydroxyphenylethanol (DOPET) or by further oxidation to its corresponding acid 3, 4-dihydroxyphenylacetic acid (DOPAC). Extracellular DA can be taken up by surrounding glial cells where it is degraded by MAO and catechol-O-methyl transferase (COMT). In glial cells, DOPAC produced by MAO activity can undergo a further methylation step by COMT resulting in homovanillic acid. COMT can also directly act on DA to form 3-methoxytyramine, which is then oxidized by MAO to produce homovanillic acid (Fig. 1.3)

(Delcambre et al., 2016; Meiser et al., 2013; Muñoz et al., 2015). Besides its own role as a neurotransmitter, DA is the precursor of norepinephrine, another monoamine neurotransmitter. In noradrenergic and adrenergic cells, DA-β-hydroxylase converts DA to norepinephrine (Meiser et al., 2013).

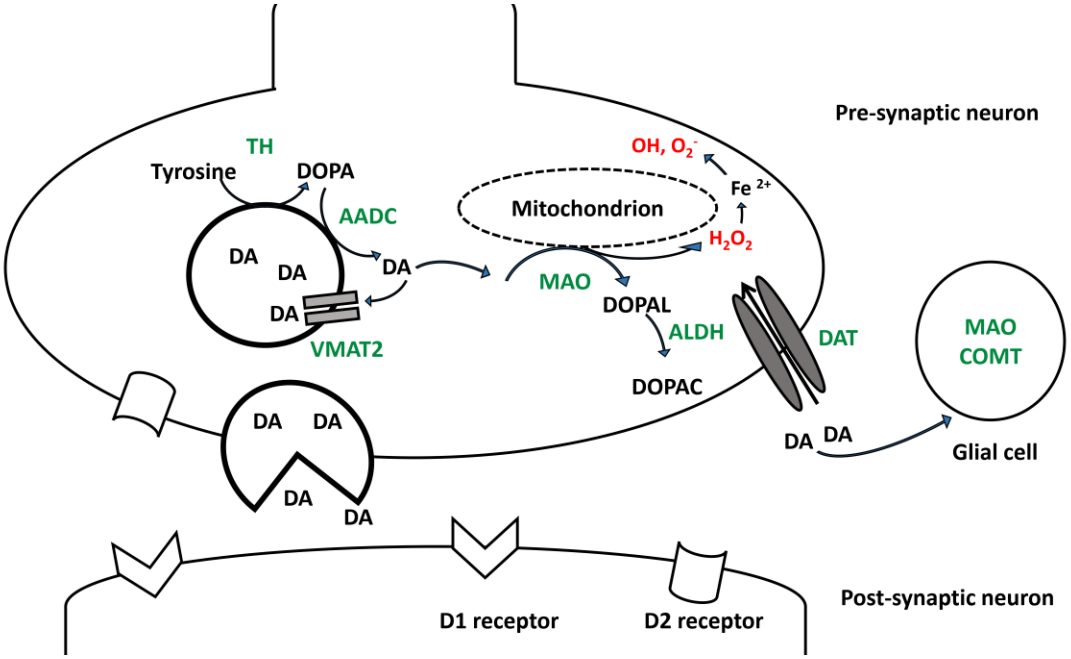

Figure 1.2. DA synthesis, sequestration, transport, and metabolism. DA is produced by combined action of TH and AADC and imported into synaptic vesicles by VMAT2. DA leaking from the vesicles is deaminated by MAO. H_2O_2 is a by-product of oxidative deamination of DA, which in the presence of reduced metals such as ferrous iron (Fe^{2+}), can be converted into the highly reactive superoxide anion and hydroxyl radicals. Abbreviations: TH, tyrosine hydroxylase; DOPA, dihydroxyphenylalanine; AADC; DA, dopamine; VMAT2, vesicular monoamine transporter 2; DAT, dopamine transporter; MAO, monoamine oxidase; ALDH, aldehyde dehydrogenase; DOPAL, dihydroxyphenylacetaldehyde, DOPAC, dihydroxyphenylacetic acid; COMT, catechol-O-methyl transferase (Figure modified from Meiser et al., 2013).

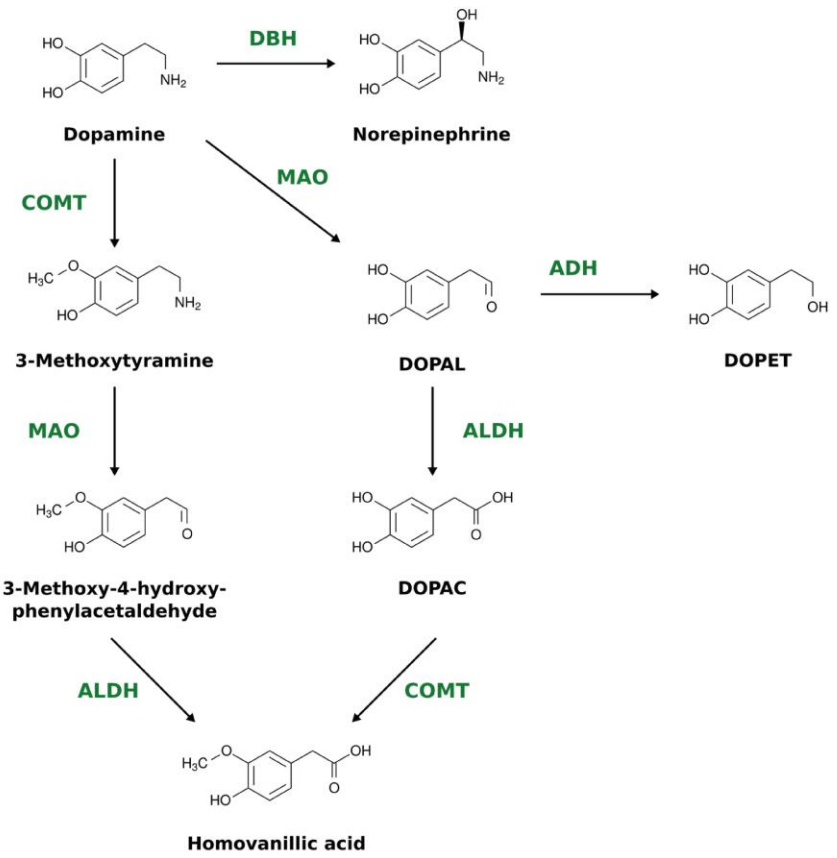

Figure 1.3. Enzymatic degradation of DA. Abbreviations: DBH, DA-β-hydroxylase; MAO, monoamine oxidase; ALDH, aldehyde dehydrogenase; DOPAL, dihydroxyphenylacetaldehyde, DOPAC, dihydroxyphenylacetic acid; COMT, catechol-O-methyl transferase; DOPET, 3, 4-dihydroxyphenylethanol; ADH, alcohol dehydrogenase (Figure modified from Delcambre et al., 2016).

The first evidence of the existence of DA receptors came from a series of experiments in 1972 showing that DA stimulates adenylyl cyclase (AC) activity (Brown and Makman, 1972; Kebabian et al., 1972). In 1978, it was established that DA receptors could exist in two distinct populations: one is positively coupled to AC and other is independent of AC (Spano et al., 1978). This finding subsequently led to the classification of DA receptors into 2 major families, D1-like receptor and D2-like receptor (hereafter referred to as D1 and D2 receptors, respectively), which was mostly based on their ability to regulate cyclic adenosine monophosphate (cAMP) (Kebabian and Calne, 1979). Currently, there are five known DA receptors, which on the basis of their structural, pharmacological, and biochemical properties grouped into either D1 and D2 receptor families (Beaulieu and Gainetdinov, 2011). The D1 receptors comprise the D1 and D5 receptor subtypes (hereafter referred to as DRD1 and DRD5), and the D2 family includes D2, D3, and D4 (hereafter referred to as DRD2, DRD3, and DRD4). All these receptors are G-protein coupled and exhibit a

heterogeneous distribution within the brain. Functionally, the D1 receptors couple to $G\alpha_{s/olf}$ family of G-proteins to stimulate AC resulting in subsequent stimulation of cAMP formation and activation of PKA. PKA has a variety of intracellular targets that influence cellular excitability. For example, PKA can increase ionotropic glutamate receptors (AMPA and NMDA), L-type calcium channel (Ca^{2+}), and sodium channel currents. Taken together, the activation of D1 receptors leads to an increase in neuronal excitability (Beaulieu and Gainetdinov, 2011; Missale et al., 1998; Neve et al., 2004). In contrast, D2 receptors are inhibitory. These receptors are coupled to $G\alpha_{i/o}$ which inhibits AC and subsequently reduces the formation of cAMP and PKA. D2 receptors can also activate potassium channels and inhibit Ca^{2+} channels. Activation of D2 receptors decreases the expression and activity of NMDA and AMPA receptors and diminishes pre-synaptic glutamate release. Such actions of D2 receptors are in line with the notion of D2 receptor signalling as "inhibitory" (Beaulieu and Gainetdinov, 2011; Ford, 2014). It is to be noted that while DA receptor functions have typically been associated with the regulation of cAMP and PKA, some of the DA receptor subtypes may also function through different signalling pathways, depending on the expressing cells (Beaulieu and Gainetdinov, 2011; Meneses, 2013). There are also differences in the localization of the DA receptors. D1 receptors are typically found post-synaptically, functioning to increase DA release. However, D2 receptors are located both post-synaptically (on dopaminergic target neurons) and pre-synaptically (on DA neurons), functioning to inhibit DA release (Meneses, 2013).

1.3.2. The role of dopaminergic system in learning and memory

DA receptors have a widespread distribution pattern throughout the CNS, particularly in those areas that are critically involved in cognition, such as the hippocampus, prefrontal cortex (PFC), amygdala, nucleus accumbens, and striatum (Mansour and Watson, 1995). The localization of DA receptors in these brain regions sheds light on the functional role of this catecholamine neurotransmitter in various aspects of learning and memory. A great body of evidence indicates that DA receptors contribute to the acquisition, consolidation, and retrieval of different types of learning and memory (El-Ghundi et al., 2007; Meneses, 2013). For example, elevated DA levels in the PFC has been reported in non-human primates and rodents while performing working memory tasks (Sweatt, 2009; Williams and Goldman-Rakic, 1995). D1 receptors play a crucial role in this form of learning. While activation of D1 receptors using specific agonists improved working memory in mice and monkey, antagonism of these receptors have been shown to impair

this type of short-term memory. Moreover, D2 receptors via modulation of D1 receptor functions may participate in working memory (El-Ghundi et al., 2007). DA transmission also plays a fundamental role in the acquisition, consolidation, and recall of spatial learning which are considered to be largely hippocampal- and nucleus accumbens dependent processes. Manipulation or dysfunction of the dopaminergic systems in these brain regions has been shown to cause deficits in spatial learning performance in rodents. For example, acquisition, consolidation, and recall of spatial learning improved in mice and rats exposed to D1 receptor agonists (Bach et al., 1999; da Silva et al., 2012). In contrast, blockade of this receptor family during spatial learning impaired memory formation and persistence (Bethus et al., 2010; da Silva et al., 2012; Rinaldi et al., 2007). Impaired acquisition of spatial tasks has also been reported in D2 receptor knockout mice (Le Nguyen et al., 2014; Tran et al., 2002). While D2 receptor agonists improved consolidation of spatial learning, antagonism of this receptor family has been shown to impair this process (Packard and McGaugh, 1994; Rinaldi et al., 2007). The levels of DA within the hippocampus and striatum are crucial for encoding of declarative memories, episodic memories in particular (Kamiński et al., 2018; Scimeca and Badre, 2012). For example, when animals are exposed to new environments, DA levels increase and facilitate LTP in the hippocampus. However, this memory enhancement is lost when hippocampal D1 receptors are blocked (Li et al., 2003). Moreover, there is some evidence that blocking D2 receptors impairs episodic memory in humans (Guarnieri et al., 2016; Rammsayer et al., 2000). As mentioned earlier (section 1.2.3), several protein kinases are involved in memory retrieval. The activity of these kinases including PKA, MAPK, and PKC is modulated by DA receptors. Therefore, alteration in the activity of DA receptor may have disruptive effects on memory retrieval (Szapiro et al., 2002). Collectively, these results show that dopaminergic neurotransmission plays an important role in both short- and long-term memories.

In addition to the above-mentioned examples, numerous studies have shown that the midbrain dopaminergic system is a key player in associative learning (Keiflin and Janak, 2015). Although there is a common belief (as early theories emphasized) that associative learning involves the encoding of relationships between two or more stimuli/events, contemporary theories emphasize that associative learning requires the subject to detect a discrepancy between an expected reward and the reward that is actually obtained. This discrepancy is called the reward prediction error. When animals experience a reward that they did not expect in the presence of a cue, a prediction error is elicited and acts as a teaching signal to correct inaccurate predictions and strengthen cue-

reward association (Keiflin and Janak, 2015; Nasser et al., 2017). In essence, when an animal for the first time encounters a cue followed by an unexpected reward, there would be a large discrepancy between what is expected and what actually occurs. However, this discrepancy decreases by multiple cue-reward pairing events and then the animal can trust the cue in which it reliably predicts a motivationally significant event. Therefore, the reward prediction error acts to drive learning about reward-predictive cues and facilitate the anticipation of future rewards. The midbrain dopaminergic system is thought to encode the reward prediction signal (Holland and Schiffino, 2016). Dopaminergic neurons produce two different activity states: a slow tonic firing pattern and a phasic burst firing (Grace, 1991). Electrophysiological studies in rodents and non-human primates have demonstrated that the phasic dopaminergic activity is responsible for coding the reward prediction error signal. When DA neurons are recorded during early training of associative learning, phasic bursts of DA firing are initially observed at the time of reward delivery, but as the animal is repeatedly exposed to the task (CS and US pairing), this response transfers to the earliest reliable predictor of that reward (Nasser et al., 2017; Schultz, 2016). Phasic DA release primarily affects D1 receptors, while D2 receptor occupancy is less affected (Dreyer et al., 2010). Therefore, the activity of D1 receptors plays a critical role in associative learning. While the increase in the activity of D1 receptors enhances associative learning, blockade or suppression of D1 receptors in the PCF, nucleus accumbens, and striatum impairs this form of information acquisition in non-human primates and rodents (Abraham et al., 2016; Di Ciano et al., 2001; Higa et al., 2017; Puig and Miller, 2012). Although less compelling, there is also evidence that D2 receptors are involved in associative learning. For example, impaired associative learning was reported in D2 receptor knockout mice (Tran et al., 2002). Moreover, antagonism of D2 receptors in the amygdala, PFC, and nucleus accumbens led to associative learning impairment in mice, rats, and monkey (Berglind et al., 2006; Di Ciano et al., 2001; Koch et al., 2000; Puig and Miller, 2014). Accumulating evidence suggests that D2 receptors through regulation of tonic the DA availability at post-synaptic receptors determine the responsivity of the system to phasic DA required for associative learning. Therefore, D2 receptors are involved in associative learning mainly via regulation of D1 receptor activity (Breitenstein et al., 2006; Grace, 1991).

Although DA has a well-established role in learning from reward, this neurotransmitter is also involved in unreinforced learning tasks. The dopaminergic system has long been implicated in latent learning. Previous research has shown that both depletion and over-activation of the midbrain

dopaminergic system in rodents led to latent learning impairment (Ahlenius et al., 1977; Archer et al., 2002; Mouri et al., 2007). Rodent studies have also demonstrated that D2 receptors play a more prominent role in latent learning, which is somewhat different from associative learning where D1 receptors play a more important role. For example, it has been reported that antagonism of D2 receptors improved latent learning performance in mice, while exposure to D1 receptor antagonist attenuated the acquisition of latent learning (Ichihara et al., 1989). Increased in synaptic levels of DA-induced by a DA re-uptake inhibitor led to latent learning impairment in mice. This effect was counteracted by a D2 receptor antagonist (Ichihara et al., 1993b). In addition, co-activation of D1 and D2 receptors caused by a non-selective DA receptor agonist apomorphine led to a latent learning impairment in mice, and this effect was ameliorated only by antagonism of D2 receptors but not D1 receptors (Ichihara et al., 1993a). Moreover, an abnormal increase in DA neurotransmission caused by traumatic brain injury resulted in latent learning deficits in mice. However, administration of D2 receptor antagonists (but not D1 receptor antagonist) significantly improved the deficits in task performance (Tang et al., 1997). Selective attention[1] is believed to be a core mechanism underlying the acquisition of latent learning (Ichihara et al., 1989, 1993a). This process is known to aid cognitive processing of information in complex environments by focusing on important stimuli, while ignoring irrelevant information. DA is critically involved in the mediation of selective attention (Noudoost and Moore, 2011). The effects of DA on attentional bias to certain stimuli are in large part mediated through D2 receptors (Ichihara et al., 1989, 1993a; Lissek et al., 2015). It is also noteworthy that latent learning requires an active interaction between an organism and its environment. In fact, acquisition of latent learning relies on a set of pre-existing expectancies that an organism has about its environment. Although latent learning is a type of unreinforced learning, the exposure to novelty is rewarding *per se* (Gómez-Laplaza and Gerlai, 2010). A wealth of evidence suggests that the rewarding effects of novelty depend on the activation of the midbrain dopaminergic system. Mammalian studies indicate that high novelty seeking behaviours are characterized by high levels of DA in the midbrain in comparison to low novelty responders (Costa et al., 2014). The high midbrain DA levels can be partially due to the weakened D2 autoreceptor control of midbrain DA producing neurons. In this regard, lower levels of D2 receptors have been reported in rodents, non-human primates, and humans with high novelty-

[1] The selective processing of some visual stimuli (targets) in favor of others (distracters), according to their component features, identity, location within visual space or physical salience.

seeking traits (Costa et al., 2014; Tournier et al., 2013; Zald et al., 2008). These findings shed more light on the importance of D2 receptors in the regulation of latent learning.

There is now substantial evidence that DA and the subsequent activation of DA receptors play crucial roles in the development of synaptic plasticity, specifically LTP and LTD. DA via activation of D1 receptors facilitates the induction of LTP. For example, D1 receptor agonists facilitate LTP in the hippocampal, PFC, and striatal synapses (Gurden et al., 2000; Huang et al., 2004; Kerr and Wickens, 2001). However, blockade of D1 receptors inhibits the expression and maintenance of LTP in these brain regions (Frey et al., 1991; Lemon and Manahan-Vaughan, 2006; Lisman et al., 2011). The activity of D2 receptors is more correlated with the expression of LTD. For example, genetic removal and blockade of D2 receptors impair the formation of LTD in the hippocampus, PFC, and striatum (Kreitzer and Malenka, 2005; Rocchetti et al., 2015; Xu et al., 2009). D2 receptors can also affect LTP but their effects are mainly restricted to the striatum. For example, rodent studies showed that genetic deletion or pharmacological blockade of D2 receptors enhanced LTP in the striatum (Calabresi et al., 1997; Centonze et al., 2001).

As mentioned earlier, the formation of LTP and LTD requires the activation of glutamate receptors, including the NMDA and AMPA receptors. Convergent evidence suggests that DA via activation of D1 receptors leads to stimulation of cAMP/PKA signaling pathways which subsequently increases phosphorylation and the surface expression of glutamate receptors. Furthermore, the activation of D1 receptors through the cAMP/PKA signalling pathway can lead to phosphorylation of transcription factors such as CREB, which appears to be a crucial modulator of gene transcription and induction of IEGs involved in long-term changes in synaptic plasticity (Jay, 2003; Meneses, 2013). For example, stimulation of D1 receptors can increase the expression of BDNF, which is known to play key roles in neuron health, the growth of new neurons and synapses, neural development, and formation of long-term memory (Rossato et al., 2009). In contrast, the action of D2 decreases the amount of cAMP, which subsequently attenuates the expression of LTP or favors the induction of LTD instead of LTP. Furthermore, D2 receptors are able to block the D1 receptor-mediated increase in phosphorylation of glutamate receptors (Calabresi et al., 2007; Jay, 2003).

1.3.3. Dopamine toxicity and oxidative stress

DA is one of the most redox-sensitive neurotransmitters in the vertebrate brain. In fact, DA synthesis, metabolism, and transport are all strongly linked to oxidative stress (Dias et al., 2013).

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the ability of a biological system to detoxify the reactive intermediates causing cellular damage (Finkel and Holbrook, 2000). As described in section 1.3.1, the oxidative deamination of DA by MAO generates H_2O_2. There is also some evidence that the activity of TH can lead to the production of H_2O_2 in dopaminergic neurons (Haavik and Toska, 1998). DA itself is a highly unstable molecule which under physiological conditions undergoes autoxidation to form DA quinones and free radicals, particularly H_2O_2. This process is catalyzed by molecular oxygen or metals such as manganese, copper, and iron (Dias et al., 2013; Meiser et al., 2013). Due to the iron-rich environment in dopaminergic neurons specifically in the substantia nigra, H_2O_2 resulted from the autoxidation and the MAO-mediated metabolism of DA can react with ferrous iron (Fe^{2+}) via the Fenton reaction[1] and generate hydroxyl radicals (•OH), which are considered as the most damaging free radicals in living cells (see also Fig. 1.2). DA quinones are highly reactive molecules, which can react with thiol groups of amino acids and proteins contributing to neurodegeneration. These electron-deficient products readily react with nucleophiles such as cysteines, reduced glutathione (GSH), and cysteinyl residues of proteins to form 5-cysteinyl-DA, a product of DA quinone, which is bound to cysteinyl residue on the protein and irreversibly alters or inhibits the protein function (Miyazaki and Asanuma, 2008). Moreover, DA quinones can undergo cyclization to form aminochrome, a redox-active compound which is highly reactive and can lead to the generation of superoxide anion ($O_2^{\bullet-}$). Aminochrome forms adducts with proteins such as α-synuclein, which is a small protein ubiquitously present in the brain and acts as a negative regulator of DA biosynthesis through interaction with TH. Therefore, DA oxidation can lead to the production of aminochrome which subsequently stabilizes α-synuclein and thus prevents its inhibitory effect on the DA synthesis, leading to more oxidative stress. Aminochrome is also the precursor of neuromelanin, a dark pigment found in dopaminergic neurons localized in the substantia nigra. Neuromelanin is known to act as a sink for metal ions indicating that this molecule plays a neuroprotective role. However, neuromelanin can turn detrimental if the accumulation of metal ions within the polymer becomes too high. In this situation, oxidative stress might result in the degradation of neuromelanin through peroxidation leading to the release of previously captured metal ions and causing more ROS stress (Dias et al., 2013; Meiser et al., 2013). Therefore,

[1] The Fenton reaction describes the formation of hydroxide (OH^-) and hydroxyl (•OH) radical by a reaction between Iron (II) (Fe^{2+}) and hydrogen peroxide (H_2O_2).

neuromelanin may also contribute to neurodegeneration by triggering oxidative stress. Taken together, perturbations of DA synthesis, metabolism, transport, and storage, which lead to an increase in cytosolic levels of DA, can contribute to increased ROS production and cellular dysfunction. This intrinsic sensitivity of the dopaminergic system to ROS makes it highly sensitive to external oxidative insult and a prime target for a wide range of environmental factors (Jones and Miller, 2008). Dysregulation of the dopaminergic system induced by oxidative stress is implicated in a variety of neurodegenerative and neurodevelopmental disorders such as Parkinson's disease, Alzheimer's disease, autism, and schizophrenia (Money and Stanwood, 2013).

A broad range of environmental contaminants can affect dopaminergic signalling and lead to neurological disorders. For example, prenatal exposure to mercury caused disruption in the dopaminergic system and permanent learning deficits in rats (Cagiano et al., 1990). Both in vivo and in vitro studies have shown that aluminum, zinc, and iron are able to interfere with and disturb the normal functions of DA neurons via induction of oxidative stress (Hare et al., 2017; Méndez-Álvarez et al., 2002; Sánchez-Iglesias et al., 2009; Verstraeten et al., 2008). Moreover, environmental manganese exposure represents an established risk factor for Parkinson's disease occurrence (Guilarte, 2010; Kim et al., 1999b). Several studies have also shown that manganese via induction of oxidative stress alters DA transmission and induces neurological disorders in both humans and animals (Benedetto et al., 2010; Brouillet et al., 1993; Kern et al., 2010; Montgomery Jr, 1995). Lead is another toxic metal that can affect the dopaminergic system. Several rodent studies reported that exposure to lead changes the biosynthesis and metabolism of DA and consequently alters the expression of DA receptors (Chang et al., 2014; Cory-Slechta, 1995; Cory-Slechta and Widzowski, 1991; Gedeon et al., 2001; Noureddine et al., 2005). Pesticide exposure is another strong risk factor for neurological disorders associated with the dysfunction of the dopaminergic system. Environmental and occupational exposure to specific pesticides such as chlorpyrifos, organochlorines, paraquat, and maneb has been associated with an increased risk of Parkinson's disease and attention deficit-hyperactivity disorder (Freire and Koifman, 2012; Richardson et al., 2015). Several recent studies have also shown that exposure to herbicide paraquat caused dysfunction of the dopaminergic system in larval and adult zebrafish (*Danio rerio*) by induction of oxidative stress (Bortolotto et al., 2014; Nellore and Nandita, 2015; Wang et al., 2018). All of these findings point to the dopaminergic system as a favorable target for a broad range of environmental contaminants.

1.4. Selenium

1.4.1. Properties, sources, and uses of selenium

Selenium (Se) is a chemical element that has an atomic number 34, an atomic weight of 78.96, and is located between sulfur and tellurium in Group 16 in the Periodic Table (Lenz and Lens, 2009). Se is considered as a metalloid, neither a true metal nor a non-metal, which shares the physical and chemical properties of both classes of elements. Being a member of the same periodic group as sulfur, Se shares many physicochemical properties with sulfur (and to a lesser extent with tellurium) such as similar atomic size, bond energies, ionization potentials, and main oxidation states. Therefore, Se can act as a sulfur analog in nature (Bodnar et al., 2012; Mehdi et al., 2013). Se is unevenly distributed in the Earth's crust, resulting in both seleniferous and Se deficient geo-ecosystems. The seleniferous soils are mainly derived from black shale, phosphate rocks, and coal originating from the Cretaceous and early Tertiary periods (Janz, 2012; White et al., 2004). Nonetheless, there are very few mines that exclusively extract Se (e.g., clausthalite and crooksite). Instead, it is obtained as a by-product of mining other metals including copper, iron, lead, nickel, gold, and silver ores (Mehdi et al., 2013; Reilly, 1996). Although natural processes (e.g., weathering, volcanic activity, and wildfires) make an important contribution to Se mobilization into the environment, this contribution is minor compared to the amount released from anthropogenic activities (Maher et al., 2010).

Due to specific physiochemical properties of Se, it is used in the manufacture of a variety of products. This element is commonly used in the manufacture of glass, plastics, ceramics, and paints (George, 2010; Haygarth, 1994). Its photoelectric and semiconducting properties are widely exploited in electronics, including manufacturing of solar energy panels, photographic exposure meters, photometers, and light-controlled switches (Janz, 2012). Se dietary supplements are used to counteract Se deficiency. Moreover, Se sulfide is used in fungicides and in antidandruff shampoos (Danby et al., 1993; Van Cutsem et al., 1990). Using Se in the medical industry has dramatically been increasing during the recent years, where Se is employed as semiconductor nanocrystals (quantum dots), which are extensively used in medical imaging (Bouldin et al., 2008). Se is also applied as an additive to fertilizers to raise crop Se concentrations in areas with low-Se soils (Wang et al., 2017).

1.4.2. Essentiality of selenium

Se was first discovered in 1817 by the Swedish chemist Jöns Jacob Berzelius when investigating the chemicals responsible for outbreaks of an illness among workers in a Swedish sulphuric acid plant. Berzelius named "selenium" after the Greek goddess of the moon, Selene (Oldfield, 1987). Early on, Se was considered to be very toxic, with no known health benefits. However, in 1957, Se was recognized to be essential to animals (Schwarz and Foltz, 1957). The essentiality of Se is due to the requirement for the so-called 21st amino acid, selenocysteine which is a component of selenoproteins. Selenocysteine is encoded by an UGA codon in selenoproteins, which is recognized by a specific selenocysteyl-tRNA. Since the UGA is a codon typically used for the termination of protein synthesis, its efficient suppression requires a specific recognition element within the mRNA which is called selenocysteine insertion sequence (SECIS) and an elongation factor which is specific for selenocysteyl-tRNA (Driscoll and Copeland, 2003). Proteins containing selenocysteine have been described in all three major domains of life, i.e. bacteria, archaea, and eukaryotes. Selenoproteins are essential components for several major metabolic pathways. Among the functionally characterized selenoproteins, many have a role in redox regulation. For example, glutathione peroxidase (GPX) that catalyzes the glutathione-dependent reduction of H_2O_2 and thioredoxin reductase that catalyzes the reduction of oxidized thioredoxin, using nicotinamide adenine dinucleotide phosphate (NADPH) as the electron donor. Iodothyronine deiodinases are also selenoproteins that are involved in the synthesis and metabolism of thyroid hormones (Reich and Hondal, 2016). Selenium is also vital for a variety of the brain functions. Interestingly, the majority of selenoproteins expressed in the brain areas involved in learning and memory, such as the cortical and hippocampal neurons (Zhang et al., 2007). The brain is highly prone to oxidative stress due to the low level of antioxidants, high content of polyunsaturated fatty acids, and high oxygen demand (Rayman, 2012). Since at least half of the selenoproteins are involved in reducing oxidative stress, the main role of Se in the CNS is neuroprotection via scavenging ROS and reactive nitrogen species (RNS). Moreover, Se can be involved in neuroprotection by mediating Ca^{2+} influx via ion channels and anti-inflammatory effects (Solovyev, 2015).

Besides its neuroprotective properties, Se is also involved in neurotransmission, specifically DA neurotransmission. For instance, an increase in DA turnover has been reported in the prefrontal cortex of the rats fed a Se deficient diet (Castaño et al., 1997). Se deficiency also exacerbates the dysfunction of the dopaminergic neurons caused by chemical lesions (Kim et al., 1999a; Kim et

al., 2000). A protective effect of Se supplementation on the DA neurons was also found in the rat's brain (Islam et al., 2002). In a more recent study, Khan (2010) showed that Se treatment could reverse the depletion of striatal DA in the mice brain (Khan, 2010). In addition to the dopaminergic system, Se can also affect the glutamatergic and cholinergic systems. For example, Se supplementation could prevent cholinergic neuron loss and cognitive deficits in rats (Ishrat et al., 2009). It has also been shown that Se deficiency altered the transcript levels of NMDA receptor subunits in the rat hippocampal neurons (Hagmeyer et al., 2015). Moreover, several studies in humans have established that Se deficiency leads to a lower cognitive performance and impaired motor function (Akbaraly et al., 2007; Gao et al., 2007; Shahar et al., 2010). Nowadays, Se deficiency is implicated in the pathology of several brain disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and epilepsy (Pillai et al., 2014; Solovyev, 2015). Therefore, a certain amount of this trace element is needed to perform important biological functions in the body including the normal functions of the CNS. The recommended dietary Se intakes on the basis of geographic regions are usually ranged from 20 to 70 μg Se per day. In the US and Canada, the recommended dietary allowance (RDA)[1] of Se is 55 μg per day for adult men and women (Liang et al., 2013). The European Food Safety Authority (EFSA) has proposed 70 μg Se per day as the dietary reference value for adequate Se intake of adults. The tolerable upper intake level set by the Institute of Medicine of the National Academy of Sciences of the United States and Environment and Climate Change Canada/Health Canada is 400 μg Se per day for adults (ECCC/HC, 2017; Solovyev, 2015).

Selenium is also an essential micronutrient of fundamental importance to aquatic organisms including fish. Interestingly, fish possess the largest selenoproteomes, with a maximum of 38 selenoproteins in zebrafish (compared to 25 selenoprotein genes in humans), indicating fish utilize selenoproteins to a larger extent than humans and other eukaryotic organisms (Mariotti et al., 2012). However, fish only require a narrow range of dietary concentrations of Se (0.1-0.5 μg/g dry weight (dw)) to maintain their large selenoproteome and to regulate their body functions. Dietary deficiency of Se in fish may contribute to the reduced growth rate (Hu et al., 2016), impaired

[1] The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life-stage and gender group.

immune function (Pacitti et al., 2016), reduced antioxidant status, and even mortality (Khan et al., 2017; Wang et al., 2013).

1.4.3. Selenium contamination and toxicity

Although Se is an essential micronutrient for a wide range of organisms, it can become toxic at concentrations slightly above its bio-essential levels. Anthropogenic activities including coal mining and combustion, uranium mining, oil refining, and irrigation of seleniferous soils for agricultural purposes can increase loading of Se into the environment, which eventually enters the aquatic ecosystems. In recent years, Se contamination of water bodies and its effects on aquatic organisms has become an increasing concern in many parts of the world, specifically in North America (He et al., 2018). In the environment, Se can occur at various oxidation states such as: selenate (SeO_4^{2-}, Se(VI)), selenite (SeO_3^{2-}, Se(IV)), selenide (Se^{2-}, Se(-II)) and elemental Se (Se(0)). Selenate and selenite are the dominant forms of Se in surface waters. These inorganic forms of Se are subsequently absorbed and biotransformed by primary and secondary producers (bacteria, algae, and higher plants) into a variety of organic Se species, predominantly selenomethionine (SeMet). SeMet represents 60-80% of the total Se present in contaminated aquatic food webs (Janz et al., 2014; Maher et al., 2010; Phibbs et al., 2011). Subsequently, this organic form of Se is transferred to higher trophic levels by dietary uptake. Fish and birds, which are either permanent or transient residents in these systems are particularly affected. In fact, these aquatic consumers are at risk of ingesting Se-enriched preys and accumulating toxic levels of this element (Hamilton, 2004). Moreover, SeMet can non-specifically incorporate into egg-yolk protein precursor vitellogenin in place of amino acid methionine. This leads to maternal transfer of SeMet to developing embryos causing deleterious effects in early life stages of development in animals (Janz, 2012). Therefore, these oviparous vertebrates and their offspring are highly vulnerable to Se toxicity and teratogenicity, respectively.

While fish require a trace amount of Se to maintain their Se-dependant biological functions, they are among the most sensitive organisms to excess dietary Se. When Se concentrations exceed 3 µg/g dw, this element can be rapidly bioaccumulated in fish tissues to reach toxic concentrations (Janz, 2012). Both environmental and laboratory studies have shown that exposure to Se (specifically SeMet) can cause a broad range of abnormalities in various fish species. For example, pathological alterations in different organs (such as liver, gill, kidney, and heart), reproductive

failure, developmental deformities, impaired growth, and mortalities have been reported in northern pike (*Esox lucius*), bluegill (*Lepomis macrochirus*), carp (*Cyprinus carpio*), green sunfish (*Lepomis cyanellus*), red shiners (*Notropis lutrensis*), channel catfish (*Ictalurus punctatus*), and cutthroat trout (*Oncorhynchus clarki lewisi*) inhibiting Se-contaminated ecosystems (Fan et al., 2002; Lemly, 2002; Muscatello et al., 2006; Rudolph et al., 2008; Woock et al., 1987). Moreover, it has been demonstrated that maternal exposure to excess SeMet increased mortality and deformities in larval zebrafish as well as impaired swim performance and led to the abnormal respiration and metabolic rates in F1-generation zebrafish (Thomas and Janz, 2014, 2015). Parental dietary exposure to SeMet also led to an increased embryo mortality, decreased hatching rate, and altered phenotypes in Japanese medaka (*Oryzias latipes*) offspring (Chernick et al., 2016). SeMet-induced developmental abnormalities in these studies typically included craniofacial deformities, spinal curvatures, missing or deformed fins, and edema. The endocrine system is another target of SeMet toxicity in fish. Chronic dietary exposure to SeMet has been associated with increased blood plasma concentrations of steroid hormones including estradiol, testosterone, and cortisol in female rainbow trout (*Oncorhynchus mykiss*) (Wiseman et al., 2011a; Wiseman et al., 2011b).

Due to its chemical similarity to sulfur, Se toxicity has most often been attributed to its ability to substitute for sulfur during the assembly of proteins (Lemly, 2002; Maier and Knight, 1994). In fact, it was proposed that Se can replace sulfur in the amino acids methionine and cysteine to form selenomethionine and selenocysteine, respectively. Since the formation of disulfide linkages (S-S) is necessary to maintain the normal tertiary structure of the proteins, substitution of Se for S in protein synthesis could disrupt the structural and functional integrity of various proteins (Lemly, 2004; Maier and Knight, 1994). Incorporation of SeMet in place of methionine in yolk proteins was believed to cause fish larval deformities (Lemly, 1997). However, this proposed mechanism of Se toxicity has been criticized in the literature because the incorporation of selenocysteine into proteins is a highly regulated process which requires an UGA codon, SECIS, and a specific tRNA (Stadtman, 1996). Furthermore, the Se residue in SeMet is shielded by the terminal methyl group in the amino acid structure and thereby prevents the formation of covalent bridges. This indicates that substitution of methionine with SeMet can not influence the tertiary structure or function of the protein (Egerer-Sieber et al., 2006; Mechaly et al., 2000). Instead, there is now a general consensus that oxidative stress is the main mechanism of Se toxicity, as various Se species can cause oxidation of cellular thiols (Lavado et al., 2012; Palace et al., 2004). Specifically, it has been

suggested that SeMet through the action of methioninase enzyme generates highly reactive metabolites such as methylselenol and selenide anion. Subsequently, oxidation of these reactive metabolites in the presence of GSH results in the production of $O_2^{\bullet-}$ (Fig. 1.4) (Palace et al., 2004; Spallholz et al., 2004). Both in vitro and in vivo studies confirm that SeMet via induction of oxidative stress can cause toxicity in fish. For example, SeMet induced oxidative stress and loss of cell viability in isolated hepatocytes of rainbow trout (Misra et al., 2012a; Misra et al., 2010). Moreover, developmental exposure to this organic form of Se led to an elevated level of oxidative stress in zebrafish and medaka embryos (Arnold et al., 2016; Lavado et al., 2012; Thomas and Janz, 2016).

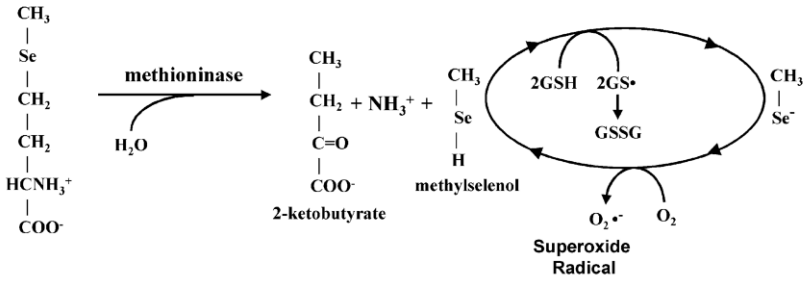

Figure 1.4. SeMet conversion to methylselenol via enzyme methioninase and the subsequent redox cycling of methylselenol which leads to the generation of ROS (Figure adapted from Palace et al., 2004).

1.4.4. Selenium criteria for the protection of freshwater aquatic life

Owing to a variety of adverse effects of Se for aquatic organisms, several environmental regulatory agencies have set limits on concentrations of Se to protect aquatic organisms (freshwater organisms in particular). For example, the Canadian Council of Ministers of the Environment (CCME, 2007) has set a limit of 1µg Se/l for the protection of freshwater aquatic life. Recently, the United States Environmental Protection Agency (USEPA, 2016) has recommended a new water quality criterion for Se to protect freshwater life. It proposes a limit of 1.2 and 3.1 µg Se/l for monthly average exposure to Se in lentic and lotic systems, respectively (USEPA, 2016). Moreover, USEPA also states that Se concentrations in fish egg-ovary, whole-body, and muscle should not exceed 15.1, 8.5, and 11.3 mg/kg dw, respectively (USEPA, 2016). Thomas and Janz (2015) also established egg Se thresholds for developmental toxicities in the early life-stages of zebrafish. They proposed egg Se thresholds (effective concentration; EC10) of 7.5 and 7.0 µg Se/g dw for mortality and

deformities in larval zebrafish, respectively. EC50 values for larval zebrafish mortality and deformities were 39.1 and 29.2 μg Se/g, respectively (Thomas and Janz, 2015).

1.4.5. Selenium neurotoxicity

Oxidative stress is the common pathogenic mechanism underlying a variety of neuropsychiatric disorders (Wells et al., 2009). This phenomenon has been implicated as a potential cause of cognitive impairments in both humans and animals (Praticò et al., 2002). For example, it has been documented that the elevated level of oxidative stress in the brain leads to an impairment of associative and spatial learning in adult zebrafish (Ruhl et al., 2016). Due to its prooxidant nature, it is not a surprise that Se can interfere with and disrupt the normal functions of the CNS. There is a growing body of evidence indicating that supranutritional levels of Se may contribute to several neurological disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Gerhardsson et al., 2008; Qureshi et al., 2006; Vinceti et al., 2010; Vinceti et al., 2017). Moreover, acute Se poisoning in humans has been associated with a suite of neurological symptoms such as the headache, ataxia, tingling, confusion, anxiety, and memory loss (MacFarquhar et al., 2010). An in vitro study has also shown that low levels of Se compounds, including sodium selenite, sodium selenate, and SeMet triggered apoptotic degeneration in a human neuronal cell line via induction of oxidative stress (Maraldi et al., 2011). As mentioned earlier (section 1.4.2), the dysfunction of the dopaminergic system is strongly linked to Se deficiency in the brain. However, there is also conclusive evidence that Se in excess can lead to malfunction of this neurotransmitter system. Rasekh et al. (1997) observed that Se (as selenite) could cause neurotoxicity through over-potentiation of DA function in the striatum and nucleus accumbens of rats (Rasekh et al., 1997). Likewise, the increase in DA levels of the striatum was reported in rats treated with sodium selenite (Tsunoda et al., 2000). Elevated levels of DA and increased activity of both D1 and D2 receptors were also reported in mice brains treated with methyl-phenyl-selenide (Oliveira et al., 2012). These data indicate that the dopaminergic system is a potential target for Se neurotoxicity. However, other neurotransmitter systems may also be affected. It has previously been shown that exposures to high levels of sodium selenite led to the dysfunction of the cholinergic and GABAergic signalling in *Caenorhabditis elegans* (Estevez et al., 2014; Estevez et al., 2012). The exposure to supranutritional levels of Se may also bring about neurobehavioural abnormalities in animals. For example, prenatal exposure to high doses of sodium selenite decreased brain levels of acetylcholine and impaired active avoidance learning in

mice (Ajarem et al., 2011b; Al Basher et al., 2011). Interestingly, Se exposure could also impair learning in fish. It has been reported that developmental exposure to SeMet led to a persistent spatial learning impairment in adult zebrafish (Smith et al., 2010). Collectively, these studies suggest that Se can disrupt the normal functions of the CNS and lead to behavioural abnormalities in animals.

1.5. Zebrafish

1.5.1. An introduction to zebrafish

Zebrafish (*D. rerio*) are small tropical freshwater teleost native to South-East Asia, where they inhabit streams and rivers, silt-bottomed well-vegetated pools, and rice paddies adjacent to streams (Spence et al., 2008). Zebrafish belong to the family of freshwater fishes Cyprinidae which is the most species-rich vertebrate family (Nelson et al., 2016). The name "Danio" originates from the Bengali name ''dhani'', meaning ''of the rice field''(Talwar, 1991). They are relatively small fish (3-4 cm long as an adult) that possess a fusiform and laterally compressed body shape. They also have a terminal oblique mouth pointing upwards. The eyes in zebrafish are central and not visible from above. Their characteristics also include two pairs of barbels and five to seven dark blue longitudinal the zebra-like stripes extending from the operculum to the caudal fin. The anal fin is similarly striped, while the dorsal fin only has a dark blue upper edge. Body colouration in males and females are almost the same, although males tend to have larger anal fins with a more yellow colouration. In their natural environment, they can tolerate a wide range of temperatures from as low as 6 °C in winter to over 38 °C in summer. They are omnivorous and their diet consists primarily of zooplankton, insects, phytoplankton, filamentous algae, invertebrate eggs, and arachnids. Zebrafish have a short generation time (3 to 4 months) and are easy to maintain in the laboratory where they breed all year round. Females can spawn every 2-3 days and produce several hundred eggs which are optically transparent (Spence et al., 2008). The zebrafish life cycle includes several distinct stages: the embryonic stage (0-72 hours post-fertilization; hpf), larval stage (4-29 days post-fertilization; dpf), juvenile fish (30-89 dpf), adult zebrafish (90 dpf to 2 years), and aging/aged zebrafish (2 years onwards). Importantly, zebrafish embryonic development is very rapid. At 24 hpf all major organs are formed and within 2-3 dpf fish hatch and start looking for food (Kalueff et al., 2014). Due to these characteristics and beginning with the pioneering work of Streisinger et al. (1981), who used zebrafish as a model organism to study the genetic basis of

vertebrate development, zebrafish have become a promising model in many research areas of biological sciences (Kalueff et al., 2014; Streisinger et al., 1981). Zebrafish are highly social animals and express strong preferences towards their conspecifics. Shoaling behaviour is established early during zebrafish ontogenesis (6 dpf) and during the first 21 dpf they show strong social preferences, based on visual stimuli from their conspecifics (Dreosti et al., 2015; Mahabir et al., 2013). This unique feature of zebrafish has increasingly been employed in modeling neurobehavioural disorders involving deficits in social behaviours, including autism and schizophrenia (Meshalkina et al., 2017; Morris, 2009). Zebrafish also show high genetic similarity to mammals. For example, nearly 70% of zebrafish genes have at least one human orthologue indicating that zebrafish are an ideal model to study human disease-related genes in fish (Howe et al., 2013).

1.5.2. Learning and memory in zebrafish

In recent years, there has been a surge of interest in utilizing the zebrafish model to study different aspects of learning and memory. Despite considerable differences in gross morphology of the brain between the mammals and the teleost, zebrafish possess a complex brain that shares many organizational features with the brain in mammals (Friedrich et al., 2010). Similar to mammals, the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain) are the major subdivisions of the zebrafish brain. The telencephalon of the zebrafish brain is divided into dorsal (area dorsalis) and ventral (area ventralis) areas as pallium and subpallium of other vertebrates. Distinct areas of the zebrafish pallium functionally resemble cortical brain areas in mammals involved in different forms of learning and memory. For example, the ventrolateral zone of the dorsal telencephalic area (medial pallium) in zebrafish is homologous to the mammalian hippocampus. Moreover, the medial zone of the dorsal telencephalic area (ventral pallium) and the central zone of the dorsal telencephalic area (dorsal pallium) in zebrafish correspond to the mammalian amygdala and neocortex (Friedrich et al., 2010; Mueller, 2012; Portavella et al., 2004; Rodríguez et al., 2002). There is now compelling evidence that the fish telencephalon plays a crucial role in different forms of learning and memory, including latent learning and associative learning (Friedrich et al., 2010; López et al., 2000; Saito and Watanabe, 2006; Salas et al., 2006; Salas et al., 1996). Some studies have also shown that the cerebellum may also be involved in the acquisition of associative learning in fish (Aizenberg and Schuman, 2011; Salas et al., 2006).

Learning and memory functions have been successfully quantified in zebrafish using either learning tasks adapted from rodent learning tasks or zebrafish-specific assays. For example, zebrafish have been found to perform well in a variety of learning tasks including, among others, spatial learning (Karnik and Gerlai, 2012), shuttle box active appetitive conditioning (Pather and Gerlai, 2009), place conditioning (Eddins et al., 2009), appetitive choice discrimination (Bilotta et al., 2005), and active avoidance conditioning (Xu et al., 2007). Specifically, several studies have shown that this small vertebrate is also capable of performing well in associative learning and latent learning tasks (Gerlai, 2016; Gómez-Laplaza and Gerlai, 2010; Sison and Gerlai, 2010). In the associative learning task which is normally conducted in a plus-shaped maze or a T-shaped maze, fish are required to perceive a link between two stimuli: a reward (unconditioned stimulus) and a visual cue (conditioned stimulus) (Gerlai, 2016). However, latent learning is conducted in a more complex maze which consists of a start chamber, a reward chamber, and two tunnels that connect these two chambers together. In this form of learning, subjects are required to learn about the spatial layout of the maze after being allowed to freely explore it for several days in the absence of the reward. While they are provided with a reward, fish use the acquired information to reach the reward more quickly than fish that were not given unreinforced exploration (Gómez-Laplaza and Gerlai, 2010). Several studies have also shown that exposure to environmental contaminants and pharmaceutical compounds can impair these cognitive functions in zebrafish. For example, zebrafish exposure to SeMet, mercury, arsenic, lead, bisphenol A, chlorpyrifos, and alcohol impaired different forms of learning and memory in zebrafish including associative learning and latent learning (de Castro et al., 2009; Fernandes et al., 2014; Levin et al., 2003; Luchiari et al., 2015; Saili et al., 2012; Smith et al., 2010; Xu et al., 2016). Nonetheless, few studies have so far attempted to determine the neural mechanisms underlying toxicant-induced learning deficits in zebrafish. In this regard, there is no study found in the literature that investigates direct and/or transgenerational effects of exposure to dietary SeMet on learning and memory in zebrafish.

1.5.3. The zebrafish dopaminergic system

Brain neurochemistry is highly conserved across vertebrate species. Zebrafish possess all major neuromodulatory systems, including neurotransmitters, their receptors, enzymes of synthesis and metabolism, and transporters, similar to those observed in the CNS of mammals (Panula et al., 2010). The dopaminergic system in zebrafish shares a high degree of similarity to the respective mammalian system. However, in contrast to mammals, there is no midbrain dopaminergic system

in zebrafish and other teleosts. Instead, dopaminergic cell groups are mainly located in the zebrafish telencephalon (including the olfactory bulb and dorsal, central and ventral nuclei of the ventral telencephalic area) and diencephalon (mainly in the periventricular nucleus of the posterior tuberculum and paraventricular organ, in the posterior tuberal nucleus, and in the caudal hypothalamus) (Rink and Wullimann, 2001; Wullimann and Mueller, 2004). The functional homologue of the midbrain dopaminergic system is currently unknown. However, immunohistochemical and tracer studies have suggested that the dopaminergic cell groups in the ventral diencephalon (the periventricular nucleus of the posterior tuberculum) projecting to the subpallium (the ventral telencephalon) can be homologous to the mammalian nigrostriatal and mesolimbic pathways (Fig. 1.5). Moreover, it has been suggested that the posterior tuberal nucleus that projects to the pallium (the dorsal telencephalon) may represent the zebrafish homologue of the mesocortical pathway found in mammals (Rink and Wullimann, 2001, 2002a). In a more recent study, Tay et al. (2011) demonstrated that the posterior tuberculum in the larval zebrafish brain also sends projections into the diencephalon, hindbrain, and spinal cord. However, the telencephalic projection from DA cells of the posterior tuberculum is scarce and can be homologous to the mammalian diencephalic dopaminergic cell group (located in the posterior hypothalamus), rather than to the midbrain dopaminergic neurons in mammals. Instead, Tay et al. (2011) showed that most subpallial dopaminergic inputs originate in a local subpallial system that also connects to the ventral diencephalon. In other words, the predominant telencephalic DA source in zebrafish may be derived locally (Fig. 1.5) (Tay et al., 2011). In line with this, it has been suggested that the ventral diencephalic-subpallial DA system plays an important role in the neuromodulatory integration of sensory function, neuroendocrine state and physiology, motor control, as well as control of behaviour and cognition (Haehnel-Taguchi et al., 2018; Tay et al., 2011). The dopaminergic system in the zebrafish brain begins to form at about 15-18 hpf in the telencephalon and diencephalon with projections that terminate locally. D2 receptors, TH, and DAT are first dopaminergic cell markers appear in the CNS of developing zebrafish (Boehmler et al., 2004; Souza and Tropepe, 2011). By 5 dpf, dopaminergic neurons and all DA receptor subtypes are expressed throughout the zebrafish brain, specifically in the telencephalon. The early appearance of dopaminergic neurons in the zebrafish brain indicates the important role of this neurotransmitter in the development of the CNS and in the formation of neural circuits involved in a variety of behaviours and physiological processes (Panula et al., 2010; Souza and Tropepe, 2011).

Therefore, early transient alterations in the dopaminergic system may produce persistent alterations in the brain functions and behavioural abnormalities in adult zebrafish (Formella et al., 2012).

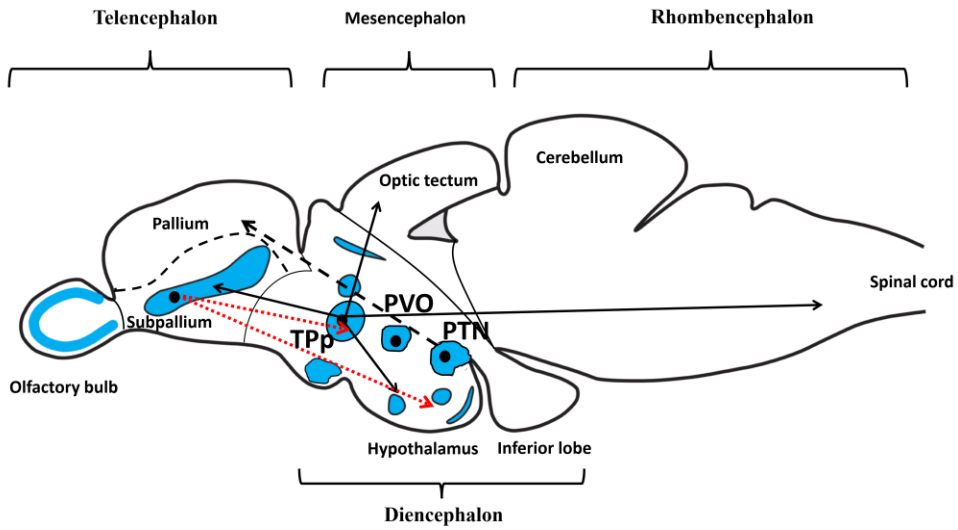

Figure 1.5. Schematic representation of dopaminergic pathways in zebrafish. Black circles indicate main dopaminergic nuclei in the zebrafish brain. Arrows denote ascending and descending DA projections to different brain regions. Abbreviations: TPp, periventricular nucleus of the posterior tuberculum; PVO, paraventricular organ; PTN, posterior tuberal nucleus (Figure modified from Tay et al., 2011).

Zebrafish possess all genes involved in DA synthesis, re-uptake, storage, and metabolism. As a result of the teleost gene duplication event, zebrafish have two paralogous genes encoding TH. TH1 is mainly expressed in the telencephalon, ventral thalamus, and posterior tuberculum while TH2 more broadly distributed in the hypothalamus and posterior tuberculum as well as in peripheral tissues such as kidney and liver (Maximino et al., 2016). Some studies have shown that TH2 gene encodes a tryptophan hydroxylase instead of TH, and it is also co-localized with serotonin in the ventral diencephalon and caudal hypothalamus in the zebrafish brain (Ren et al., 2013). Therefore, TH1 is referred to as TH in zebrafish brain (Horzmann and Freeman, 2016). Zebrafish possess two VMAT genes: VMAT1 which is localized exclusively in peripheral neuroendocrine cells and VMAT2 which is found in the telencephalon and diencephalon (Maximino et al., 2016). Zebrafish also carry a single copy of DAT (solute carrier family 6 (neurotransmitter transporter), member 3; SLC6A3) which is responsible for the re-uptake of DA from the synaptic cleft back to the pre-synaptic neuron and is highly expressed in the telencephalon, preoptic area, posterior tuberculum, and hypothalamus. Contrary to mammals which have two MAO genes (MAO-A and MAO-B), only one gene related to MAO enzyme is found in the

zebrafish genome. The highest level of MAO activity is detected in the zebrafish hindbrain as well as in the ventral and dorsal parts of the telencephalon (Maximino et al., 2016). In the zebrafish brain, MAO appears to be more important though in the metabolism of serotonin than DA (Anichtchik et al., 2006). COMT, another enzyme involved in DA metabolism, was not well characterized in the zebrafish until recently. Zebrafish possess two COMT genes: COMTA and COMTB. The expression of these genes (COMTB in particular) was detected in the zebrafish brain (the areas near the telencephalic and diencephalic ventricles) (Semenova et al., 2017). However, it is still not clear whether COMT is involved in DA metabolism in the zebrafish brain.

Zebrafish also possess all DA receptor subtypes corresponding to the mammalian D1 and D2 receptor families. In zebrafish, seven copies of the D1 receptor gene have been described. However, only the DRD1b subtype shares high similarity with the mammalian D1 receptor and is expressed mainly in the telencephalon, diencephalon, and hindbrain. The other six D1 receptors were inferred from sequence analysis and structure prediction, hence next to nothing is known about their functions in the zebrafish brain (Li et al., 2007; Maximino et al., 2016). Three D2 receptor genes have been identified in zebrafish: DRD2a, DRD2b, and DRD2c. In zebrafish, DRD2a is expressed in the hypothalamus, pituitary gland, hindbrain, and spinal cord. DRD2b and DRD2c receptors are mainly localized in the telencephalon, diencephalon, and hindbrain. A single copy of the DRD3 gene has been described in the zebrafish brain with low to moderate expression in the telencephalon and hindbrain, and high expression in the diencephalon. Three D4 receptor subtypes have been described in zebrafish. DRD4a and DRD4b subtypes are highly expressed in the telencephalon, diencephalon, hindbrain, and retina. However, the expression of DRD4c is restricted to the spinal cord and retina (Boehmler et al., 2007; Boehmler et al., 2004; Maximino et al., 2016).

In zebrafish, DA is thought to be involved in the regulation of social interaction. For example, the development of shoaling has been found to be associated with rising DA levels in the zebrafish brain (Buske and Gerlai, 2011). Moreover, dopaminergic neurotransmission has been shown to control agonistic interactions in zebrafish (Dahlbom et al., 2012). It has also been demonstrated that antagonism of D1 receptors disrupted social preference in zebrafish (Scerbina et al., 2012). In a recent study, it has been found that developmental social isolation reduced dopaminergic response to social stimuli (Shams et al., 2018). In addition to social behaviours, DA can also affect motor activity in these small tropical fish. DA receptor (both D1 and D2) agonists and antagonists have

previously been found to alter zebrafish larvae swimming patterns. While DA receptor agonists increased locomotion, the selective antagonists decreased zebrafish activity (Irons et al., 2013). Tran et al. (2015) also showed that antagonism of the D1 receptor decreased locomotor activity (total distance traveled by the fish) in adult zebrafish. However, zebrafish exposed to a D2 receptor antagonist exhibited a decreased and increased activity with low and high concentrations of the drug, respectively (Tran et al., 2015). There is also some evidence that DA is involved in the regulation of learning and memory in zebrafish. For instance, the exposure to nicotine increased a DA metabolite (DOPAC) concentrations in the brain and improved learning performance of zebrafish tested in a spatial discrimination task (Eddins et al., 2009). Moreover, antagonism of D2 receptors, but not D1 receptors, impaired conditioned place preference in zebrafish (Darland et al., 2012). However, there is still a paucity of information about the role of DA receptors in the regulation of other forms of learning and memory in zebrafish including associative learning and latent learning.

1.6. Research objectives

My research was primarily designed to investigate the effects of chronic exposure to environmentally relevant concentrations of dietary SeMet on learning and memory in zebrafish and its underlying mechanisms. DA is one of the most important neurotransmitters in the brain, which is involved in the regulation of different forms of learning and memory. Moreover, previous studies have shown that the normal functions of the dopaminergic system are strongly linked to Se concentrations in the brain. However, due to the prooxidant nature of Se, supranutritional levels of this element can lead to the dysfunction of the dopaminergic system, which is highly sensitive to oxidative stress. Therefore, this research was primarily focused on alterations in the dopaminergic neurotransmission in the zebrafish brain as a possible mechanism underlying Se neurotoxicity. Dietary Se can also be deposited into the eggs, transferred to the embryos, and deleteriously affect early life stages of fish. DA is one of the first neurotransmitters that appear in the developing CNS of zebrafish modulating the development and functions of neural circuits involved in fish cognition and behaviour. Therefore, maternal exposure to dietary Se via changes in the dopaminergic system during early development may cause persistent behavioural abnormalities into adulthood. It has previously shown that developmental exposure to SeMet impaired spatial learning in adult zebrafish (Smith et al., 2010). However, next to nothing is known about its underlying mechanisms. Although DA has been shown to be involved in the regulation of a number of behaviours in fish,

its role in mediating learning and memory is yet to be elucidated. Accordingly, before examining the effects of Se on the dopaminergic system and its consequences in zebrafish learning, it was important to elucidate the role of DA receptors in the regulation of different forms of learning and memory.

Fish are among the most sensitive organisms to Se toxicity. However, the majority of studies have been restricted to the reproductive and developmental effects of Se on fish. Since learning and memory affect a variety of behaviours including foraging behaviour, mate choice, and anti-predatory behaviour their impairment may directly or indirectly influence fish health and survival. Furthermore, the dysfunction of the dopaminergic system is implicated in several neurological disorders such as Parkinson disease, schizophrenia, and attention-deficit-hyperactivity disorder. Accordingly, this study can also provide an understanding of how the exposure to environmental contaminants such as Se may contribute to brain disorders.

Hypothesis: This research project is based on the principal hypothesis that "chronic dietary exposure to environmentally relevant concentrations of SeMet leads to learning impairment in zebrafish via induction of oxidative stress and disruption of the dopaminergic system".

Research objectives: My research has five main objectives which are as follows:

1. To get insights into the role of DA receptors in the acquisition and consolidation of latent learning (unreinforced spatial learning) in adult zebrafish (Chapter 2).

In this study, adult zebrafish were exposed to both specific and non-specific DA receptor agonists and antagonists before and after the training in a latent learning paradigm. For latent learning tasks, fish were trained in a complex maze for a period of 16 days. During this period fish were required to acquire information about the spatial layout of the maze and use this information once provided with a reward in a probe trial.

2. To provide insights into the role of DA receptors in the acquisition, consolidation, and recall of associative learning (reward-related learning) in adult zebrafish (Chapter 3).

In this study, adult zebrafish were exposed to specific DA receptor agonists and antagonists. Moreover, fish were trained and tested in a plus-shaped maze where they were required to associate a reward with a reward-predictive cue. To examine the effects of dopaminergic drugs on the acquisition, consolidation, and recall of associative learning performance, fish were exposed to

each drug before training, after training, and before the probe trial. Finally, fish were tested in a probe trial.

3. To investigate the effects of chronic dietary exposure to environmentally relevant concentrations of Se on latent learning in adult zebrafish (Chapter 4).

In this study, adult zebrafish were exposed to different dietary concentrations of SeMet (low, medium, and two high concentrations) for 30 days and then tested in a latent learning task. Total Se concentrations were measured in food, water, and whole-body tissues. To uncover mechanism(s) underlying Se neurotoxicity, the change in oxidative status and alterations in dopaminergic neurotransmission in the zebrafish brain were determined. The GSH:GSSG ratio and lipid peroxidation content of the brain were used as biomarkers of oxidative stress. Moreover, the mRNA expression of DA receptor genes, as well as the expression of genes involved in the DA synthesis, re-uptake, and metabolism were evaluated. In addition to these, the expression level of two IEGs including BDNF and EGR-1 as markers of neuronal activity was assessed in the zebrafish brain.

4. To investigate the effects of chronic dietary exposure to Se on associative learning in adult zebrafish (Chapter 5).

In this study, adult zebrafish were exposed to environmentally relevant concentrations of dietary SeMet for a period of 60 days. Then, fish were trained and tested in a plus-shaped maze similar to the second objective (Chapter 2). Total Se concentrations were measured in fish diet, water, and whole-body tissues. The induction of oxidative stress was evaluated by measuring GSH:GSSG ratio, lipid peroxidation, and the expression of antioxidant enzymes (glutathione peroxidase 1a (GPX1a), catalase (CAT), manganese superoxide dismutase (Mn-SOD), and copper/zinc superoxide dismutase (Cu/Zn-SOD)). DA levels of the brain were quantified using a DA enzyme-linked immunosorbent assay kit. The expression of genes associated with DA receptors, DA synthesis, storage, transportation, and metabolism was quantified in the zebrafish brain. Moreover, the expression of genes involved in neural plasticity and neurogenesis including BDNF, neuronal PAS domain protein 4a (NPAS4), and neuronal differentiation 1(NEUROD 1) was also examined in the zebrafish brain. At the end of this experiment, female fish treated with different concentrations of Se were crossed to unexposed adult male fish in order to produce embryos required for the final experiment.

5. To study the effects of maternal exposure to environmentally relevant concentrations of Se on learning and memory in F1-generation adult zebrafish (Chapter 6).

In this experiment, zebrafish embryos obtained from the fourth experiment were raised in clean water and fed with a normal diet until adulthood (6 months). F1-fish were then trained and tested in a latent learning paradigm. Mortalities and developmental abnormalities in early life stages of zebrafish were recorded. Total Se concentrations were measured in fish diet, water, and whole-body tissues of F0-fish. To evaluate the oxidative status of the brain several biomarkers including the GSH:GSSG ratio, lipid peroxidation, and the mRNA expression of genes related to antioxidant enzymes were measured in the brain of F1-fish, as described in the fourth objective. DA levels and the expression of genes involved in DA neurotransmission including DA receptors, TH, DAT, VMAT2, and MAO were assessed in the brain of F1-fish. Moreover, the transcript levels of immediate early genes including BDNF, EGR-1, and NEUROD 1 were also determined in the brain of F1-fish.

Chapter 2: Dopamine receptors participate in the acquisition and consolidation of latent learning of spatial information in zebrafish (*Danio rerio*)

Preface

The aim of this chapter is to address the first objective of my doctoral research work, which is to provide insights into the role of dopamine receptors in the acquisition and consolidation of latent learning in adult zebrafish. To this end, fish were exposed to D1 and D2 receptor agonists and antagonists for 30 min before and after training in a latent learning task for 16 days. The results of this study show that both D1 and D2 receptors are critically involved in the regulation of latent learning in zebrafish, with a more prominent role for D2 receptors. Moreover, the findings of this chapter provide a basis for Chapters 4 and 6 where I have investigated the effects of chronic and maternal exposure to selenomethionine on the dopaminergic system and latent learning performance in zebrafish.

The content of Chapter 2 was reprinted (adapted) from Progress in Neuro-Psychopharmacology and Biological Psychiatry (DOI: 10.1016/j.pnpbp.2016.01.002). Naderi, M., Jamwal, A., Ferrari, M.C.O., Niyogi, S., Chivers, D.P. "Dopamine receptors participate in acquisition and consolidation of latent learning of spatial information in zebrafish (*Danio rerio*)". Copyright 2016, with permission from Elsevier Inc.

Author contribution

Mohammad Naderi (University of Saskatchewan) designed and conducted the experiment, generated and analyzed the data, prepared all the figures, and drafted and revised the manuscript.

Ankur Jamwal (University of Saskatchewan) provided technical assistance with behavioural tests.

Maud Ferrari (University of Saskatchewan) assisted in data analysis and also edited the manuscript.

Som Niyogi and Doug Chivers (University of Saskatchewan) provided inspiration, scientific input and guidance, commented on and edited the manuscript, and provided funding for the research.

2.1. Introduction

Learning and memory are fundamental functions of the brain that enable animals to respond plastically to their environmental needs (Gerlai, 2011). During recent years, a growing body of evidence has highlighted a number of different hormonal and neurotransmitter systems involved in these cognitive processes (Sweatt, 2009). Dopamine (DA) is one of the major neurotransmitters in the central nervous system (CNS) of vertebrates which plays vital roles in a variety of brain functions including locomotor activity, emotion, positive reinforcement, neuroendocrine integration, and cognition (Cropley et al., 2006; Girault and Greengard, 2004; Iversen and Iversen, 2007). Dysfunctions in the homeostatic control of brain dopaminergic neurotransmission are implicated in a number of neurological and psychiatric disorders, including Parkinson's disease, schizophrenia, drug addiction, and attention-deficit hyperactivity (Bowton et al., 2010; Goodman, 2008; Lodge and Grace, 2011a; Missale et al., 1998).

This catecholamine neurotransmitter is largely found in the mesolimbic, mesocortical, nigrostriatal, and tuberinfundibular pathways where its effects are governed by two distinct families of G protein-coupled receptors: the D1-like receptors family (D1 and D5 subtypes) and the D2-like receptors family (D2, D3, and D4 subtypes). The D1-like receptors (henceforth referred to simply as D1 receptors) are $G\alpha_{s/olf}$-coupled receptors that are positively coupled to adenylyl cyclase activity (AC) to increase intracellular levels of cyclic adenosine monophosphate (cAMP). In contrast, the D2-like receptors (hereafter D2 receptors) are $G_{i/o}$-coupled receptors which inhibit AC and reduce intracellular levels of cAMP (Dalley and Everitt, 2009; Meneses, 2013; Missale et al., 1998). DA receptors are widely present in a number of DA-innervated regions of the brain, which are critically involved in cognition, such as the hippocampus, prefrontal cortex (PFC), amygdala, and ventral and dorsal parts of the striatum (El-Ghundi et al., 1998). Abundant evidence shows that DA receptors are critically involved in many forms of learning and memory (Dalley and Everitt, 2009; El-Ghundi et al., 1998; Puig et al., 2014). Spatial learning and memory is one of the advanced neurophysiologic functions of the animal brain, which allows individuals to navigate their surrounding environment and learn to associate particular spatial cues or places with particular responses or events which are crucial for the survival and fitness (Sweatt, 2009). Several lines of evidence have demonstrated a profound effect of DA neurotransmission in the control of different forms of spatial learning and memory in rodents (da Silva et al., 2012; Granado et al., 2008; Tran et al., 2015). However, the specific role of DA receptors in mediating the different phases (i.e.

acquisition, consolidation, and retrieval) of spatial learning and memory remains poorly understood.

Most of our knowledge about the behavioural effects of DA receptors and other neurotransmitters stems from studies using mammalian models (Sweatt, 2009). However, it is widely believed that mammalian organisms *per se* have become a bottleneck in the implementation of neurobehavioural research. Requiring lots of space, high costs of maintenance, and displaying complex behaviours which are difficult to measure and interpret are some drawbacks with mammalian models (Bowman and Zon, 2010; Newman et al., 2011). Among vertebrates, zebrafish (*Danio rerio*), which have proven to be an ideal model organism in genetics and developmental biology, is solidifying its spot as a prolific and cost-effective model organism for neuropharmacological and behavioural research (Bailey et al., 2015; Kalueff et al., 2013). It is an excellent alternative to mammalian models because it enjoys sequence identity to most human genes, it has a rich behavioural repertoire, it is highly malleable, and accessible (Newman et al., 2011). Despite its small size, zebrafish possess a complex brain patterning with the structure and function of neurochemical systems similar to those of mammals. For example, different parts of pallium and subpallium in zebrafish forebrain have been implicated as functional homologues of cortical and sub-cortical structures in mammals, including the hippocampus, amygdala, and striatum (Cheng et al., 2014; Friedrich et al., 2010). All major neurotransmitter systems in mammals are also represented in the zebrafish brain (Kalueff et al., 2014; Panula et al., 2006). Specifically, subpallial DA neurons and ventral diencephalic DA neurons of the zebrafish posterior tuberculum are identical to the midbrain dopaminergic pathways in mammals (Rink and Wullimann, 2002b; Schweitzer and Driever, 2009; Tay et al., 2011). More interestingly, all DA receptor subtypes have been identified and cloned in zebrafish, which resemble those of mammalian organisms (Boehmler et al., 2007; Boehmler et al., 2004; Li et al., 2007).

Along with the adaptation of rodent learning paradigms, significant strides have also been made to develop robust and replicable techniques to specifically investigate neurocognitive functions in zebrafish (Blaser and Vira, 2014). Although they are considered as newcomers in studies of learning and memory (Sison et al., 2006), zebrafish cognitive abilities have been successfully compared to that of mammalian counterparts in a broad range of learning and memory paradigms (Al-Imari and Gerlai, 2008; Chacon and Luchiari, 2014; Colwill et al., 2005; Pather and Gerlai,

2009; Sison and Gerlai, 2011). Recent studies have also shed light on the capability of zebrafish for solving complex spatial learning tasks (Gómez-Laplaza and Gerlai, 2010; Karnik and Gerlai, 2012; Luchiari et al., 2015), further advocating the competency of this model organism to study memory functions in vertebrates.

Despite the fact that zebrafish model has opened a new avenue towards high-throughput assays of cognitive functions, there is still a paucity of information on the involvement of different neuromodulatory systems in learning and memory processes in this species. The cumulative evidence provides a firm foundation that DA underlies a variety of neurobehavioural functions in zebrafish such as locomotor activity, anxiety, and social interaction (Irons et al., 2013; Savio et al., 2012; Scerbina et al., 2012). Using a three-chamber task of spatial discrimination, it has been demonstrated that nicotine administration improves learning performance in zebrafish via the mediation of the dopaminergic system (Eddins et al., 2009). Furthermore, Darland et al. (2012) have shown that antagonism of D2 receptors, but not D1 receptors, impaired cocaine-induced conditioned place preference in zebrafish (Darland et al., 2012). However, to our knowledge next to nothing is known about the direct effect of DA receptors manipulation on different aspects of spatial learning and memory in zebrafish. In the present study, we sought to clarify the role of DA receptors in two different phases of learning and memory: acquisition and consolidation of memory. To this end, we used a spatial latent learning paradigm that has specifically been developed to study learning and memory processes in zebrafish (Gómez-Laplaza and Gerlai, 2010; Luchiari et al., 2015). Unintentional or unplanned acquisition of information forms a major part of normal learning and memory in animals and humans. This paradigm is based on the principles of latent learning in which fish incidentally learn about the spatial construction of a complex maze by exploring and wandering, in the absence of reinforcer. Once a relevant reinforcer or reward is introduced into that environment, subjects use the spatial map to find the reinforcer more quickly than those individuals that were not given the unreinforced exploration (Jensen, 2006).

2.2. Materials and methods

2.2.1. Animals and maintenance

A total of 136 adult (130 experimental fish plus 6 fish as stimulus fish) wild-type zebrafish (1-year-old, ~ 1:1 male: female ratio) were used in this study. The animals were obtained from the University of Saskatchewan Health Sciences vivarium and were given at least 14 days to acclimate

to the animal facility. The fish were housed in groups of 16 in glass tanks (50 cm × 30 cm × 25 cm, length × width × depth; 50-l) at the Aquatic facility of the Dept. of Biology. All tanks were filled with filtered and de-chlorinated Saskatoon tap water with room and water temperatures maintained at 26 ± 1 °C. The illumination of holding tanks was provided by overhead fluorescent light tubes (23 W) mounted directly above the tanks on a 12-hr cycle (08.00–20.00 hr light) consistent with the zebrafish standard of care (Westerfield, 2007). Fish were fed two times a day (at approximately 10:00 a.m. and 4:00 p.m., 2% body weight/day ration) with a mixture of ground flake food (Nutrafin Max flakes, Hagen, Germany) and frozen brine shrimp (Sally's, San Francisco Bay Brand Inc., USA). Each alternate day, 30% of the water was replaced with fresh system water. The housing conditions were identical for both experimental fish and stimulus group.

2.2.2. Drugs and pharmacological manipulations

All drugs were purchased from Sigma-Aldrich (St. Louis, MO). DA receptor agonists included apomorphine hydrochloride hemi-hydrate (APO, non-selective DA receptors), (±)SKF-38393 hydrochloride (SKF, selective D1 receptors), and (−)quinpirole hydrochloride (QUIN, selective D2 receptors). DA receptor antagonists consisted of R-(+)-SCH-23390 hydrochloride (SCH, selective D1 receptor antagonist), and eticlopride hydrochloride (ETIC, selective D2 receptor antagonist). Based on previous studies on fish and rodents, we used doses of 0.1 mg/l of APO, 10 mg/l of SKF, 1 mg/l of QUIN, 1 mg/l of SCH, and 0.5 mg/l of ETIC (Ahlenius et al., 1977; Boehmler et al., 2007; Irons et al., 2013; Mora-Ferrer and Gangluff, 2002; Scerbina et al., 2012).

Experimental fish (n = 130) were randomly assigned to thirteen 30-l glass tanks in order to make groups of 10 fish per tank and were subjected to drug treatment and learning and memory tests. The sex ratio in each tank was approximately 50:50%. There was no significant effect of sex. Therefore, the data for the sexes were pooled. The fish were allowed to acclimate to their new tanks for 3 days prior to the initiation of the experiment. A solution-immersion method was applied according to drug delivery protocols previously proposed for zebrafish (Scerbina et al., 2012; Sison and Gerlai, 2011). For this, the appropriate amount of each drug was dissolved in a 3-l rectangular beaker contained system water. Afterward, fish were gently netted out from their respective tanks and placed in the beaker which was slightly aerated. Experimental fish were allowed to explore the beaker for 30 min to uptake the drug through the mouth, gills, and skin (Sison and Gerlai, 2011). The efficacy of this exposure period has already been established, where dopaminergic drugs and

other neurotransmitter-targeted agents have shown to reach and affect the brain in zebrafish (Scerbina et al., 2012; Sison and Gerlai, 2011; Tran et al., 2015). The effects of dopaminergic drugs persist up to 260 min (Irons et al., 2013), while the exploration period for each training trial did not exceed 30 min. In order to target different phases of learning and memory (including acquisition and consolidation) we applied two different drug administration time points: before the start of training to target the acquisition process and after the training trial of each day to target consolidation of memory. Therefore, for evaluating the possible impact of DA receptors on the acquisition process, 10 fish in each group treated with APO, SKF, QUIN, SCH, and/or ETIC prior to each training. However, for the investigation of the consolidation process, fish received these drugs immediately after the end of each training trial. Moreover, two groups of 10 fish were served as controls for either acquisition or consolidation process. Control fish experienced the same manipulation and training sessions similar to their corresponding groups (either acquisition or consolidation), but fish received no chemical exposure. A group of 10 fish also served as untrained controls which were exposed to neither drugs nor training sessions. (5 chemicals × 2 times = 10 treatment groups + 2 trained control groups + 1 untrained control group).

2.2.3. Apparatus and learning procedure

The test apparatus used in this study was identical to one recently introduced by Gómez-Laplaza and Gerlai (2010). The maze was constructed from 2 mm thin transparent Plexiglas and contained two side and one straight tunnel which were branched from a start chamber and led to a reward chamber, making a 4-way intersection (see Fig. 2.1). The straight tunnel was blocked in order that physical access to the reward chamber was only possible via left and right tunnels. The maze was filled with system water to reach a 10 cm water depth with the same temperature as experimental tanks. Transparency of the maze provided the experimental fish with visual access to all parts of the maze, from any location. The maze was located on a wooden table that covered by a white plastic tablecloth. The front path of the start chamber was closed using a transparent Plexiglas sheet and once was open the experimental fish had three choices: swim back into the start chamber, choose the right and/or left tunnels that take them to the reward chamber.

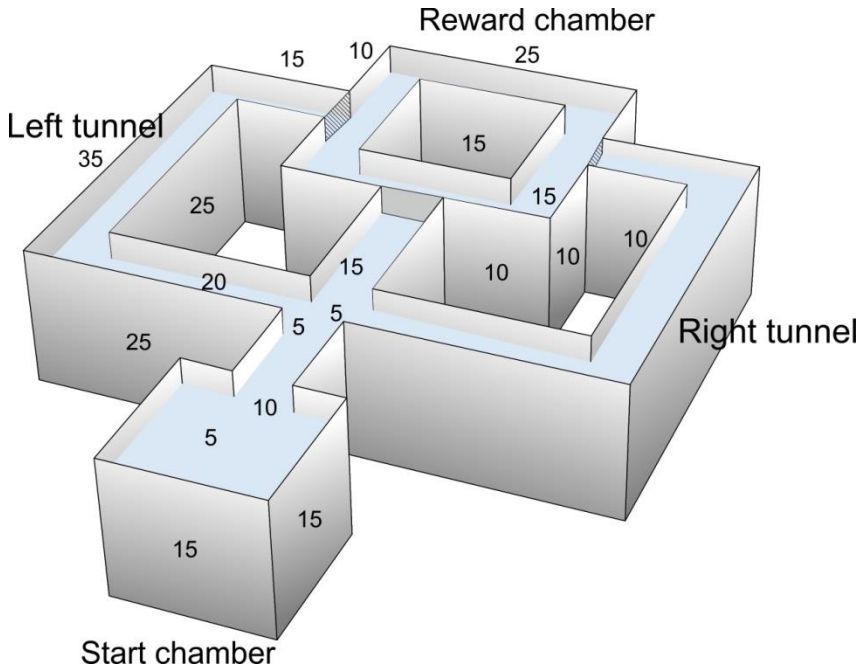

Figure 2.1. Schematic drawing of the latent learning apparatus used in this study. The maze is made of transparent Plexiglas similar to what was employed before (Gómez-Laplaza and Gerlai, 2010). The start chamber, left and right tunnels, and reward chamber are illustrated in the figure. The numbers indicate the dimensions of the maze in cm. Striped walls point to removable doors leading to the reward chamber. Note that the reward chamber contained stimulus fish only during the probe trial.

During the training period, groups of 10 fish from their respective treatments were netted out and placed in the start chamber to acclimate for 30s, thereafter they were released and allowed to freely explore the maze. Fish in groups that received APO, SKF, and QUIN were allowed to explore the maze with the right tunnel open while the left tunnel was closed. However, fish in the control groups and those treated with SCH and ETIC were trained to reach the reward chamber via the left tunnel open. This training pattern was randomly chosen based on a coin flip. Finally, in order to certify the process of learning in our maze, one group of 10 fish was underwent a manipulation and handling procedure similar to other groups, but these fish were exposed to a rectangular Plexiglas tank that was filled with water to a depth identical to the maze (hereinafter referred to as other tank group or untrained group). Each training trial lasted 30 min and fish were allowed to explore the maze. During the training sessions the reward chamber was kept empty (i.e. no stimulus group was presented). This process repeated for 16 consecutive days.

At the conclusion of the training sessions, we performed a probe trial in order to evaluate learning performance in experimental fish. To this end, a single fish was placed in the start chamber of the maze for 30s and then released to explore the maze for 10 min. During the probe trial, both the

right and left tunnels were open. More importantly, the reward chamber contained 6 stimulus fish. Given the highly social nature of zebrafish (Spence et al., 2008), the sight of conspecifics has been shown to have robust and insatiable rewarding properties (Al-Imari and Gerlai, 2008; Gerlai, 2011). Therefore, we assumed that the experimental fish according to their training choose either the right or left tunnels to reach the close proximity of their conspecifics. The behaviour of subjects was recorded using an HD webcam (Logitech c310, USA) mounted above the maze. Footages were analyzed using image processing and vision techniques utilizing MATLAB (Academic version R2015a) and the parameters of interest were extracted (MATLAB codes were specifically developed for this experiment). The behavioural variables quantified included the latency to leave the start chamber, the time spent in the left and the right tunnel leading to the reward chamber, the latency to enter the reward chamber, the time spent in the reward chamber, and locomotion (total distance traveled).

2.2.4. Statistical analysis

Data were analyzed parametrically using SPSS software (version 22.0, IBM SPSS Inc., USA) and are expressed as mean ± the standard error of the mean (SEM). To compare the performance of fish in the trained (the groups with one tunnel open) and untrained (other tank) groups independent sample *t*-tests were conducted. One-way ANOVA with planned contrasts was performed to compare group differences in cognitive performance before and after exposure to DA agonists and antagonists. We used simple contrasts, hence comparing each drug-treated group to the control group only. In all comparisons, the significance level was set at $p < 0.05$.

2.3. Results

Zebrafish actively explored the maze during both the training sessions and probe trial, and exhibited no signs of fear such as freezing, erratic movement or jumping (Parra et al., 2009; Speedie and Gerlai, 2008).

2.3.1. Trained control groups versus the untrained control group

In order to certify the process of learning in experimental fish, we compared different measures of cognitive performance between the other tank trained group (untrained in the maze) and the trained control groups (the groups with one tunnel open which served as control groups for the acquisition and consolidation processes). As shown in Fig. 2.2A and demonstrated by the *t*-test, fish in control groups that were trained in the maze exhibited significantly lower latency to leave the start chamber

compared to fish of the untrained group (acquisition: $t_{10.14} = 2.32$, $p = 0.041$; consolidation: $t_{9.37} = 2.28$, $p = 0.045$), indicating a higher motivation of these groups to explore the maze. Next, we examined the route bias in the experimental fish by quantifying the amount of time they spent in a certain tunnel according to their past training experience. As Fig. 2.2B depicts, fish in the control groups (both acquisition and consolidation) trained with one tunnel open appeared to spend more time in their respective tunnel. Independent sample *t*-test confirmed this observation and showed that fish in control groups trained with one route open spend significantly more time in that tunnel compared to the untrained fish (acquisition: $t_{18} = 2.63$, $p = 0.016$; consolidation: $t_{18} = 2.27$, $p = 0.035$). Latency to enter the reward chamber (Fig. 2.2C) was served as another factor which reflects the motivation and motor function of experimental fish to reach the reward chamber (Gómez-Laplaza and Gerlai, 2010). Independent sample *t*-test showed a significant effect of training, since fish in the trained control groups reached the reward chamber significantly sooner than the untrained group (acquisition: $t_{18} = 4.79$, $p = 0.001$; consolidation: $t_{13.11} = 4.64$, $p = 0.001$). We also quantified the duration of time that subjects spent in the reward chamber. This measure indicates the shoaling tendency of our experimental fish to stay with their conspecifics. As depicted in the Fig. 2.2D, the *t*-test showed no significant differences among the trained control groups and untrained control group in the amount of time the spent in the reward chamber (acquisition: $t_{11.89} = 0.53$, $p = 0.59$; consolidation: $t_{13.18} = 0.13$, $p = 0.89$). The level of activity of experimental fish we also quantified as the total distance traveled by fish. This factor represents either motivation of fish to explore the maze to find the reward chamber or level of reluctance and fear. Fig. 2.2E presents the total distance traveled by experimental fish over the probe trial. Independent sample *t*-test found no significant difference in locomotor activity between the trained and untrained control groups (acquisition: $t_{12.24} = 0.19$, $p = 0.85$; consolidation: $t_{18} = 0.55$, $p = 0.58$). Taken together, these results emphasize the efficiency of learning paradigm used in this study. Moreover, there was no significant difference in learning performance between males and females.

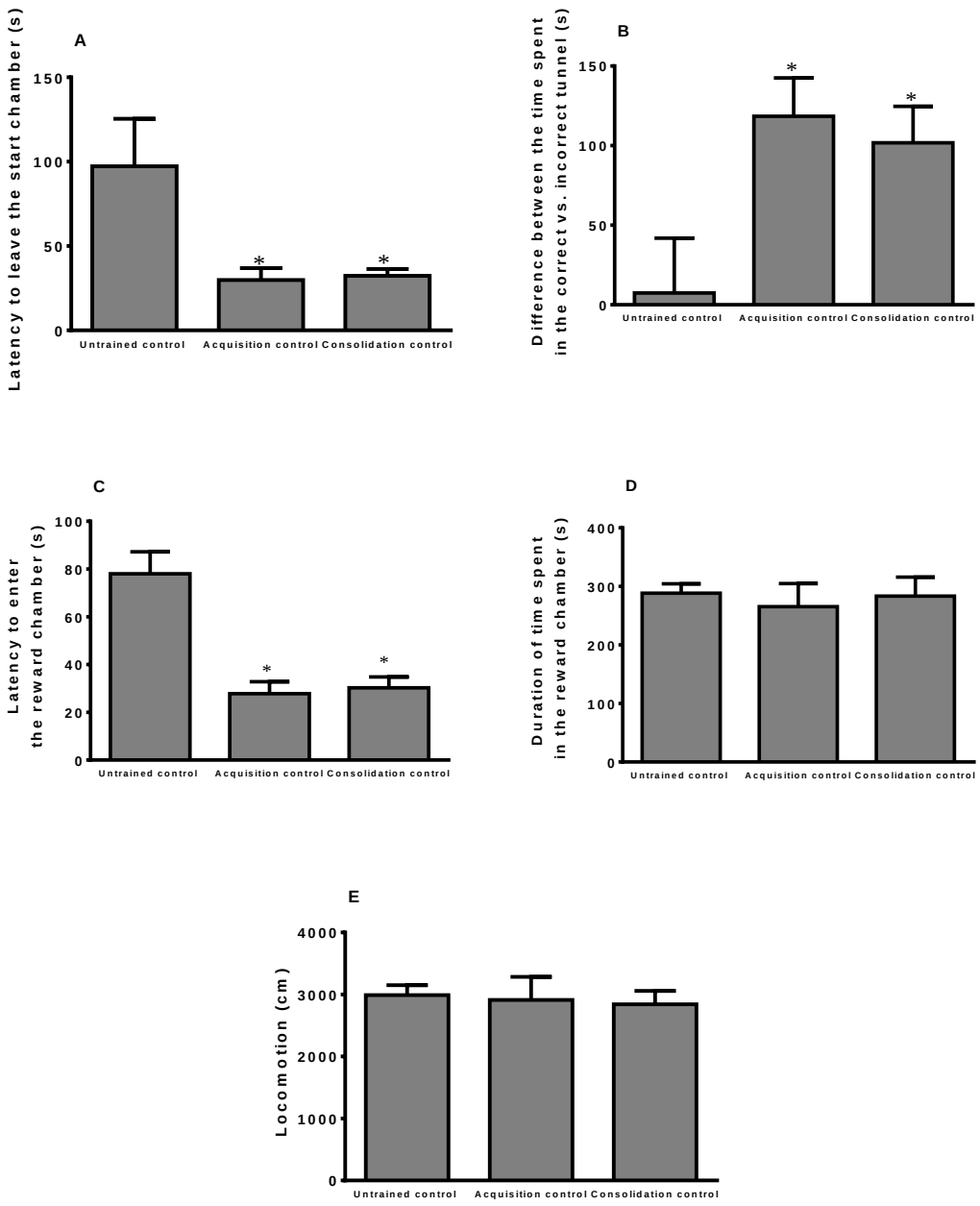

Figure 2.2. The effect of training trials on the performance of adult zebrafish in latent learning task in comparison to the untrained fish. The latency to leave the start chamber (A), difference between the time spent in the correct vs. incorrect tunnel according to the training condition (B), the latency to enter the reward chamber (C), duration of time the fish spent in the reward chamber (D), and locomotion (E). Asterisks above error bars denote a significant difference at $p < 0.05$ ($n = 10$).

2.3.2. Effects of dopaminergic drugs on the acquisition of latent learning

In Fig. 2.3 the effects of different dopaminergic drugs on the acquisition of latent learning task in zebrafish are shown. As illustrated in Fig. 2.3A and confirmed by one-way ANOVA, there was a

significant difference ($F_{5, 54} = 8.62$, $p = 0.001$) in latency to leave the start chamber among treatment groups. Planned contrasts recognized that groups treated with APO ($t_{13.89} = -2.60$, $p = 0.021$), SKF ($t_{11.94} = -3.99$, $p = 0.002$), and QUIN ($t_{11.66} = -2.09$, $p = 0.023$) showed remarkably longer latencies to leave the start chamber, while fish in the group received ETIC exhibited significantly ($t_{10.60} = 2.59$, $p = 0.026$) shorter latency to leave the box compared to the control group. There was no statistically significant difference in the latency to leave the start chamber between the group exposed to SCH ($t_{17.08} = 0.67$, $p = 0.50$) and the control group. After leaving the start chamber fish appeared to have the route bias (Fig. 2.3B). ANOVA confirmed this observation ($F_{5, 54} = 12.83$, $p = 0.001$) and planned contrasts showed that fish in groups treated with APO ($t_{54} = 3.15$, $p = 0.003$), SKF ($t_{54} = 3.10$, $p = 0.003$), and QUIN ($t_{54} = 4.64$, $p = 0.001$) spent significantly less time in their corresponding tunnel. In contrast, dwelling time in the correct tunnel significantly increased in fish given ETIC ($t_{54} = -2.34$, $p = 0.023$) compared to the control group irrespective of the tunnel they were trained with. No significant preference in dwelling time was found in the group treated with SCH ($t_{54} = 1.32$, $p = 0.192$). The latency to enter the reward chamber after leaving the start box is depicted in Fig. 2.3C. ANOVA demonstrated a significant effect of dopaminergic drugs ($F_{5, 54} = 17.30$, $p = 0.001$). Planned contrasts showed that exposure to APO ($t_{15.13} = -9.54$, $p = 0.001$), SKF ($t_{11.74} = -4.65$, $p = 0.001$), and QUIN ($t_{10.43} = -3.11$, $p = 0.010$) significantly prolonged the latency to reach the reward chamber. In contrast, fish that were given ETIC reached the reward chamber significantly sooner ($t_{13.04} = 2.19$, $p = 0.046$) compared to the control group. However, latency to enter the reward chamber was statistically indistinguishable between fish that received SCH ($t_{14.64} = 0.36$, $p = 0.72$) and the control group. Furthermore, the tendency of fish to shoal with their conspecifics was quantified as the amount of time the experimental fish spent in the reward chamber (Fig. 2.3D). ANOVA showed statistically significant differences ($F_{5, 54} = 6.20$, $p = 0.001$) among groups in the amount of time the experimental fish stayed in the reward chamber. Subsequently, planned contrasts identified that the fish treated with APO spend significantly lower time ($t_{54} = 2.66$, $p = 0.010$) in the reward chamber. Nevertheless, fish that were given SKF ($t_{54} = -1.82$, $p = 0.074$), QUIN ($t_{54} = 1.75$, $p = 0.85$), SHC ($t_{54} = -1.32$, $p = 0.190$), and ETIC ($t_{54} = 1.08$, $p = 0.281$) stayed the same amount of time near the stimulus group in comparison to the control group. Additionally, ANOVA did not show any significant alteration in locomotor activity (Fig. 2.3E) among different groups ($F_{5, 54} = 2.33$, $p = 0.056$).

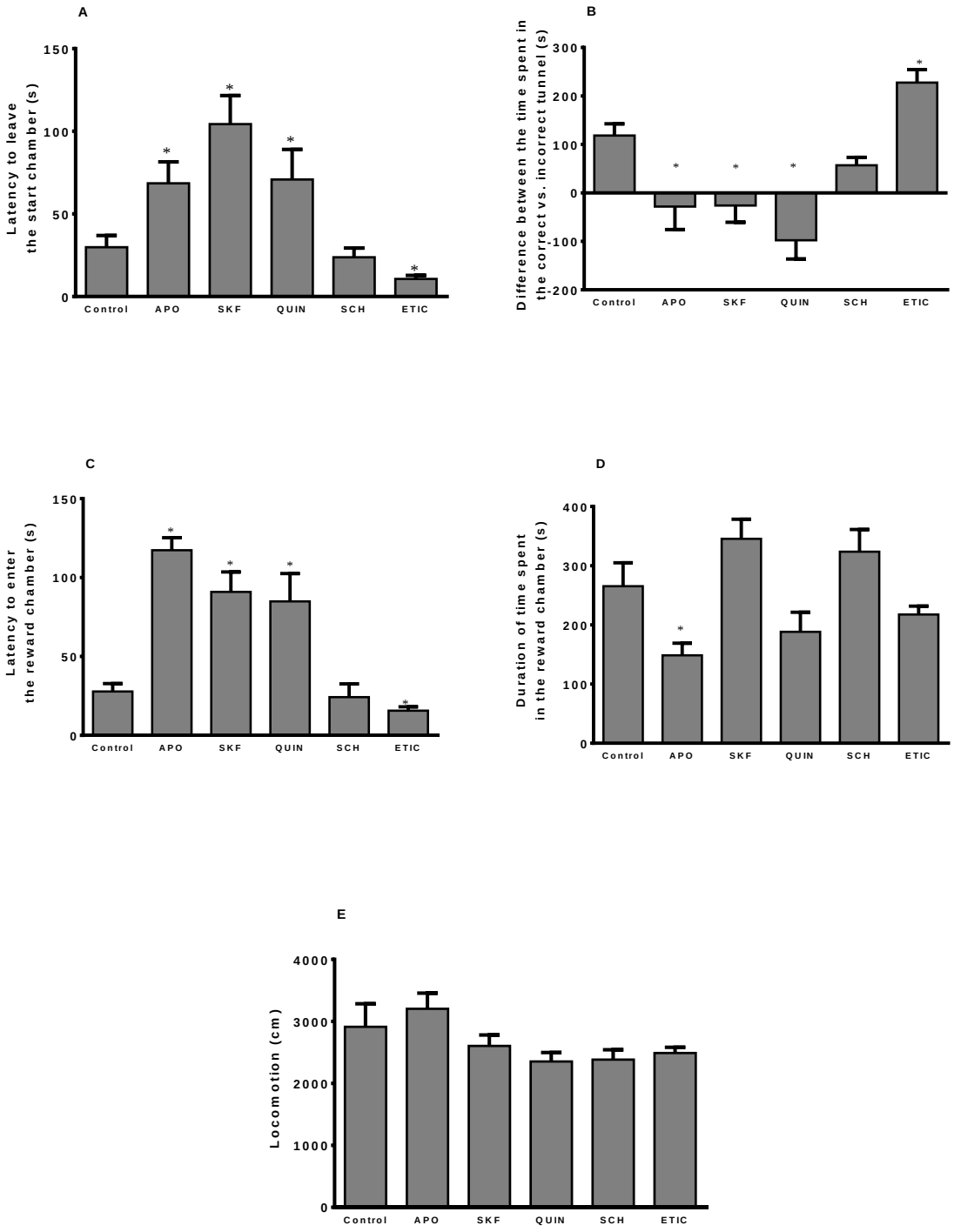

Figure 2.3. The effects of dopaminergic agonists and antagonists on the acquisition of latent learning in adult zebrafish. The latency to leave the start chamber (A), difference between the time spent in the correct vs. incorrect tunnel according to the training condition (B), the latency to enter the reward chamber (C), duration of time the fish spent in the reward chamber (D), and locomotion (E). Asterisks above error bars denote a significant difference at $p < 0.05$ ($n = 10$).

2.3.3. Effects of dopaminergic drugs on consolidation of latent learning

In the last stage of the present study, we investigated the effect of dopaminergic drugs on the consolidation process of latent learning in zebrafish. As shown in Fig. 2.4A, there was a trend towards higher latencies to leave the start chamber for some drug-treated groups. In this regard, ANOVA found a significant treatment effect ($F_{5, 54} = 5.54$, $p < 0.001$). Planned contrasts further showed that fish in groups received APO ($t_{9.89} = -2.97$, $p = 0.014$), SKF ($t_{9.35} = -2.46$, $p = 0.035$), and QUIN ($t_{11.76} = -4.18$, $p = 0.001$) exhibited a significantly higher latency to leave the start chamber compared with the control group. In contrast, fish that were given ETIC had significantly ($t_{14.66} = 3.92$, $p = 0.001$) shorter latency to exit the start chamber. However, exposure to SCH did not change the latency to leave the start chamber ($t_{11.78} = -0.53$, $p = 0.60$). As represented in Fig. 2.4B, the time spent in the correct tunnel irrespective of left or right tunnel training appeared to be different among groups. ANOVA showed this significant difference ($F_{5, 54} = 11.77$, $p < 0.001$) and planned contrast attributed that to the groups exposed to SKF ($t_{54} = -2.13$, $p = 0.037$) and QUIN ($t_{54} = -4.80$, $p < 0.001$) which preferred to spend significantly less time in their respective tunnels when compared to the control group. In contrast, dwelling time in the correct tunnel significantly ($t_{54} = 2.50$, $p = 0.016$) increased in the group treated with ETIC. The time spent in the correct tunnel, however, did not significantly differ between the groups that received APO ($t_{54} = -1.71$, $p = 0.092$) and SCH ($t_{54} = -0.823$, $p = 0.414$) compared to the control group. Fig. 2.4C shows the latency to enter the reward chamber as measured after the experimental fish left the start box. ANOVA recognized a significant main effect of drug treatment ($F_{5, 54} = 11.82$, $p = 0.001$). Planned contrasts showed that it took significantly more time for the fish treated with APO ($t_{12.04} = 6.07$, $p < 0.001$), SKF ($t_{9.99} = 2.67$, $p = 0.023$), and QUIN ($t_{11.32} = 3.69$, $p = 0.003$) to reach the reward chamber while this time significantly reduced ($t_{16.01} = -2.60$, $p = 0.019$) in group exposed to SCH. However, no significant effect of treatment on the latency to enter the reward chamber was found in the group exposed to ETIC ($t_{17.86} = -1.47$, $p = 0.15$). Fig. 2.4D presents the duration of time zebrafish spent in the reward chamber during the probe trial. ANOVA identified a significant effect of drug treatment ($F_{5, 54} = 4.90$, $p = 0.001$) and planned contrasts showed that exposure to APO ($t_{10.40} = -4.43$, $p = 0.001$) and ETIC ($t_{17.04} = -2.19$, $p = 0.042$) significantly reduced the time that experimental fish stay close to the stimulus group. However, the tendency to swim in close proximity of the stimulus fish did not statistically differ between groups treated with SKF ($t_{16.08} = -0.61$, $p = 0.54$), QUIN ($t_{13.84} = -2.05$, $p = 0.060$), and SCH ($t_{17.56} = -0.23$, $p = 0.81$) compared to

the control group. As depicted in Fig. 2.4E we also quantified the distance fish swam. However, ANOVA did not show any significant effect of drug treatment on this parameter ($F_{5, 54} = 0.80$, $p = 0.55$).

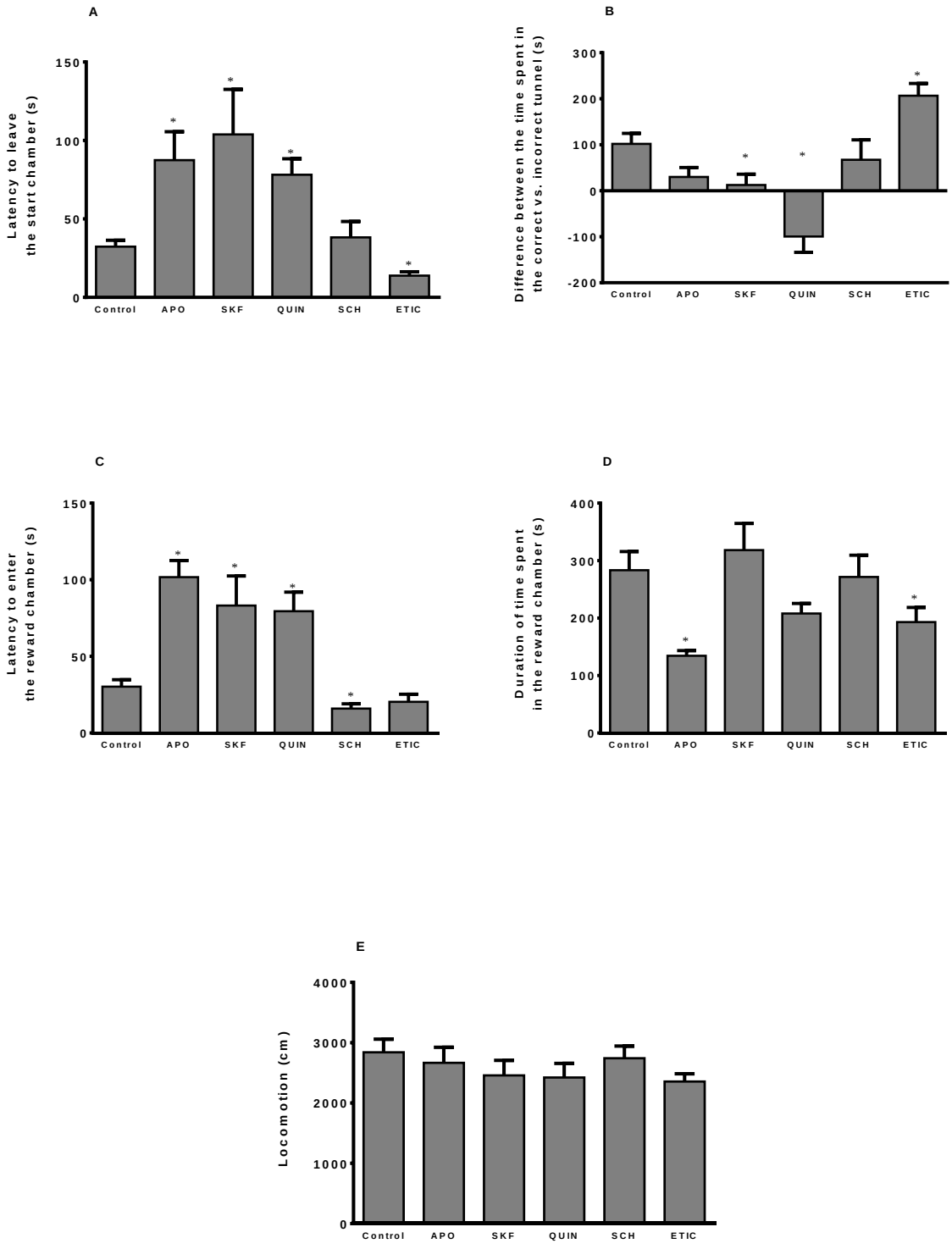

Figure 2.4. The effects of D1/D2 receptor agonists and antagonists on the consolidation process of latent learning in adult zebrafish. The latency to leave the start chamber (A), difference between the time spent in the correct vs. incorrect tunnel according to the training condition (B), the latency to enter the reward chamber (C), duration of time the fish spent in the reward chamber (D), and locomotion (E). Asterisks above error bars denote a significant difference at $p < 0.05$ ($n = 10$).

2.4. Discussion

In latent learning, animals learn in the absence of reinforcement. The manifestation of the acquired information is not displayed at the time of learning, but rather is evident only when a suitable reward appears (Jensen, 2006). This type of learning is not principally different from associative learning tasks, where a reinforcer is paired with a conditioned stimulus. In fact, the interface between these learning paradigms is the presence of reinforcement which evokes a learned response. It is also noteworthy that the novelty of the maze *per se* can act as a reinforcer or reward (Gómez-Laplaza and Gerlai, 2010).

In this study, the experimental fish were given an exploration phase in a spatial environment by which they incidentally learned the spatial layout of the maze in the absence of a reinforcer. Once the sight of conspecifics emerged as a reward, the subjects were expected to be able to recall and use the spatial map to find the stimulus group in a more efficient way than those fish that were not given the unreinforced exploration. As seen in Fig. 2.2, the maze-trained fish exhibited significantly lower latency to leave the start chamber, higher dwelling time in the tunnel that was open during their training, and shorter latency to find the stimulus group compared to the untrained fish, indicating that latent learning had taken place. However, all fish spent about the same length of time in the reward chamber supporting the high motivation provided by the conspecific school. In other words, in spite of reduced exploration tendency in the untrained fish, they still had the motivation to stay in close proximity to their conspecifics.

DA receptors have been shown to modulate different aspects of learning and memory in animals. There is very strong experimental support for the involvement of this neurotransmitter in information acquisition and consolidation of various types of learning and memory (reviewed in El-Ghundi et al., 2007; Dalley and Everitt, 2009). In this study, we have demonstrated that stimulation of the D1/D2 receptors impaired both acquisition and consolidation of latent learning in zebrafish. Administration of APO before and after training trials was associated with the prolonged latency to leave the start chamber, increased dwelling time in the incorrect tunnel, and increased latency of the experimental fish to reach the reward chamber (Fig. 2.3 and 2.4). These findings clearly suggest that exposure to APO impaired acquisition and consolidation of latent learning in zebrafish. Previous studies on rodents have also demonstrated the disruptive effects of apomorphine, a non-selective DA receptor agonist on the acquisition and consolidation of memory

in different learning paradigms (Carrera et al., 2011; Hale and Crowe, 2001; Pitsikas and Markou, 2014; Shao et al., 2010). More importantly, there is also compelling evidence that this DA receptor agonist disrupts latent learning in mice. Ahlenius et al. (1977) found that administration of APO prior to the training trial significantly suppressed the exploratory activity of mice in a latent learning task. Moreover, it has also been reported that increased DA transmission induced by the DA receptor agonist APO, the DA receptor blockers (GBR 12909 and nomifensine), and DA releaser (methamphetamine), attenuated latent learning in the water-finding task (Ichihara et al., 1993b). Similar results were found in a study by Nabeshima et al. (1994), where administration of APO before the training trial produced latent learning impairment in mice (Nabeshima et al., 1994).

Our results also show that pre- and post-training exposure to APO significantly decreased the tendency of shoaling in zebrafish (Fig. 2.3D and 2.4D) which was indicated by a decline in the time spent in the reward chamber. Recently, using a protocol similar to the present study, Luchiari et al. (2015) have reported a marked decline in the duration of time zebrafish spent in the reward chamber after exposure to acute doses of alcohol. Dysfunction of the dopaminergic system which is critically important for different aspects of reward processing has been suggested as a possible reason for the reduced shoaling tendency of alcohol-treated fish (Adinoff, 2004; Hyman et al., 2006). Moreover, several studies in rodents highlight the importance of DA for a broad range of socially motivated behaviours (Krach et al., 2010). It has been demonstrated that the activation of D2 receptors is particularly important in social reactivity (Aragona and Wang, 2009; Cibrian-Llanderal et al., 2012; Gingrich et al., 2000). In addition, the behavioural effects of APO are thought to be mediated in large part via D2 receptors (Ichihara et al., 1993a; Seeman, 1980). Therefore, it is highly likely that the reduction in group preference of APO treated fish is mediated via D2 receptors.

As our results indicate (Fig. 2.3E and 2.4E), pre- and post-training administration of APO did not alter the locomotor activity of fish. Although the evidence regarding the locomotor effect of APO seems inconsistent, previous studies suggested that the effect of APO on motor activity is both dose- and context-dependent (Gunzler et al., 2007; Irons et al., 2013). In line with previous reports (Coelho et al., 2011; Garcia et al., 2005), our results show that the effect of low dose of APO was not significant on motor activity of zebrafish. Additionally, it has been shown that effect of low doses of APO similar to that used in this study, on locomotion is transient having the maximal

effect within 20 min of administration (Gunzler et al., 2007; Iron et a., 2013). On the other hand, it has been demonstrated that APO impairs latent learning by disrupting the animals' attention to a novel stimulus (Ichihara et al., 1993a; Nabeshima et al., 1994). Therefore, we argue that impaired ability to swim is not a potential factor underlying the attenuated memory performance in fish, instead, the observed cognitive deficit is ascribable to the disruptive effects of APO neural mechanisms of learning.

APO is a non-selective dopaminergic receptor agonist at both D1 and D2 receptors (Seeman, 1980). Thus, to identify which receptor specifically mediates the disruptive effects of APO on latent learning, we further examined the effects of selective DA receptor agonists and antagonists on zebrafish learning performance. The results show that pre- and post-training administration of D1/D2 agonists significantly attenuated the learning and memory in zebrafish. Administration of SKF and QUIN remarkably increased latency to leave the start chamber, the time spent in the wrong tunnel, and latency to reach the reward chamber, indicating impaired acquisition and consolidation of latent learning in zebrafish. However, antagonism of D2 receptors by administration of ETIC before or after training trials resulted in an enhancement of latent learning ability in zebrafish. Our results, including the shortened latency to leave the start chamber, longer swimming time in the tunnel route correspond to their prior experience, and diminished latency to find and reach the reward chamber clearly imply an improvement in the cognitive performance of zebrafish. It is of note that antagonism of D2 receptors by ETIC influenced both acquisition and consolidation of memory while administration of SCH did not affect the acquisition of latent learning. In fact, post-training administration of SCH just reduced the duration of time that fish reach the reward chamber (Fig. 2.4C). Rodent studies demonstrated that the DA receptor agonists impair latent learning while the DA antagonists improve it. Ichihara et al. (1993a) found that pre-training administration of the D2 receptor antagonist sulpiride fully counteracted the disruptive effect of APO in a latent learning task in mice and subsequently improve their cognitive performance whereas SCH failed to antagonize APO effects. Using a water-finding task, it has also been shown that D2 receptor antagonist sulpiride (but not D1 receptor antagonist) effectively counteracted impaired latent learning in mice induced by GBR 12909, a selective DA uptake inhibitor. Additionally, both D1 and D2 receptor agonists (SKF and QUIN) has been revealed to impair latent learning in mice (Ichihara et al., 1993b). Administration of a D2 receptor antagonist

(sulpiride) but not D1 receptor antagonist (SCH) significantly improved the deficits in latent learning performance in mice induced by mild traumatic brain injury (Tang et al., 1997).

Our results are in agreement with these studies in which pre- and post-training exposure to D1 and D2 agonists attenuated cognitive performance of zebrafish in latent learning task while D2 antagonist produced a significant memory enhancement. Also of note is the fact that the administration of SCH had no effect on acquisition of learning in zebrafish while its stimulatory effect was evident on the consolidation process. One might argue that the dose of SCH that we used was ineffective to produce significant enhancements of latent learning when administrated after training. However, the same dose led to a marked effect on memory consolidation, making this possibility remote. An intriguing speculation is that a functional interaction between D1 and D2 receptors may to some extent be involved. In this case, it has been postulated that D1 receptor antagonists such as SCH are also able to block the effects of D2 receptor agonists, QUIN in particular (Andersen and Nielsen, 1986; Byrnes et al., 1994; Gioanni et al., 1998). This raises the possibility that the effects of D1 receptors on latent learning in zebrafish were mediated through D2 receptors. Taken together, our findings indicate that stimulation of both D1 and D2 receptors is important, but the effects of DA neurotransmission on acquisition and consolidation of latent learning are predominantly mediated by activation of D2 receptors.

Selective attention has strongly been implicated in the acquisition of latent learning by enhancing perceptual sensitivity to stimuli at an attended location. An optimum DA level in the brain is crucial for attentional focus towards important stimuli (Noudoost and Moore, 2011). Hence, D2 autoreceptor regulation of extracellular DA in latent learning seems to be necessary. In line with this, previous studies have demonstrated a negative correlation between activity level of D2 receptors and selective attention to novel stimuli (Ichihara et al., 1989, 1993a; Lissek et al., 2015; Tang et al., 1997). Therefore, antagonism of D2 receptors might improve latent learning performance in zebrafish by enhancement of selective attention during unreinforced exploration. Converging evidence from animal and human studies implicates that DA plays a fundamental role in novelty-seeking behaviour and exploratory excitability (Balleine et al., 2007; Norbury and Husain, 2015). It is widely believed that novelty can increase DA activity in the hippocampus and the medial prefrontal cortex of animals (Hamilton et al., 2010; Ihalainen et al., 1999). Neuropharmacological studies have also shown that direct manipulations of the DA system in

rodents and mammals alter the exploration of novel objects (Costa et al., 2014; Pogorelov et al., 2005; Tournier et al., 2013; Watson et al., 2012). The heightened DA release in rodent brains, which is accompanied by higher responsivity to the novel environment, appears to be at least partially due to the weakened autoreceptor control of the midbrain DA producing cells (Marinelli and White, 2000). Moreover, a recent study has posited that there is an inverse relationship between novelty seeking personality traits in humans and D2 receptor availability in the lower midbrain (Zald et al., 2008).

DA agonist QUIN at low doses increase pre-synaptic activation of D2 autoreceptors which subsequently leads to the reduction in DA neurotransmission while at higher doses affects post-synaptic receptors (Benaliouad et al., 2009; Nader and LeDoux, 1999a; Shilliam and Heidbreder, 2003). A delicate balance in DA signal is crucial for latent learning paradigm (Beninger and Miller, 1998). Therefore, it seems that impaired latent learning in fish treated with QUIN resulted from an extreme decrease in DA signal. D2 receptor antagonists attenuate the inhibition caused by stimulation of autoreceptors (White and Wang, 1984). Thus, we speculate that the stimulatory effects of ETIC on latent learning performance in zebrafish are a direct consequence of D2 autoreceptor inhibition followed by an optimum level of DA increase in the brain. Although DA receptor activation and inactivation markedly affected two different phases of latent learning, mechanisms downstream of these effects remain to be elucidated.

Consistent with the view that distinct DA receptors are able to modulate reward-related processes, our results show that the blockade of D2 receptors, by post-training administration of a D2 receptor antagonist, considerably reduced social preference in zebrafish. However, the antagonism of D1 receptors produced no effects on the shoaling tendency. In line with rodent studies (Aragona and Wang, 2009), these findings further support our early assumption that the reduced shoaling response in APO treated fish is mediated via D2 receptors. Contrary to the present results, Scerbina et al. (2012) reported a reduction of the social preference in the AB strain of zebrafish induced by the D1 antagonist SCH. Since the dose of SCH and procedure of exposure was identical to that of the present study, we assume that a difference in experimental methods is at the root of this discrepancy. For instance, in that study fish were tested in a social preference paradigm which is totally different from the task employed in our research which was specifically designed to examine the cognitive performance of zebrafish. In the task of Scerbina et al. (2012), computer-animated

images of female zebrafish applied as a synthetic shoal while we used mixed genders of live fish as stimulus group. Alternatively, strain-dependent effects of SCH as stated by Scerbina et al. (2012) might be another feasible justification for this inconsistency. On the other hand, previous reports have described an important role for D2 receptors in reward-related behaviours. For instance, blockade of D2 receptors or deletion of its gene brought about to the reduction of reward-potentiating effects of different stimuli in rodents (Johnson and Kenny, 2010; Fish et al., 2014). In zebrafish, Darland et al. (2012) showed that antagonism of a potent D2 receptor blocked the rewarding effect of cocaine in a conditioned place preference task, while blockade of D1 receptors had no effect. As a result, our present results suggest that, regarding reward-seeking behaviour in zebrafish, D2 receptors may contribute more than D1 receptors. Finally, the doses of dopaminergic drugs used in the present study had no significant effect on the locomotor and exploratory activity of zebrafish in the latent learning task. This finding helps us to ensure that any effects D1 and D2 receptors on learning the tasks were unlikely to be confounded by effects on general behavioural activity.

2.5. Conclusion

In summary, the present data show a striking convergence between zebrafish and rodents in the dopaminergic modulation of the latent learning task, perhaps by mediating D2 (auto)receptor activity. Given that the spatial latent learning encompasses a large proportion of animal and human learning, disruption of this advance cognitive ability via imbalance of DA neurotransmission subsequently contributes to a variety of neurobehavioural disorders like what manifest in schizophrenia and Parkinson's disease. Furthermore, our data indicate that D2 receptors, possibly govern the reward-potentiating effect of the social stimulus in zebrafish. However, exactly how D2 receptor signalling in the zebrafish brain is involved in this process remains to be determined. To our knowledge, this is the first report demonstrating the involvement of DA receptors in regulating latent learning in zebrafish. Considering the fact that this small tropical species has provided novel insights into the processes of learning and memory in vertebrates, the current study opens a new avenue of research for studying the neurological bases of these advanced cognitive functions

Chapter 3: Modulatory effects of dopamine receptors on associative learning performance in zebrafish (*Danio rerio*)

Preface

The aim of this chapter is to address the second objective of my doctoral research work, which is to provide an understanding of the role of dopamine receptors in the acquisition, consolidation, and recall of associative learning in adult zebrafish. For this purpose, fish were exposed to D1 and D2 receptor agonists and antagonists for 30 min before and after training in the associative learning paradigm, and also for 30 min before the probe trial. The results of this study show that D1 receptors play a more important role in the acquisition and consolidation of associative learning in zebrafish, while D2 receptors are critically involved in the regulation of memory recall. Moreover, the findings of this chapter provide a foundation for Chapter 5 where I have investigated the effects of chronic exposure to dietary selenomethionine on the dopaminergic system and associative learning performance in adult zebrafish.

The content of Chapter 3 was reprinted (adapted) from Behavioural Brain Research (DOI: 10.1016/j.bbr.2016.01.034). Naderi, M., Jamwal, A., Chivers, D.P., Niyogi, S. "Modulatory effects of dopamine receptors on associative learning performance in zebrafish (*Danio rerio*)". Copyright 2016, with permission from Elsevier Inc.

Author contribution

Mohammad Naderi (University of Saskatchewan) designed and conducted the experiment, generated and analyzed the data, prepared all the figures, and drafted and revised the manuscript.

Ankur Jamwal (University of Saskatchewan) provided technical assistance with behavioural tests and data analysis.

Doug Chivers and Som Niyogi (University of Saskatchewan) provided inspiration, scientific input and guidance, commented on and edited the manuscript, and provided funding for the research.

3.1. Introduction

Learning and memory are basic pillars of advanced cognition that allow individuals to adapt to their environmental needs and consequently increase their chance of survival. To date, there has been mounting evidence for involvement and recruitment of different hormonal and neuromodulatory systems in these fundamental higher brain functions. Among the modulatory systems involved in the regulation of information acquisition and synaptic plasticity in the central nervous system (CNS), the dopaminergic system has received particular attention (Wise, 2004). Dopamine (DA) is the most important catecholaminergic neurotransmitter in the vertebrate brain (Ma and Lopez, 2003). It not only plays a critical role in various physiological processes (Beaulieu and Gainetdinov, 2011; Dufour et al., 2005), but also regulates a wide array of cerebral functions related to cognition such as fear, mood, motivation, attention, locomotion, reward, and memory (Girault and Greengard, 2004; Vidal-Gadea et al., 2011). Therefore, it is not surprising that dysfunction in dopaminergic neurotransmission is associated with a variety of psychiatric and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, schizophrenia, Tourette syndrome, and attention deficit-hyperactivity disorder, as well as drug and alcohol abuse (Buse et al., 2013; Lodge and Grace, 2011b; Martorana and Koch, 2014).

Two pharmacologically and physiologically distinct categories of G protein-coupled receptors mediate DA neurotransmission in the brain of vertebrates. In mammals, these receptors are classified as D1-like (D1 and D5; henceforth referred to as D1 receptors) and D2-like (D2, D3, and D4; henceforth referred to as D2 receptors) receptors. D1 receptors stimulate the production of intracellular 3'-5'-cyclic adenosine monophosphate (cAMP) by adenylyl cyclase (AC) induction and positively regulate protein kinase A (PKA) activity or intracellular calcium (Ca^{2+}) signalling. In contrast, the D2 receptors inhibit AC which results in the reduction of intracellular messenger cAMP as well as PKA activity and intracellular Ca^{2+} levels (Sweatt, 2009). DA receptors have a widespread distribution pattern in different brain regions, particularly areas that are critically involved in cognition, such as the hippocampus, prefrontal cortex, amygdala, and ventral and dorsal parts of the striatum. Clearly, localization of DA receptors in these brain regions sheds more light on the functional role of this catecholamine neurotransmitter in various aspects of learning and memory (Beaulieu and Gainetdinov, 2011; Dalley and Everitt, 2009; El-Ghundi et al., 2007). In this regard, a growing body of evidence indicates that DA receptors functionally contribute to the

acquisition, consolidation, retrieval, reconsolidation, and extinction phases of different types of learning and memory (El-Ghundi et al., 2007; Puig et al., 2014).

Mammals are still pioneering organisms in the fields of toxicology, teratology, behaviour, and neuroscience. However, they bear several drawbacks and limitations (e.g., require a lot of space for maintenance, high maintenance and breeding costs, slow growth, etc.), which has triggered the search for finding more useful alternatives (Linney et al., 2004; Newman et al., 2011). Since being introduced for biomedical research purposes by Streisinger (1981), zebrafish are increasingly taking the place of more complex animals in several disciplines such as genetics, developmental biology, and pharmacology (Streisinger et al., 1981). This species enjoys many favorable properties relative to its mammalian counterparts, which make it a powerful experimental model (Kalueff et al., 2014). Importantly, zebrafish possess sufficient anatomical complexity with high physiological and genetical (~ 70%) homology to humans–allowing effective modeling of human diseases and neurobehavioural disorders (Howe et al., 2013; Newman et al., 2011; Terriente and Pujades, 2013). Despite the relatively long phylogenetic distance, basic features of the CNS in fish resemble to those of mammals with the engagement of all major neurotransmitters, neurohormones, and receptors (Kalueff et al., 2014).

Given evolutionarily conserved features of the vertebrate catecholaminergic system (Smeets and González, 2000), the zebrafish has also emerged as a valuable model organism for studying different aspects of these important signalling molecules. Dopaminergic pathways in this species, in particular, display remarkable similarities to the corresponding mammalian systems (Maximino and Herculano, 2010). Nonetheless, there are some differences in the localization pattern of DA neurons between mammals and zebrafish. The majority of zebrafish DA neurons are located across the diencephalon and telencephalon, while the midbrain is the main center of dopaminergic domains (substantia nigra and ventral tegmental area) in mammals. However, previous studies have suggested that zebrafish DA neurons in the posterior tuberal nucleus, DA neurons in posterior tuberculum (periventricular nucleus of posterior tuberculum), and subpallial dopaminergic neurons can be functional equivalents of the main dopaminergic pathways in the mammalian brain (Rink and Wullimann, 2002a; Schweitzer and Driever, 2009; Tay et al., 2011). This similarity is more striking since all types of mammalian DA receptors have also been identified in zebrafish (Boehmler et al., 2007; Boehmler et al., 2004; Li et al., 2007). Several neurobehavioural

abnormalities associated with dysfunction of the dopaminergic system have been reported in zebrafish (Anichtchik et al., 2004; Chatterjee and Gerlai, 2009; Powers et al., 2011). Additionally, behavioural alterations as a result of acute exposure to dopaminergic drugs have been observed in this species (Boehmler et al., 2007; Irons et al., 2013; Scerbina et al., 2012; Tran et al., 2015).

Zebrafish model is also gaining momentum in the field of learning and memory (Colwill et al., 2005; Gerlai, 2011; Gómez-Laplaza and Gerlai, 2010). A number of studies showed robust cognitive abilities of zebrafish in a range of learning tasks such as visual choice discrimination learning (Colwill et al., 2005), shuttle box learning (Pather and Gerlai, 2009), place conditioning (Zala and Määttänen, 2013), active avoidance learning (Aoki et al., 2015), spatial memory tasks (Luchiari et al., 2015), and plus-maze non-spatial and spatial associative learning paradigms (Fernandes et al., 2014; Grossman et al., 2011; Sison and Gerlai, 2010, 2011). Nonetheless, there is still a dearth of literature regarding the particular role of different neurotransmitters in these cognitive functions. Specifically, next to nothing is known about the involvement of the specific DA receptor subtypes in modulating different aspects of learning and memory. Mammalian studies show that DA plays a central role in coding reward-associated prediction during associative learning. Dopaminergic cells produce two different activity states: a slow tonic firing and phasic burst firing. There is a broad consensus that phasic DA firing derives associative learning through activation of post-synaptic D1 receptors (Dreyer et al., 2010; Hazy et al., 2010). Therefore, the purpose of the present study was to investigate the effects of pharmacological manipulation of D1/D2 receptors on the acquisition, consolidation, and recall of associative learning in zebrafish using a plus-shaped maze. To this end, fish were exposed to D1 and D2 receptor agonists and antagonists before the training trial in an associative learning task, immediately after the training, and just before the probe trial.

3.2. Materials and methods

3.2.1 Animals and housing

Wild-type adult zebrafish (*Danio rerio*, 1-year-old) were obtained from the University of Saskatchewan Health Sciences vivarium and maintained in the Aquatic facility of the Dept. of Biology. A total of 128 zebrafish (~ 50-50% sex ratio) were housed in groups of ~ 20 in 50-l glass tanks and acclimatized for three weeks before their use in the experiment. All tanks were filled with de-chlorinated Saskatoon tap water which was under constant aeration. The water temperature

was maintained at 25-27 °C by using 75-W thermostat controlled aquarium heaters (AQUATOP model GH75, China). Illumination was provided by fluorescent light tubes (23 W) mounted on top of the tanks in 12/12 hr light/dark (08.00-20.00 hr light) cycle according to the standards of zebrafish care (Westerfield, 2007). A 30% exchange of water was carried out in each tank every alternate day. Fish were fed twice a day (at approximately 10:00 a.m. and 4:00 p.m., 2% body weight/day ration) with a mixture of fish flake food (Nutrafin Max flakes, Hagen, Germany) and frozen brine shrimp (Sally's, San Francisco Bay Brand Inc., USA).

3.2.2. Drugs and dosing procedure

All drugs were obtained from Sigma-Aldrich (Saint Louis, MO, USA). DA receptor agonists used in this study included (±)SKF-38393 hydrochloride (SKF; a selective D1 receptor agonist), and (−)quinpirole hydrochloride (QUIN; a selective D2 receptor agonist). DA receptor antagonists used were R-(+)-SCH-23390 hydrochloride (SCH; a selective D1 receptor antagonist), and eticlopride hydrochloride (ETIC; a selective D2 receptor antagonist). Due to their water solubility, all drug stock solutions were made by dissolving the chemicals in system water and stored in the dark at –20 °C until use. Final concentrations of SKF (10 mg/l), SCH (1 mg/l), QUIN (1 mg/l), and ETIC (0.5 mg/l) were based on previous studies in fish (Boehmler et al., 2007; Irons et al., 2013; Mok and Munro, 1998; Scerbina et al., 2012; Tran et al., 2015) and rodents (Brady and O'Donnell, 2004; Pruitt et al., 1995).

The treatment procedure used in this study was adapted from Sison and Gerlai (2011) with some modifications. Experimental fish were randomly distributed to sixteen 30-l tanks to make groups of 8 fish per tank. Fish were exposed to each drug at three time points: immediately before the first training trial of the day (targeted the memory acquisition), immediately after the last training trial of the day (targeted the memory consolidation), and immediately before the probe (targeted the memory recall). One control group was allocated to each phase. Thus, we had three control groups that received paired CS-US training and no drug treatment. Moreover, fish in these control groups experienced a manipulation procedure similar to their corresponding treatments. There was also another control group (from now on referred to as the unpaired control group) in which fish received no drug exposure and were trained in the maze while CS and US presented separately (i.e. there was no association between the US and the CS).

Fish were treated with the dopaminergic drugs, which involved immersion of the entire fish in the drug solution (Sison and Gerlai, 2011; Scerbina et al., 2012; Tran et al., 2015). In short, the appropriate amount of each compound was dissolved in a 3-l rectangular beaker containing ambient water; then individual fish was gently netted out of the tank and placed into the beaker which was provided with proper aeration. Fish were then allowed to swim freely within the beaker for 30 min. The rationale behind choosing 30 min exposure period was based on previous studies that have shown this period is enough for the drug to influence the zebrafish brain (Scerbina et al., 2012; Irons et al., 2013; Tran et al., 2015). Moreover, it has been shown that significant effects of these dopaminergic drugs persisted at least for 4 hours post-dosing (Irons et al., 2013), while each trial in this study lasted only up to 30 min.

3.2.3. The associative learning task; apparatus and procedure

The test apparatus used in this study to evaluate learning and memory performance in zebrafish was identical to that previously employed by others (Al-Imari and Gerlai, 2008; Fernandes et al., 2014; Sison and Gerlai, 2010). It was a transparent plus-shaped maze made of Plexiglas (2 mm thin), an adapted version of rodent radial-arm maze (Schwegler and Crusio, 1995). The dimensions for maze were: 35 cm arm's length, 11 cm width, and 20 cm wall height, which was interconnected by a 11 cm×11cm central platform, into which a transparent plastic was placed that served as the start box (Fig. 3.1A). The maze was placed on a wooden table that was covered by a white plastic tablecloth. At the end and adjacent to the left side of each arm, there was a small clear plastic tank (stimulus or reward tank, 17 cm × 9 cm ×10 cm, length × width × height). Visual access to the content of stimulus tanks was confined by placing white opaque plastic sheets on 3 sides of each tank (except for the side facing the maze). Hence, the presence or absence of stimulus group was determined only if experimental fish swim into the arm. Owing to the social nature of zebrafish, the sight of conspecifics (unconditioned stimulus; US) was chosen as the stimulus, which is established as a highly potential rewarding factor (Al-Imari and Gerlai, 2008; Sison and Gerlai, 2011; Gerlai, 2011). Six fish served as the stimulus group, whose origin, housing, and feeding conditions were identical to the experimental fish. Furthermore, four plastic cue cards with two different colors (conditioned stimulus; CS) were placed at the end of each arm, indicating the presence or absence of the stimulus group. During the experiment with paired groups, a red cue card always presented with the stimulus fish, while 3 white cue cards at the end of other arms served as the indicator of empty stimulus tanks. The same procedure was employed for the unpaired

control group but the association of red cue card with the stimulus groups was done in a randomized manner. The maze was rotated randomly across training trials to make extra-maze spatial cues on the ceiling irrelevant. To acclimate the experimental fish to the maze and reduce the novelty and handling the stress of the test to a minimum, a series of 2 hrs habituation trials were conducted in which initial groups of 20 fish were introduced to the maze and allowed to explore freely. The numbers of fish were gradually reduced to 10, 5, and finally 1 fish on the fourth day of the habituation phase. Zebrafish in these trials were not exposed to either the CS or the US.

The learning trials were initiated after the habituation period and each experimental fish underwent 16 training sessions, each lasting for 5 min for 4 consecutive days (4 trials per day with 2 min inter-trial intervals, Fig. 3.1B). During this phase, one of the stimulus tanks contained 6 zebrafish (US) and co-presented with the red cue card (CS), whereas other stimulus tanks were empty and accompanied by white cue cards. This arrangement (location of US and CS) was carried out in a randomized manner once fish in the unpaired control group were transferred to the maze. For each trial, a single fish was netted from its respective tank and placed into the start box. After 30s, the start box was gently lifted and the fish was allowed to explore the maze for 5 min.

To assess the efficacy of memory and learning performance in zebrafish, training sessions were followed by a final probe trial. During the probe trial, a single fish was placed in the plus-maze for 5 min. At this time, no stimulus group was presented and only the CS (red and white cue cards) positioned at the end of the arms. The behaviour of experimental fish was monitored using an HD webcam (Logitech c310, USA) mounted above the maze. Video files were analyzed using image processing and vision techniques utilizing MATLAB (Academic version R2015a) and the parameters of interest were extracted. The behavioural variables quantified were daily average of the percentage of time that the subject fish spent in the target arm (Time in Target Arm: TTA), daily average of the number of entries to the target arm (Target Arm Entries: TAEs), daily average of the number of incorrect arm entries (Incorrect Arm Entries: IAEs), and the daily average of locomotion (total distance moved).

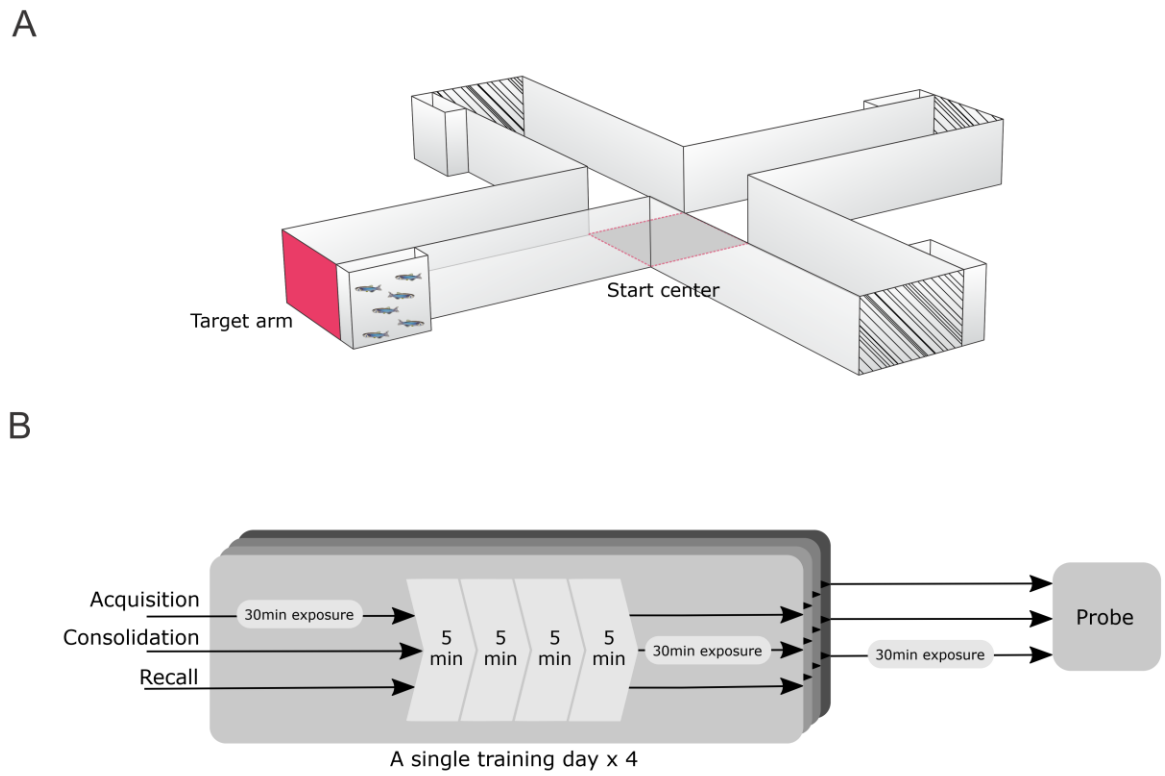

Figure 3.1. Panel A. Schematic of the plus-maze apparatus used in this study. There was a stimulus fish tank close to the end of each arm. The end of one of the arms was covered by a red plastic cue card (+ CS) indicating the presence of stimulus fish in the stimulus tank (US). Three white plastic sheets (− CS) were also positioned at the end of other arms served to predict the empty stimulus tanks (indicated by diagonal lines at the end of the arms). Panel B. Experimental design for the associative learning task in the plus-maze apparatus. The training and probe sessions of experiment include training trials by which experimental fish train to associate the CS with the US (4 consecutive trials each lasts 5min for a period of 4 days), and the probe trial which was conducted 24 hrs after the last training trial. There were 2 min long inter-trial intervals between each training trial. Three different experiments based on drug administration periods and targeted mechanism are shown (adapted from Sison and Gerlai, 2011).

3.2.4. Statistical analysis

Data were analyzed using SPSS for Windows (version 22.0, IBM SPSS Inc., USA). To evaluate the efficacy of training protocol in zebrafish learning, the performance of individuals in each paired control group (acquisition, consolidation, and recall) was compared with the unpaired control group using separate independent sample *t*-tests. Separate variance analyses (ANOVA) with planned contrasts were performed for each experiment (i.e. acquisition, consolidation, recall) to investigate the effect of dopaminergic drugs on different aspects of associative learning in zebrafish. Data are presented as mean ± SEM. The significance level was set at $p < 0.05$.

3.3. Results

Zebrafish during both training and probe trials actively explored the maze and no signs of fear or stress (e.g., erratic movement, jumping or freezing) were observed. In order to certify the efficacy of associative learning paradigm used in this study, we compared the cognitive performance of fish in the unpaired control group with other paired controls for the acquisition, consolidation, and recall processes. The percentage of time that the experimental fish spent in the target arm (i.e. the arm that contained just the CS) could be an important indicator of cognitive capabilities in the trained fish. As shown in Fig. 3.2A and demonstrated by *t*-test, fish in the control groups for acquisition ($t_{10.001} = -3.104$, $p = 0.011$), consolidation ($t_{14} = -4.086$, $p = 0.001$), and recall ($t_{14} = -5.461$, $p = 0.001$) spent significantly more time in the target arm compared with the unpaired control group. Fish in the control groups that received the CS-US pairing exhibited significantly more TAEs compared to the unpaired control group (acquisition: $t_{14} = -2.531$, $p = 0.001$; consolidation: $t_{14} = -2.532$, $p = 0.024$; recall: $t_{14} = -2.531$, $p = 0.024$, Fig. 3.2B). As shown in Fig. 3.2C, fish in the control groups that experienced paired CS-US training displayed significantly fewer IAEs compared with the unpaired control group (acquisition: $t_{14} = 2.328$, $p = 0.035$; consolidation: $t_{14} = 2.892$, $p = 0.012$; recall: $t_{14} = 2.412$, $p = 0.030$). However, independent sample *t*-test did not find any significant alteration in locomotor activity in each paired control group when compared to the unpaired control group (acquisition: $t_{14} = -0.125$, $p = 0.902$; consolidation: $t_{14} = 0.128$, $p = 0.900$; recall: $t_{14} = 0.115$, $p = 0.910$, Fig. 3.2D). Taken together, these results emphasize the efficiency of learning paradigm used in this study.

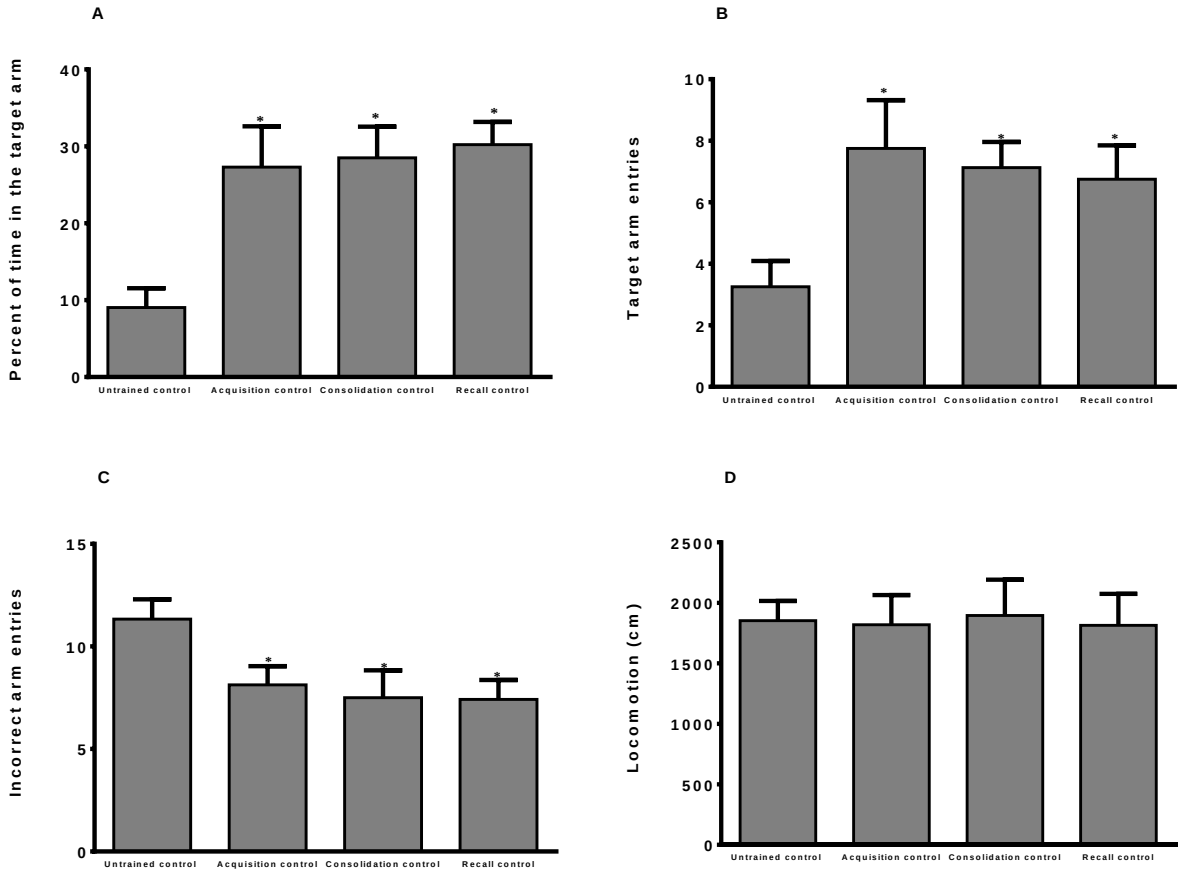

Figure 3.2. The effect of training on zebrafish performance in the associative learning task. The performance was assessed by measuring the percentage of time zebrafish spent in the target arm during the probe (A), target arm entries (B), incorrect arm entries (C), and total distance traveled by the fish (D). Note that separate independent sample *t*-tests were performed to compare each paired control group with the unpaired control group. Asterisks above data bars indicate significance vs. control group ($p < 0.05$).

3.3.1. Effects of dopaminergic drugs on memory acquisition

The effects of different dopaminergic drugs on the acquisition of associative learning task in zebrafish are shown in Fig. 3.3. The percentage of time that experimental fish spent in the target arm appeared to differ among some treatment groups (Fig. 3.3A). One-way ANOVA showed a significant treatment effect ($F_{4, 35} = 2.771$, $p = 0.040$), demonstrating that some group(s) differed from the control group. Planned contrasts disclosed that fish treated with SKF ($t_{35} = -2.525$, $p = 0.016$), SCH ($t_{35} = -2.141$, $p = 0.038$), and QUIN ($t_{35} = -2.292$, $p = 0.028$) spent significantly more time in the target arm. There was no statistically significant difference in TTA between the group exposed to ETIC ($t_{35} = -0.401$, $p = 0.691$) and the control group. One-way ANOVA also showed that TAEs significantly differed among groups ($F_{4, 35} = 6.300$, $p = 0.001$, Fig. 3.3B). Planned

contrasts showed that TAEs significantly increased in fish treated with SCH (t_{35} = -2.058, p = 0.040), while decreased in fish treated with ETIC (t_{35} = 2.390, p = 0.022). There was no statistically significant difference in TAEs in the groups treated with SKF (t_{35} = 0.797, p = 0.431) and QUIN (t_{35} = 1.992, p = 0.073) compared to the control group. As shown in Fig. 3.3C, IAEs differed between groups. One-way ANOVA showed a significant treatment effect ($F_{4, 35}$ = 4.158, p = 0.007) and planned contrasts showed that the fish in the groups treated with SKF (t_{35} = 2.469, p = 0.019) and QUIN (t_{35} = 3.968, p = 0.001) had fewer IAEs compared with the control group. IEAs did not significantly differ in groups treated with SCH (t_{35} = 1.526, p = 0.136) and ETIC (t_{35} = 1.969, p = 0.060) when compared to the control group. As reported in Fig. 3.3D, one-way ANOVA found no significant difference among groups in the total distance moved by fish ($F_{4, 35}$ = 0.734, p = 0.575).

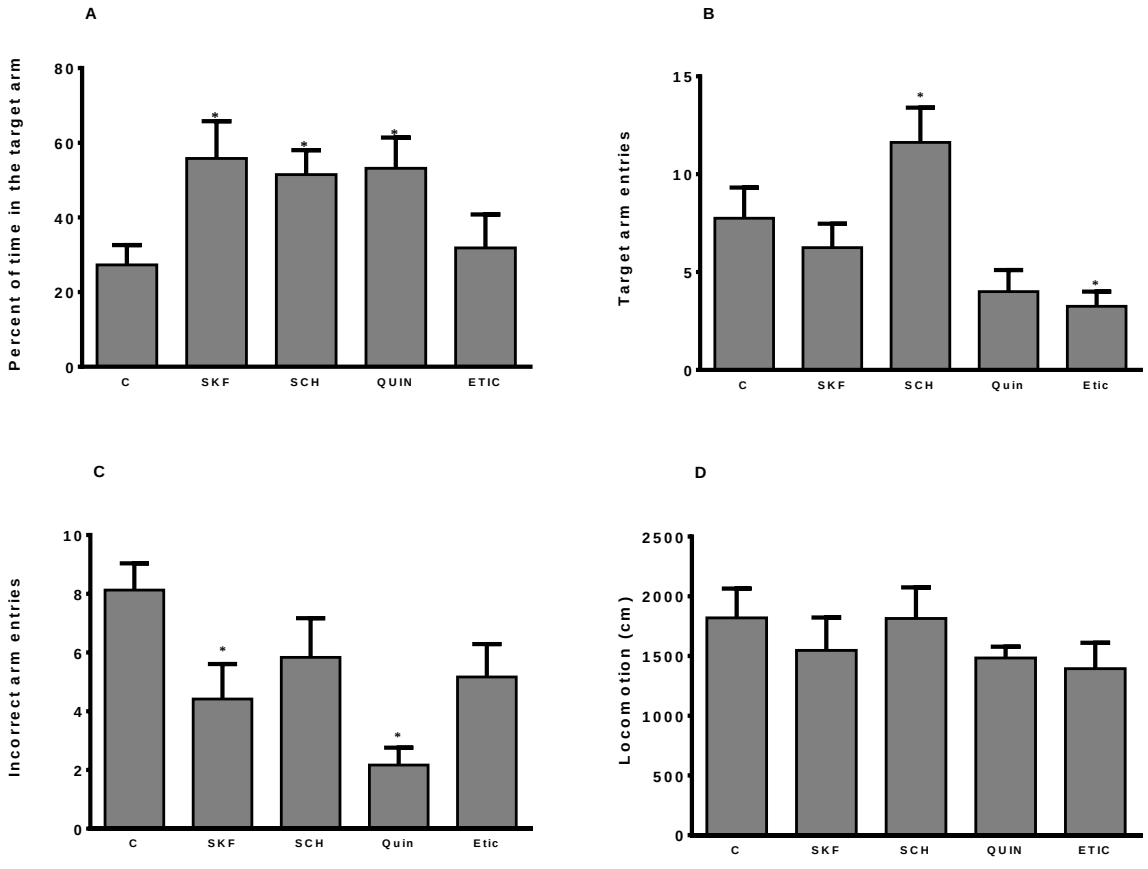

Figure 3.3. The effects of dopaminergic agonists and antagonists on the acquisition of associative learning in zebrafish as measured by: the percentage of time zebrafish spent in the target arm during the probe (A), target arm entries (B), incorrect arm entries (C), and total distance traveled by the fish (D). Asterisks above data bars indicate significance vs. control group ($p < 0.05$).

3.3.2. Effects of dopaminergic drugs on memory consolidation

We also assessed the effects of DA receptor agonists and antagonists on consolidation of associative learning in zebrafish (Fig. 3.4). One-way ANOVA showed a significant treatment effect on the percentage of time that fish spent in the target arm ($F_{4, 35} = 2.812$, $p = 0.039$). Planned contrasts showed that fish in groups received SKF ($t_{35} = -2.357$, $p = 0.024$) and SCH ($t_{35} = -2.353$, $p = 0.024$) spent significantly more time in the target arm compared with the control group. However, no statistically significant difference was found in TTA between groups treated with QUIN ($t_{35} = -0.329$, $p = 0.744$) and ETIC ($t_{35} = -0.278$, $p = 0.783$), when compared to the control group. Fig. 3.4B shows TAEs made by the experimental fish during the probe trial. One-way ANOVA demonstrated a significant treatment effect ($F_{4, 35} = 5.740$, $p = 0.001$). Planned contrasts showed that fish exposed to SCH ($t_{35} = -2.438$, $p = 0.020$) made significantly more TAEs, while fish treated with ETIC ($t_{35} = 2.195$, $p = 0.035$) exhibited significantly fewer TAEs when compared with the control group. TAEs did not significantly differ in groups treated with SKF ($t_{35} = -1.036$, $p = 0.307$) and QUIN ($t_{35} = -0.061$, $p = 0.952$) compared to the control group. One-way ANOVA also showed that IAEs significantly differed between groups ($F_{4, 35} = 2.841$, $p = 0.038$, Fig. 3.4C). Planned contrasts further indicated that fish in group treated with SKF made fewer IAEs ($t_{35} = 2.251$, $p = 0.031$) compared to the control group. No statistically significant difference in IAEs was found in groups treated with SCH ($t_{35} = -0.534$, $p = 0.597$), QUIN ($t_{35} = 1.856$, $p = 0.073$), and ETIC ($t_{35} = 1.206$, $p = 0.236$) relative to the control group. One-way ANOVA did not find any significant difference in locomotion ($F_{4, 35} = 1.112$, $p = 0.366$, Fig. 3.4D) among groups.

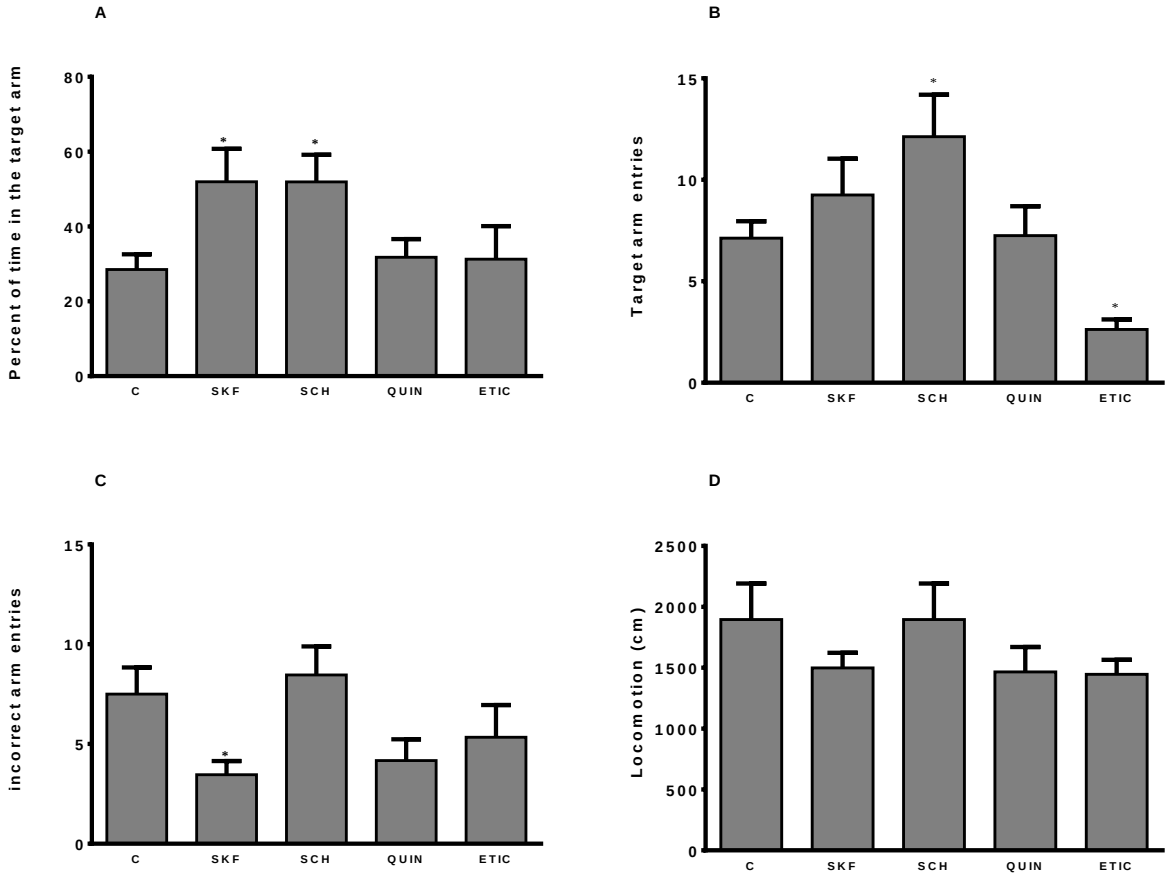

Figure 3.4. The effects of dopaminergic agonists and antagonists on the consolidation of associative learning in zebrafish as measured by: the percentage of time zebrafish spent in the target arm during the probe (A), target arm entries (B), incorrect arm entries (C), and total distance traveled by the fish (D). Asterisks above data bars indicate significance vs. control group ($p < 0.05$).

3.3.3. Effects of dopaminergic drugs on memory recall

Administration of dopaminergic drugs 30 min before the probe trial appeared to influence the cognitive performance of experimental fish during the probe (Fig. 3.5). The percentage of time fish spent in the target arm is shown in Fig. 3.5A. One-way ANOVA demonstrated a significant treatment effect ($F_{4, 35} = 5.505$, $p = 0.002$). Planned contrasts showed that fish in the group treated with QUIN ($t_{7.864} = -2.321$, $p = 0.040$) spent significantly more time in the target arm compared to the control group. In contrast, TTA significantly decreased in fish given ETIC ($t_{13.494} = 4.786$, $p = 0.001$) 30 min before the probe trial. No statistically significant difference in TTA was found in groups treated with SKF ($t_{9.281} = -0.975$, $p = 0.354$) and SCH ($t_{9.504} = -0.393$, $p = 0.703$) compared to the control group. Fig. 3.5B depicts TAEs in different groups during the probe trial. One-way ANOVA, however, did not detect any significant treatment effect ($F_{4, 35} = 1.497$, $p = 0.224$). Fig.

3.5C shows IAEs in different groups during the probe trial. One-way ANOVA determined a significant treatment effect ($F_{4, 35} = 3.915$, $p = 0.010$). Planned contrasts subsequently showed that IAEs were significantly reduced ($t_{35} = 3.372$, $p = 0.002$) in fish that received QUIN before the probe. However, there was no significant difference in IAEs in groups treated with SKF ($t_{35} = 0.664$, $p = 0.511$), SCH ($t_{35} = 0.512$, $p = 0.612$), and ETIC ($t_{35} = 0.061$, $p = 0.951$) when compared to the control group. Finally, we measured total distance traveled by the fish in different groups (Fig. 3.5D). One-way ANOVA, however, showed no significant treatment effect ($F_{4, 35} = 0.943$, $p = 0.451$) indicating locomotor activity of the experimental fish did not change during the probe trial.

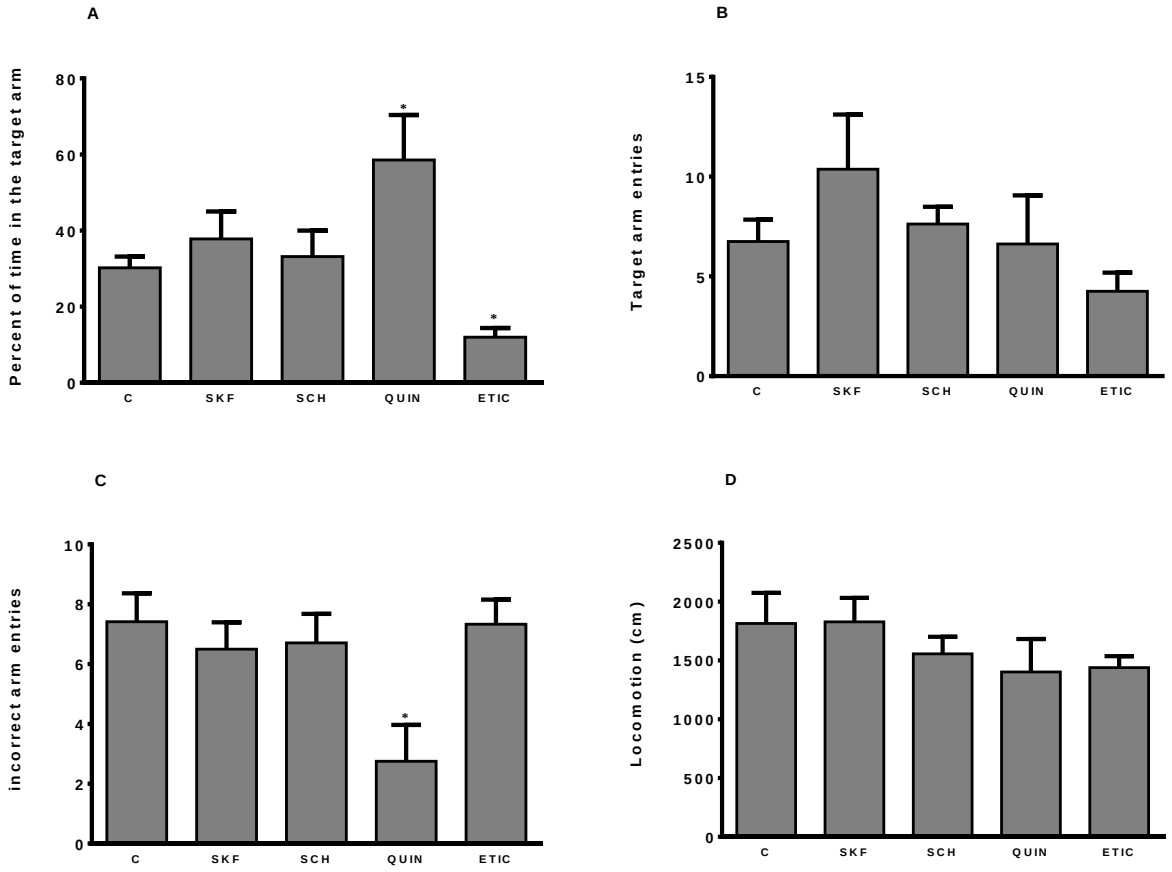

Figure 3.5. The effects of dopaminergic agonists and antagonists on recall of associative learning in zebrafish as measured by: the percentage of time zebrafish spent in the target arm during the probe (A), target arm entries (B), incorrect arm entries (C), and total distance traveled by the fish (D). Asterisks above data bars indicate significance vs. control group ($p < 0.05$).

3.4. Discussion

Associative learning is a fundamental learning mechanism enabling animals to predict vital environmental stimuli through experience. Results of this study show the capability of zebrafish to associate CS and US in the plus-maze as quantified by different variables (Fig. 3.2). Fish in all control groups that received the paired presentation of the CS and US spent more time in the target arm relative to the unpaired control group, which did not receive the CS-US pairing. Moreover, fish in control groups that experienced paired CS-US during training made more TAEs and had fewer IAEs compared with the unpaired control group. Therefore, we conclude that this specific preference for the target arm in fish that received the CS-US pairing is due to the manifestation of associative learning.

Rodent studies have demonstrated the importance of D1 receptors in the acquisition of different forms of learning and memory. For example, administration of D1 receptor agonist SKF facilitated the acquisition of fear conditioning in rats (Biedenkapp and Rudy, 2009; Péczely et al., 2014). In contrast, impaired acquisition of conditioned taste aversion, appetitive instrumental learning, conditioned place preference, and contextual fear conditioning following exposure to D1 receptor antagonists have previously been reported in rats (Baldwin et al., 2002; Heath et al., 2015; Young et al., 2014). Our results are in agreement with previous research in which pre-training administration of D1 receptor agonist improved learning performance of fish during the probe trial. As shown in Fig. 3.3, the administration of D1 agonist SKF prior to the beginning of training trials led to an increase in TTA, while attenuated the IAEs in fish during the probe. Increase in the target arm dwelling time and the decline in incorrect arm entries both imply enhanced cognitive performance in the group treated with D1 agonist. Our results also revealed an enhanced learning performance in the group received pre-training administration of D1 receptor antagonist SCH. This group exhibited an increase in TTE and TAEs during the probe trial (Fig. 3.3A,B). This finding, however, is in contradiction with a majority of investigations demonstrating the negative impact of the D1 receptor blockade on the learning process (reviewed in El-Ghundi et al., 2007). Previous studies have suggested an inverted U-shaped dose/response effect of dopaminergic drugs on performing different cognitive tasks in mice (Lidow et al., 2003), rats (Chudasama and Robbins, 2004), and monkeys (Vijayraghavan et al., 2007), where low doses improved learning performance while higher concentrations disrupted it. Moreover, several studies have shown that under certain conditions D1 receptor antagonist SCH may have D1 agonist-like effects (González et al., 2016;

Olsen and Duvauchelle, 2001; Racké et al., 1988). Therefore, it seems that employed dose of SCH in the present study had a stimulatory effect on the dopaminergic activity of the brain, which eventually resulted in the increased cognitive performance of the experimental fish. It has also been shown that blockade of the medial prefrontal cortex dopaminergic activity via infusion of SCH enhanced mesolimbic DA transmission in rat brain (Olsen and Duvauchelle, 2001). Activation of other compensatory pathways that elevate DA neurotransmission even after blockade of D1 receptors can be another explanation for our obtained results. It is also notable that SCH has a weak affinity for serotonergic and adrenergic receptors (Bischoff et al., 1988; Ramos et al., 2005), which have been strongly implicated in mediating learning and memory (Meneses et al., 2011; Olvera-Cortés et al., 2008; Sweatt, 2009). Therefore, the activation of other neurotransmitter pathways after SCH administration may also be involved in the regulation of cognitive processes. SCH can also increase the density of post-synaptic D1 receptors (Giorgi et al., 1993; O'boyle et al., 1993), which can subsequently enhance post-synaptic responses to phasic DA release required for associative learning. Differences in drug administration, training procedures, and/or type of testing apparatus may also account for the observed discrepancy.

Previous studies identified a differential involvement of D2 receptor signalling in the acquisition of different types of memory. The facilitatory effects of D2 receptor agonists on the acquisition of several learning tasks such as the Morris water task (Brown et al., 2000) and the conditioned place preference (Merritt and Bachtell, 2013) have previously been reported. In contrast, deleterious effects of D2 agonists on the acquisition of operant conditioning (Kurylo, 2004) and reward-based learning in a four-arm cross maze (Yawata et al., 2012) have been reported in rats. Furthermore, blockade of D2 receptors was found to be associated with impairment of acquisition of several forms of learning tasks in mice such as the fear conditioning (Ponnusamy et al., 2005) and the conditioned place preference (Young et al., 2014).

Our findings are in agreement with previous studies reporting the stimulatory effects of D2 receptor agonists on the acquisition of novel information. Pre-training administration of QUIN led to an increase in TTA, while decreased IAEs (Fig. 3.3). Moreover, our results showed that pre-training administration of D2 receptor antagonist ETIC reduced fish performance during the probe trial. However, our observations are only limited to the reduction in TAEs, since changes in TTA and IAEs did not reach a significant level. Consequently, we can conclude that the activation of D2

receptors accelerates the memory acquisition in the associative learning task, while their blockade leads to a reduction in the learning performance. Perhaps TTA is the most robust measure of learning performance in the present study, as also recommended by previous studies (Al-Imari and Gerlai, 2008; Sison and Gerlai, 2011). ETIC exposure did not remarkably alter this parameter. This finding raises another possibility in which D2 receptors play a complementary role in the acquisition of associative learning in zebrafish. It is possible that D2 receptors influenced the acquisition process via interaction with D1 receptors. Previous studies advocate this hypothesis in which D2 receptor effects are mediated in part by stimulation of D1 receptors (Bergstrom et al., 1987; Keefe and Gerfen, 1995; Nolan et al., 2007). Further research is required to determine the exact role of D2 receptors in the acquisition of associative learning in zebrafish.

Newly acquired information is consolidated progressively over time from an initially labile state to a more stable form (Dudai, 2012). The causal involvement of dopaminergic pathways in memory consolidation has been substantiated by various studies (see a review by Balderas et al. (2015)). For instance, administration of D1 receptor agonists enhanced consolidation of an inhibitory avoidance response in rats and mice (Bernabeu et al., 1997; Castellano et al., 1991). Nevertheless, post-training administration of D1 antagonist (SCH) has been observed to disrupt the consolidation of novelty-preference learning, object recognition, and inhibitory avoidance memory in rats (Balderas et al., 2015; Furini et al., 2014; Rossato et al., 2013).

Consistent with these findings, our results also indicate the involvement of D1 receptors in the consolidation of memory in fish, as observed in mammals. Enhanced memory consolidation in fish that received post-training SKF was exhibited by an increase and decrease in TTA and IAEs, respectively (Fig. 3.4). We also found that administration of D1 receptor antagonist immediately after training trials improved the performance of experimental fish in the plus-maze. In this regard, fish received SCH exhibited higher TTA and TAEs relative to the control group. This stimulatory effect of SCH, however, was not consistent with previous studies. The excitatory effect of SCH on the dopaminergic system, in conjunction with other neurotransmitter pathways, could be possible explanations for the obtained results, as described before. It is also worth noting that SCH can modulate the consolidation of different cognitive tasks under certain conditions. For example, Maroun and Akirav (2009), using an object recognition paradigm, reported that D1 receptor antagonist SCH impaired 24 hrs retention in rats that were well habituated before the training,

whereas enhanced 24 hrs consolidation in rats that did not experience the habituation process (Maroun and Akirav, 2009). More recently, it has been shown that systemic administration of SCH impaired contextual fear conditioning, but had no effect on fear memory consolidation in rats (Heath et al., 2015). Based on these findings, it can be suggested that the D1 receptors may adopt differential involvement in memory consolidation, depending on the type of learning tasks which include qualitatively and quantitatively different habituation and training sessions.

The evidence regarding the role of D2 receptors in the consolidation of newly-acquired information seems inconsistent. Immediate post-training administration of D2 agonists has been shown to facilitate memory consolidation in rats using the cued version of the Morris water maze task (Packard and McGaugh, 1994) and the Y-maze discrimination task (Gasbarri et al., 1997). However, D2 receptor agonists have shown to attenuate consolidation of fear conditioning in rats (Nader and LeDoux, 1999a). Blockade of D2 receptors, however, has been shown to impair consolidation of memory in mice tested in the water maze learning task (Morice et al., 2007) and in the novel object preference paradigm (França et al., 2014). Neither the effect of DA receptor agonists nor of antagonists on consolidation window has been found in other studies (de Lima et al., 2011; Kramar et al., 2014).

Our findings are consistent with those reports that suggest D2 receptor agonist does not alter consolidation of memory, while the D2 receptor antagonist disrupts this phase of memory formation. Pharmacological activation of D2 receptors in the present study did not produce any significant change in TTA, TAEs, and/or IAEs (Fig. 3.4). However, post-training blockade of D2 receptors by administration of ETIC led to a decrease in TAEs. The most parsimonious interpretation of the results is that D2 receptors are involved in the consolidation of associative learning in zebrafish. A possible explanation for our obtained results may be found in the synaptic-level effects of QUIN. Although pre-training administration of QUIN (1 mg/l) enhanced the acquisition of associative learning, this dose of D2 receptor agonist seems to be insufficient to exert the distinct effect on the consolidation process. This may be due to a striking distinction between acquisition and consolidation phases. Unlike to the acquisition process, consolidation requires extensive alterations of synaptic protein synthesis, activation of intracellular transduction cascades, and transcription factors that lead to changes in gene expression (Rosenberg et al., 2014). Therefore, it is plausible that a higher level of D2 receptor activation is required to accelerate

consolidation of acquired information. In support of this view, Sommer et al. (2014) have recently reported that D2 neurons in the dorsolateral striatum of mice (known to be increasingly engaged with skill consolidation) show a progressive reduction in their ability to respond to dopaminergic input after a prolonged training in the accelerated rotarod task (Sommer et al., 2014). Another possible explanation may reside in the interaction between D1 and D2 receptors. In fact, it is likely that blockade of D2 receptors influences the activity of D1 receptors via the change in the DA release. Therefore, the effects of D2 receptors on memory consolidation in zebrafish might be mediated through D1 receptors.

Memory retrieval is considered a dynamic readout of established neuronal plasticity associated with the recognition of previously presented items (Buchanan, 2007). There are a few studies that investigated the effects of dopaminergic drugs on recall of previously acquired information. For instance, both facilitatory and inhibitory effects of a selective D1 agonist (SKF-81297) on information retrieval have been previously observed in rats tested in object recognition, object location, and object temporal order tasks (Hotte et al., 2005). Impairment of spatial memory and reward memory retrieval has also been reported in rats after administration of the selective D1 antagonist SCH (Hotte et al., 2006; Ting-A-Kee et al., 2013). The results of the present study indicated, however, that the administration of either D1 agonist or antagonist immediately before probe trial did not affect recall of associative learning in fish. In this regard, we did not find any significant effect of SKF and SCH administration on TTA, TAEs, and/or IAEs (Fig. 3.5). One possible explanation for this inconsistency with previous reports could be the concentration of drugs used in this study, which were not sufficiently high enough to influence the recall of memory. As explained for consolidation, enhancement of memory recall may also require an optimum level of dopaminergic activity. This assumption can be supported by previous studies indicating that the effects of dopaminergic drugs on the recall process are highly dose-dependent (de Lima et al., 2011; Hotte et al., 2005). Alternatively, the absence of any tangible effect of D1 receptor agonist and antagonist on recall of memory might be due to the lack of D1 receptor effect on this cognitive ability in fish.

Although relatively little strides have been made toward elucidating the role of D2 receptors in the process of memory recall, a few studies have demonstrated the importance of these receptors in the retrieval of stored information. Administration of low and high doses of a D2 receptor agonist

bromocriptine in mice has been shown to enhance (Kebabian and Calne, 1979) or impair (Jackson et al., 1988) memory retrieval. Ichihara et al. (1988b) have reported that D2 receptor antagonist sulpiride disrupted memory retrieval in mice tested in the passive avoidance learning (Ichihara et al., 1988b). In a fear conditioning paradigm, administration of QUIN impaired the retrieval of a learned association between CS and a footshock US in rats (Nader and LeDoux, 1999b). Moreover, it has been shown that the systemic administration of D2 receptor antagonists can attenuate the glucocorticoid-induced impairment of memory retrieval in rats (Pakdel and Rashidy-Pour, 2007). The results of the present study are in line with previous reports indicating the important role of D2 receptors in the recall process. Administration of QUIN 30 min before the probe trial enhanced TTA while strikingly reduced IAEs in experimental fish (Fig. 3.5). In contrast, blockade of D2 receptors by ETIC resulted in a marked decline in TTA. TAEs also showed a considerable decrease in fish treated with ETIC, but it was not statistically distinguishable from the control group. A delicate balance of DA level is crucial for the brain to retrieve cue-triggered patterns learned during associative learning. DA imbalance in either direction (up or down) causes the inability to retrieve memory patterns during associative memory recall (Li et al., 2010). Pre-synaptic activation of D2 autoreceptors at low concentrations and early after the administration of QUIN reduces DA neurotransmission (Benaliouad et al., 2009; Nader and LeDoux, 1999a). However, QUIN at higher doses contributes to post-synaptic receptor activation (Benaliouad et al., 2009; Packard and McGaugh, 1994). Therefore, it is likely that the excitatory effect of QUIN on recall of memory in the present study results from pre-synaptic activation of D2 receptors followed by an optimum level of DA release in the brain. As observed in previous studies (Bracs et al., 1984; Ichihara et al., 1988a), antagonism of D2 receptors by ETIC increases pre-synaptic DA efflux. As a result, an extreme increase in synaptic levels of DA might be responsible for the impaired memory recall in fish treated with ETIC.

The phasic and tonic activities of DA neurons are thought to mediate different aspects of associative learning. While phasic activity facilitates the acquisition and consolidation of cue-reward association, tonic activity is involved in behavioural flexibility and maintaining alertness during learning. Phasic and tonic signals are mediated through D1 and D receptors, respectively (Breitenstein et al., 2006; Clopath, 2012; Floresco et al., 2003). Therefore, it seems that SKF and SCH by activation of D1 receptors potentiate the intensity of post-synaptic responses to phasic DA leading to the improved acquisition and consolidation of associative learning in zebrafish. On the

other hand, D2 receptor agonist via stimulation of D2 autoreceptors can decrease tonic DA activity. Tonically decreased activation (and thus receptor occupation) of post-synaptic receptors can potentiate the subsequent phasic DA release triggered by rewarding stimuli and heighten neural responses in the post-synaptic neuron. In contrast, antagonism of autoreceptors by ETIC may increase DA release and consequently reduce phasic DA signalling. Therefore, it is possible that D2 receptors indirectly and by regulation of the phasic-tonic balance of DA releasing influence associative learning in zebrafish (mainly acquisition and consolidation phases).

The dopaminergic system is also critically involved in motor function (Bowton et al., 2010; Irons et al., 2013). In this study, we quantified total distance traveled by experimental fish which is a precise indicator of locomotion. However, our results showed no significant difference in the exploratory activity of treated fish during the probe trial. This finding appears to be convincing enough to suggest that observed changes in zebrafish performance were most likely due to the effects of dopaminergic drugs on neural substrates involved in cognition.

3.5. Conclusion

To our knowledge, this is the first study showing the modulatory effects of DA receptors on different aspects of learning and memory in fish. Collectively, our results corroborate the involvement of the dopaminergic system in the acquisition, consolidation, and subsequent recall of information comparable to what occurs in mammals. Importantly, we found that although D1 receptors play more prominent roles in the acquisition and consolidation, D2 receptors appear to be more important in control of memory recall. Considering numerous advantages of zebrafish over classical vertebrate models, the results of the present study can be an important step towards better understanding of the role of different neuromodulatory systems in cognitive functions. Finally, this study provides a solid foundation for future research exploring neurobehavioural effects of dopaminergic drugs.

Chapter 4: Chronic dietary selenomethionine exposure induces oxidative stress, dopaminergic dysfunction, and cognitive impairment in adult zebrafish (*Danio rerio*)

Preface

The aim of this chapter is to address the third objective of my doctoral research work, which is to investigate the effects of chronic exposure to dietary selenomethionine (SeMet) on latent learning performance in zebrafish. To this end, fish were exposed to environmentally relevant concentrations of SeMet (0.97, 2.3, 9.7, 32.5, and 57.5 µg/g Se) for 30 days. The results of this study show that dietary exposure to the two highest concentrations of Se impairs latent learning performance in zebrafish. Moreover, impaired learning is associated with the induction of oxidative stress, alterations in dopaminergic neurotransmission, and down-regulation in the expression of immediate early genes in the zebrafish brain. The findings of this chapter complement the findings of Chapters 2 and 6 and provide a mechanistic understanding of how dietary SeMet impairs latent learning in zebrafish.

The content of Chapter 4 was reprinted (adapted) from Environmental Science and Technology (DOI: 10.1021/acs.est.7b03937). Naderi, M., Salahinejad, A., Jamwal, A., Chivers, D.P., Niyogi, S. "Chronic dietary selenomethionine exposure induces oxidative stress, dopaminergic dysfunction, and cognitive impairment in adult zebrafish (*Danio rerio*)". Copyright 2017, with permission from American Chemical Society Publications.

Author contribution

Mohammad Naderi (University of Saskatchewan) designed and conducted the experiment, generated and analyzed the data, prepared all the figures and tables, and drafted and revised the manuscript. Arash Salahinejad and Ankur Jamwal (University of Saskatchewan) provided technical assistance with behavioural tests, biochemical assays, and data analysis. Doug Chivers and Som Niyogi (University of Saskatchewan) provided inspiration, scientific input and guidance, commented on and edited the manuscript, and provided funding for the research.

4.1. Introduction

Learning and memory are fundamental higher brain functions taking place at the synaptic level to encode experiential information, which enables animals to generate adaptive behaviours essential for their survival. A wide range of environmental contaminants has been shown to interfere with different aspects of the central nervous system (CNS), which consequently manifests as learning and memory deficits in animals (Schantz and Widholm, 2001). In recent years there has been an increasing level of concern about essential trace elements since not only their deficiency may cause abnormal neurological functions, but their excessive intake due to environmental contamination may also lead to neurological dysfunctions.

Selenium (Se) is a naturally occurring metalloid element found in trace amounts in soil, water, animals, and plants. The anthropogenic redistribution of Se through agricultural runoff, coal combustion, metal mining, and oil refining activities results in elevated concentrations of Se in surface waters (Janz et al., 2010). Although Se can be extremely toxic to fish, much of its toxicity has been characterized in terms of its reproductive and developmental effects (Janz et al., 2010), and virtually nothing is known about its neurobehavioural effects on fish. The biological functions of Se are primarily implemented through its incorporation into selenoproteins. Mammalian studies have demonstrated an indispensable role of Se in the maintenance of optimal brain functions via redox regulation (Steinbrenner and Sies, 2013), as selenoproteins play a crucial role in neurodevelopment (Chen and Berry, 2003), neuroprotection (Yang et al., 2014), and signal transduction (Raman et al., 2013). Although beneficial at optimum levels, both insufficient and supranutritional levels of Se contribute to adverse health effects (Lee and Jeong, 2012). For example, the imbalance in Se concentrations in the brain has been implicated in the pathophysiology of several neurological diseases (Caito et al., 2011; Wirth et al., 2010) and cognitive decline (Shahar et al., 2010). There is now a general consensus that the primary mechanism underlying Se toxicity is oxidative stress (Jamwal et al., 2016; Misra et al., 2010), which in turn is a major contributor to the neurodegeneration of the brain leading to cognitive decline (Dröge and Schipper, 2007).

Among divergent outcomes of Se neurotoxicity, interaction with and perturbation of several neurotransmitter systems has received increasing attention in recent years (Estevez et al., 2012; Souza et al., 2010). Dopamine (DA) is the predominant catecholamine neurotransmitter in

vertebrates and has been implicated in a plethora of processes such as learning and memory, locomotion, emotion, and neuroendocrine secretion. Degeneration of dopaminergic neurons is believed to mediate neuropathological conditions such as Parkinson's disease and schizophrenia, and oxidative stress has been implicated as the major pathogenic process in all of them (Bretaud et al., 2004; Maas et al., 2017). Indeed, the biosynthesis, transport, and metabolism of DA are all strongly linked to oxidative stress (Meiser et al., 2013). Since dopaminergic neurons intrinsically bear a high oxidative load, even a moderate level of oxidative stress can trigger a cascade of events that may eventually lead to the dysfunction of the dopaminergic system and its related neurological functions. Se has been found to affect the dopaminergic system in mammals (Qureshi et al., 2006; Rasekh et al., 1997; Tsunoda et al., 2000). Although early life-stage exposure to SeMet was found to impair spatial learning in adult zebrafish (Smith et al., 2010), whether this effect is mediated by the disruption of the dopaminergic system has never been investigated.

In the natural environment, Se exposure to fish occurs predominantly via diet and in the form of selenomethionine (SeMet) (Janz et al., 2014). The primary goal of the present study was, therefore, to investigate the effects of chronic dietary Se exposure on learning and memory in adult zebrafish (*Danio rerio*), with a particular focus on the dopaminergic system. Zebrafish possess a conserved dopaminergic projection pathway that is homologous to the mammalian midbrain dopaminergic system (Yamamoto and Vernier, 2011). Moreover, we previously established the role of DA receptors in different forms of learning and memory in this species (Naderi et al., 2016a; Naderi et al., 2016b). Adult zebrafish exhibit robust learning ability in a variety of paradigms, making them a powerful model for neurobehavioural studies (Truong et al., 2014). It has been demonstrated that older adult zebrafish (2 years old) are more prone to neurobehavioural effects of neurotoxicants in comparison to younger adults (Xu et al., 2012). On the other hand, it has been suggested that juvenile zebrafish (younger than 4 weeks of age) do not possess fully developed brain functions necessary for acquisition of different learning tasks (Valente et al., 2012). With that in mind, 6 months old adult zebrafish were used in the present study. Subjects were exposed to different environmentally relevant concentrations of SeMet, and then trained in a latent learning of spatial task. To elucidate possible biochemical and molecular mechanisms underlying Se neurotoxicity, oxidative stress and expressions of different genes associated with DA receptor activity (D1 and D2 receptors), as well as the DA biosynthesis (tyrosine hydroxylase 1 (TH)), re-uptake (dopamine transporter (DAT)), and metabolism (monoamine oxidase (MAO)) were quantified in the zebrafish

telencephalon. This brain region is not only the center of dopaminergic neurons, but is also the main target of DA projections from the posterior tuberculum (homologous to the midbrain dopaminergic system of mammals), and plays a pivotal role in various forms of learning and memory in fish (Hurtado-Parrado, 2010; O'connell et al., 2011; Tay et al., 2011). Expression of two immediate early genes (IEGs; brain-derived neurotrophic factor (BDNF) and early growth response1 (EGR-1)) was also assessed in the telencephalon. These genes are involved in neural plasticity processes related to learning and memory, and their expressions are commonly used as markers of neuronal activity in the brain of vertebrates, including zebrafish (Manuel et al., 2014; Teles et al., 2016).

4.2. Materials and methods

4.2.1. Fish

Zebrafish (wild-type, 6 months old) were procured from the in-house stock of the R.J.F. Smith Center for Aquatic Ecology of the University of Saskatchewan, and maintained in the aquatic facility of the Dept. of Biology. A total number of 280 adult zebrafish (0.54 ± 0.30 g and 3.54 ± 0.07 cm) were used in this study and were randomly distributed into twenty 30-l glass aquaria (14 fish/aquarium) supplied with filtered and de-chlorinated Saskatoon tap water (total hardness 150 mg/l as $CaCO_3$, alkalinity 120 mg/l as $CaCO_3$, pH 8) at 27 ± 1°C under a 12:12 hr light: dark cycle, and fed with fish flake food (Nutrafin Max flakes, Germany).

4.2.2. Diet preparation and experimental design

Fish were fed four times per day (5% body weight/day ration) with either control food or food spiked with different nominal concentrations of Se (3, 10, 30, and 60 µg/g dw; added as Seleno-L-methionine (purity > 98%), Sigma-Aldrich, USA). These Se concentrations are environmentally relevant since a similar range of Se concentrations has been reported in aquatic invertebrates and prey fish species collected from Se-impacted sites (Driedger et al., 2009; May et al., 2008; Muscatello and Janz, 2009; Schuler et al., 1990). Different nominal concentrations of SeMet were dissolved in deionized distilled water, mixed with flake food, and freeze-dried using a freeze dryer (Labconco, USA) for 48 hrs. The control diet was treated the same way without any added SeMet. Triplicate diet samples (500 mg each) were taken from each batch of food for total Se analysis. Fish (56 fish per treatment, equally divided in 4 replicates per treatment) were exposed to either the control diet or SeMet-spiked food for a period of 30 days. This exposure period was selected

on the basis of previous observations demonstrating chronic effects of trace elements in zebrafish brains (Sarkar et al., 2014; Senger et al., 2006). Fish were allowed to feed for 1 hr, after which excess food was siphoned out. A 75% exchange of water was carried out in each tank every day. On day 15, water samples (n = 3 per treatment) were collected 3 hrs post feeding in order to determine dissolved Se concentrations.

4.2.3. Latent learning task

After the first 14 days of experimental exposure, training trials were started in which fish from each treatment were trained in a latent learning task for the subsequent 16 days. Latent learning is a complex cognitive task in which fish incidentally learn about the spatial construction of a complex maze by exploring and wandering, in the absence of a reward (Jensen, 2006). Zebrafish have been shown to perform well in this paradigm (Naderi et al., 2016b). The maze was composed of a start chamber, a reward chamber, and two tunnels which connect these two chambers to each other (see Fig. 2.1; Chapter 2). As described elsewhere (Naderi et al., 2016b), the training sessions were conducted each day by placing groups of 14 fish in the start chamber and releasing them after 30s. Fish that demonstrated normal swimming behaviour (no erratic movement, jumping, and/or freezing) were only used in the training sessions. Fish were then allowed to explore the maze for 30 min in the absence of a reward. During the training trials, only one tunnel was kept open: either the right or the left tunnel. Among the four replicate groups of fish assigned to each treatment, two groups were trained in the maze with the right tunnel open, and the other two groups were trained with the left tunnel open. In order to evaluate learning performance of fish after 16 days of training, probe trials were conducted. Due to differential mortality among treatments, the number of fish tested in the probe trial was not equal for each treatment. The number of fish used in the probe was 53, 56, 52, 44, and 36 in the control, 3, 10, 30 and 60 µg/g dietary Se treatments, respectively. During the probe trial, a single fish was placed in the start chamber while both the right and the left tunnels were open, and the reward chamber contained 6 stimulus fish (fish to which the zebrafish would respond in the test) as a reward. Given the highly social nature of zebrafish, the sight of conspecifics (or shoaling) has potent rewarding properties and thus triggers a response (Al-Imari and Gerlai, 2008). Learning performance of the subjects was monitored for 10 min using an HD webcam (Logitech c310, USA) mounted above the maze. Video footages were analyzed using image processing and vision techniques utilizing MATLAB (Academic version R2015a) and the parameters of interest were extracted. To evaluate latent learning performance in zebrafish, five

behavioural variables were quantified: the latency to leave the start chamber, the time spent in the correct versus incorrect tunnel, the latency to enter the reward chamber, the time spent in the reward chamber, and locomotion (total distance traveled by the fish). After the completion of probe trials, fish were euthanized by 10 mg/l of Aquacalm within 2 min (Syndel Laboratories, Canada). Whole-brains were dissected out from male and female zebrafish and pooled separately. Briefly, the dorsal surface of the brain was exposed. Then, the optic nerves and the medulla at the beginning of the spinal cord were cut and the brain was removed. Subsequently, the telencephalon was dissected out from a subset of brains, using a fine forceps and iridectomy scissors under a dissecting microscope that was equipped with an Axiocam camera (Zeiss, Germany). Brain and whole-body samples were stored at −80 ℃ until further analysis. Oxidative stress responses were assessed in the whole-brain, since it is generally accepted that reactive oxygen species (ROS) generated in different brain regions can affect dopaminergic neurons. More importantly, ROS generated from the auto-oxidation of DA shows widespread toxicity not only in DA neurons but also in other brain regions (Miyazaki and Asanuma, 2008). In contrast, the expression of genes related to dopaminergic neurotransmission and IEGs was evaluated specifically in the zebrafish telencephalon, as these genes can also be expressed in other parts of the brain that have no cognitive functions (Manuel et al., 2014).

4.2.4. Measurement of selenium

Total Se concentrations in water, food, and whole-body of fish were measured using a GF-AAS (PerkinElmer AAnalyst 800, USA) as described previously (Misra et al., 2012b). Briefly, water samples (n = 3) were treated with 0.2% (v/v) concentrated nitric acid and stored in 4 ℃ until Se analysis. Food and tissue samples (n = 3) were weighed and transferred into borosilicate glass vials with polypropylene screw tops (Metal free, EPA certified, VWR, Canada). Then, 1N nitric acid was added to vials (1 to 5 mass (g): volume (ml) ratio) and kept at 60 ℃ for 48 hrs. Digested samples were removed and centrifuged at 15,000 *g* for 4 min. Supernatants were collected and stored at 4℃ until Se analysis. The quality control and assurance of the analysis were maintained using appropriate method blanks and sample duplicates, a certified Se standard, and a reference material (DOLT-4; National Research Council of Canada). The recovery percentage of Se in the reference material was 96%.

4.2.5. Biochemical assays

Glutathione (GSH) is considered as the most abundant antioxidant in aerobic cells, and it plays a critical role in the protection of the brain against oxidative stress. The ratio of reduced GSH to oxidized GSH (GSSG) is employed to gauge the degree of oxidative stress in cells (Owen and Butterfield, 2010). The concentration of GSH and GSSG in zebrafish brain (pools of three, n = 4 per treatment) was measured using a fluorometric method as described previously (Jamwal et al., 2016). GSH content was measured in a final reaction mixture volume of 200 µl, which contained 180 µl of phosphate–EDTA buffer (0.1 M sodium phosphate–0.005 M EDTA, pH 8.0), 10 µl of o-phthalaldehyde (OPT, 100 µg per 100 µl methanol) and 10 µl of sample. The final reaction mixture for GSSG measurement contained 140 µl of 0.1 N NaOH, 20 µl of OPT, and 40 µl of sample. The fluorescence intensity was measured in a multimode microplate reader (Varioskan Flash, Thermo Fisher Scientific, Finland) at excitation and emission wavelengths of 350 nm and 450 nm, respectively. The Bradford method was employed for quantification of the protein content in the samples (n = 4) (Bradford, 1976). The GSH and GSSH content was expressed as µg per mg of protein. Lipid peroxidation (LPO), another index of oxidative stress, was measured by the determination of malondialdehyde (MDA) content, using a commercially available assay kit (Abcam, USA). Each replicate was a pool of 2-3 brain samples and 5 replicates per treatment were used for LPO measurement.

4.2.6. Gene expression measurement

The expression of following genes was assessed, based on their involvement in cognitive functions and their localization in the zebrafish telencephalon as reported previously: D1 receptor (DRD1, also known as DRD1b), D2 receptors (DRD2b, DRD2c, DRD3, DRD4a, DRD4b subtypes), TH, DAT, MAO, BDNF, and EGR-1. (Manuel et al., 2014; Maximino and Herculano, 2010; Panula et al., 2010). Total RNA was isolated from a pool of 4 telencephalons (n = 4 per treatment) using the RNeasy Mini Kit (Qiagen, Germany), which included a DNase treatment according to the manufacturer's protocol. The RNA concentrations and purity were verified on a Nanodrop spectrophotometer (NanoDrop, Thermo Scientific, USA) using the absorbance ratios at wavelengths of 260 and 280 nm, respectively. Subsequently, the cDNA was synthesized from 1µg total RNA using a QuantiTect Reverse Transcription® kit (Qiagen, Germany). Quantitative real-time PCR was performed on an iCycler Thermal Cycler (Bio-Rad, USA). The 20 µl reaction mixture contained 10 µl of SYBR Green PCR Master Mix (SensiFAST, SYBR No-ROX Kit,

Bioline, USA), 0.8 µl of each forward and reverse primers, and 2 µl of the cDNA and nuclease-free water. PCRs were performed in triplicate. The mRNA expression of each target gene was normalized to β-actin as the house-keeping gene. The relative quantification of gene expression among treatment groups was analyzed by the $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen, 2001). The sequences of primers are presented in Table 4.1.

Table 4.1. Primer sequences for quantitative PCR used in this study.

Target Gene	Forward primer (from 5' to 3')	Reverse primer (from 5' to 3')	GenBank accession no.
DRD1b	ACTGCATGGTTCCTTTTTGC	GGATTTGTGCTGTCCGTTTT	NM_001135976
DRD2b	GTCTCCATCTCCGTCCTCTC	TTACCGAACACCACACAGAAG	AY333791
DRD2c	ATGCTCCTGACTCTCCTC	ATCTGCCACCGCCAAG	AY333792
DRD3	TTCAGACCACCACCAACTACC	GCTCCGCCGACCACTTC	NM_183067.1
DRD4a	GGTCCCTCAATATGACCGTATG	GCGATGAACCTGTCTATGCT	AY750152.1
DRD4b	GAGCTCTGGTCGTAACCGCTCACA	ACAGGCAGCACCTTCATTGCCTTG	AY750153.1
TH1	GCCATACCAAGACCAGACTTAC	GCTTGATGCGGGTCACATA	NM_131149.1
DAT	ACTTTCTGCTGTCCGTCATC	GCTCCTCCGCCATTCTTG	NM_131755
MAO	GCAGTCAGAGCCCGAATC	CACACCCATAAACTTGAGGAATC	NM_212827
BDNF	AGAGCGGACGAATATCGCAG	GTTGGAACTTTACTGTCCAGTCG	NM_131595
EGR-1	GTGAGCCCAACCCCATCTAT	CCAGGCTGATCTCACTTTGC	NM_131248
β-Actin	AGGTCATCACCATTGGCAAT	GATGTCCACGTCGCACTTCAT	AF057040

4.2.7. Statistical analysis

Data were analyzed using SPSS software (version 23.0, IBM SPSS Inc., USA) and presented as mean ± the standard error of the mean. The data were checked for normality and homogeneity of variance using the Kolmogorov–Smirnov one-sample test and Levene's test, respectively. Statistical analysis was performed on parametric data using one-way analysis of variance (ANOVA) with Tukey's multiple comparisons. The Welch's test followed by the Games–Howell post hoc test was used if the equality of variance assumption was rejected. When nonparametric tests were required, statistical analysis was performed using the Kruskal–Wallis test with Dunn's post hoc analysis. In addition, a chi-square test (with Bonferroni's correction) was used to determine significant differences in mortality among the treatment groups. The alpha level was set at 0.05 except when a Bonferroni's correction was applied ($\alpha = 0.016$). No sex-specific differences in

learning performance of fish and the expression of dopaminergic genes were found, and therefore the data were pooled for sexes.

4.3. Results

4.3.1. Selenium concentrations

The measured Se concentrations in water, food, and whole-body of fish are outlined in Table 4.2. Concentrations of dissolved Se in water samples differed significantly among fish aquaria (one-way ANOVA: $F_{4,10} = 56.54$, $p < 0.001$). Tukey's post hoc comparison showed higher dissolved Se concentrations in water collected from aquaria in the two highest dietary treatments compared to the control aquaria ($p < 0.001$). The control food (no added SeMet) contained 0.9 ± 0.1 µg/g dw of Se. SeMet-spiked food, prepared by adding 3, 10, 30 and 60 µg/g of Se to the commercial diet, contained 2.3 ± 0.2, 9.7 ± 0.1, 32.5 ± 2.4, and 57.5 ± 3.2 µg Se/g dw, respectively. Whole-body concentrations of Se in adult zebrafish were also 0.9 ± 0.1, 0.98 ± 0.1, 4.2 ± 0.2, 12.9 ± 0.6, and 25.5 ± 0.9 µg/g wet weight in fish treated with control, 2.3, 9.7, 32.5, and 57.5 µg Se/g diets, respectively. Whole-body Se concentrations differed significantly among treatments (one-way ANOVA: $F_{4,10} = 398.26$, $p < 0.001$). Tukey's post hoc comparison confirmed that dietary exposure to 9.7, 32.5, and 57.5 µg Se/g dw resulted in significantly higher Se body burdens in fish compared to that in the control (all $p < 0.01$).

Table 4.2. Total Se concentrations in water (µg/l), food (µg/g dry weight), and fish body (µg/g wet weight), and cumulative mortality of fish in each treatment after 30 days of exposure to SeMet.

Nominal concentrations of Se in diet (µg/g)	Water (µg/l)	Food (µg/g dry weight)	Whole body (µg/g wet weight)	Cumulative % mortality
Control	0.4 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	5.3 ± 3.4
3	0.4 ± 0.1	2.3 ± 0.2	0.9 ± 0.1	0
10	0.8 ± 0.1	9.7 ± 0.1a	4.2 ± 0.2a	7.1 ± 4.1
30	1.2 ± 0.1a	32.5 ± 2.4a	12.9 ± 0.6a	21.4 ± 2.9b
60	2.1 ± 0.1a	57.5 ± 3.2a	25.5 ± 0.9a	35.7 ± 5.8b

[a] Significantly different from control group at $p < 0.05$. n = 3 for total Se concentrations per treatment.

[b] Significantly different from the control group at $p < 0.017$.

4.3.2. Mortality

Mortalities were observed in all treatment groups except the group treated with 2.3 µg Se/g dw during 30 days of exposure. The results of the chi-square test showed that there is a significant difference in fish mortality among different treatment groups ($\chi^2_4 = 39.44$, $p < 0.001$; Table 4.2). Mortalities were significantly greater in the groups treated with 32.5 (chi-square test: $\chi^2_1 = 6.23$, $p = 0.012$) and 57.5 µg Se/g diets (chi-square test: $\chi^2_1 = 15.81$, $p < 0.001$) compared to the control group.

4.3.3. Latent learning performance

The Welch test showed a significant difference in the latency to leave the start chamber among different treatments ($F_{4, 101.61} = 7.62$, $p < 0.001$). Post hoc test (Games-Howell) showed that fish fed with 9.7, 32.5, and 57.5 µg Se/g diets exhibited longer latencies to leave the start chamber (all $p < 0.02$, Fig. 4.1A). As illustrated in Fig. 4.1B and determined by one-way ANOVA ($F_{4, 236} = 9.64$, $p < 0.001$), the dietary exposure to SeMet significantly affected the length of time that fish spent in the correct versus the incorrect tunnel. Subsequently, Tukey's post hoc test showed that fish treated with 32.5 and 57.5 µg Se/g diets spent significantly less time in the correct tunnel compared to the control group ($p < 0.001$ and $p < 0.003$, respectively). Familiarity with the spatial layout of the maze was also assessed by measuring the latency to enter the reward chamber by experimental fish. A Kruskal-Wallis test indicated that there was significant variation in the latency to enter the reward chamber among treatments ($X^2_4 = 58.85$, $p < 0.001$). As depicted in Fig. 4.1C, Dunn's post hoc test showed that fish fed with 32.5 and 57.5 µg Se/g diets exhibited a prolonged latency to reach the reward chamber relative to the control group (both $p < 0.001$). The length of time that fish spent in the reward chamber is depicted in Fig. 4.1D. One-way ANOVA detected a significant alteration in this parameter ($F_{4, 236} = 9.13$, $p < 0.001$). Subsequently, Tukey's post hoc test attributed this difference to the groups that were exposed to the two highest dietary concentrations of SeMet (32.5 and 57.5 µg Se/g) compared to the control group ($p = 0.015$ and $p = 0.004$, respectively). Fig. 4.1E represents the locomotor activity of fish in different groups. One-way ANOVA found a significant difference in locomotion among different treatment groups ($F_{4, 236} = 2.98$, $p = 0.020$). Tukey's post hoc comparisons indicated that fish fed with 32.5 µg Se/g diet had lower locomotion than fish treated with 9.7 µg Se/g diet ($p = 0.012$). However, there were no significant differences in locomotor activity among other groups (all $p > 0.05$).

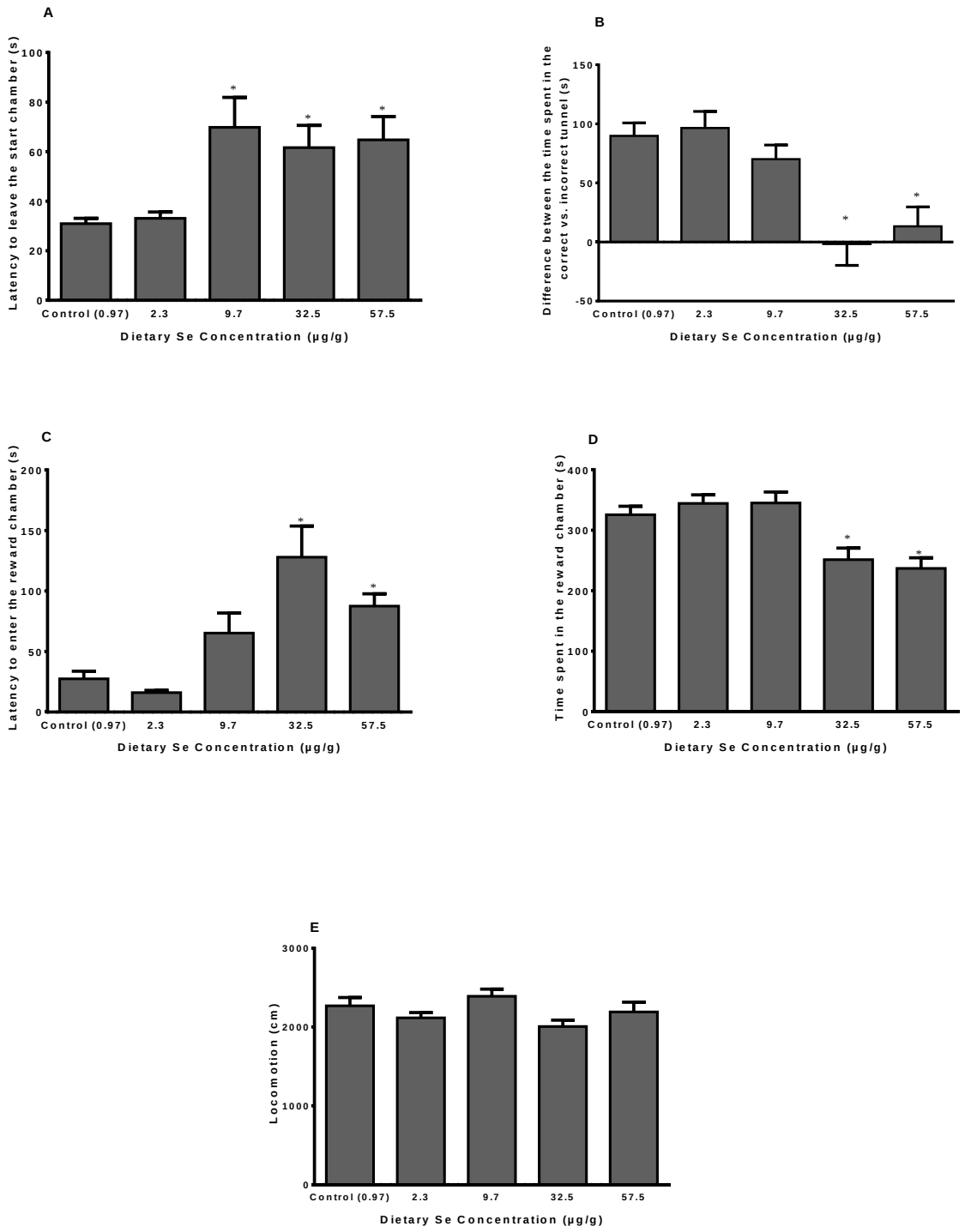

Figure 4.1. The effects of dietary Se exposure on latent learning performance of adult zebrafish measured by: the latency to leave the start chamber (A), difference between the time spent in the correct vs. incorrect tunnel, according to the training condition (B), the latency to enter the reward chamber (C), the time the fish spent in the reward chamber (D), and locomotion (E). Asterisks above error bars denote a significant difference at $p < 0.05$. The number of fish tested in the control, 2.3, 9.7, 32.5, and 57.5 µg Se/g dw was 53, 56, 52, 44, and 36, respectively.

4.3.4. Oxidative stress responses

The GSH and GSSG ratio, and lipid peroxidation (MDA content) were quantified to evaluate the degree of Se-induced oxidative stress in the zebrafish brain. As depicted in Fig. 4.2A, the GSH:GSSG ratio followed a biphasic pattern of response. One-way ANOVA detected a significant treatment effect ($F_{4, 15} = 45.72$, $p < 0.001$) and Tukey's post hoc test showed a significant increase in GSH:GSSG ratio in fish treated with 2.3 µg Se/g diet ($p < 0.001$), while a significant reduction was observed in the brain of fish treated with 32.5 and 57.5 µg Se/g diets relative to the control fish ($p = 0.012$ and $p = 0.004$, respectively). As shown in Fig. 4.2B and confirmed by ANOVA, the MDA content in the whole-brain was also altered significantly ($F_{4, 20} = 9.74$, $p < 0.001$). Tukey's post hoc test showed a significant increase in the brain MDA level in fish fed with 32.5 and 57.5 µg Se/g diets compared to the control group ($p = 0.003$ and $p = 0.001$, respectively).

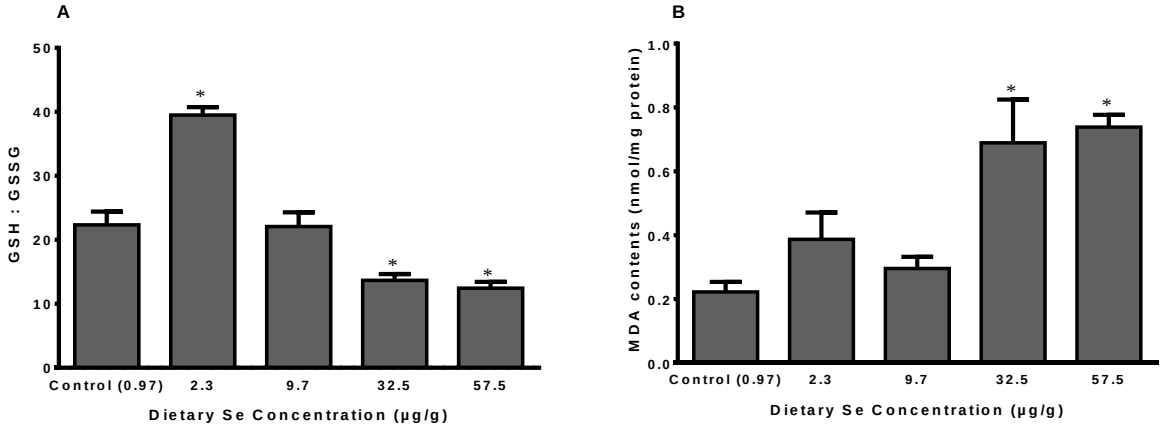

Figure 4.2. The GSH:GSSG ratio (A) and lipid peroxidation level (measured as MDA content) of the zebrafish brain (B) exposed to different concentrations of dietary Se. Asterisks above error bars denote a significant difference at $p < 0.05$. n = 4 and 5 for GSH:GSSG and LPO, respectively.

4.3.5. Responses of dopaminergic genes

In the present study, transcription status of DA receptors, as well as important genes involved in the DA biosynthesis, re-uptake, and metabolism were assessed in the zebrafish telencephalon. As shown in Fig 4.3A, the mRNA expression of DRD1 differed significantly in the telencephalon among different treatments (one-way ANOVA; $F_{4, 15} = 11.76$, $p < 0.001$). Subsequently, Tukey's post hoc test showed a significant decrease in fish fed with 32.5 and 57.7 µg Se/g diets compared to the control fish ($p = 0.019$ and $p = 0.014$). One-way ANOVA also noted a significant change in

the relative expression of the DRD2b gene among different treatments ($F_{4, 15} = 6.18$, $p = 0.004$, Fig. 4.3B). Tukey's post hoc test showed a significant up-regulation of DRD2b expression in fish treated with 32.5 µg Se/g diet ($p = 0.014$). However, dietary exposure to Se did not change the mRNA abundance of DRD2c (one-way ANOVA; $F_{4, 15} = 2.27$, $p = 0.10$; Fig. 4.3C) and DRD3 (one-way ANOVA; $F_{4, 15} = 1.67$, $p = 0.20$; Fig. 4.3D) in the zebrafish telencephalon. Fig. 4.3E shows a marked up-regulation in the mRNA expression of another D2 receptor subtype DRD4a (one-way ANOVA; $F_{4, 15} = 5.46$, $p = 0.006$) following treatment with the highest concentration of dietary Se ($p = 0.008$). However, the mRNA expression of DRD4b in different treatment groups remained unchanged compared with the control group (one-way ANOVA; $F_{4, 15} = 1.52$, $p = 0.24$; Fig. 4.3F).

In addition to the alteration in mRNA expressions of DA receptors, an apparent change in the expression of TH and DAT was also noteworthy. For the mRNA expression of TH (Fig. 4.4A), a one-way ANOVA ($F_{4, 15} = 6.88$, $p = 0.002$) followed by Tukey's post hoc test showed a significant increase in fish treated with 32.5 and 57.7 µg Se/g diets compared to the control fish ($p = 0.038$ and $p = 0.002$, respectively). A similar result was found for the DAT gene (one-way ANOVA; $F_{4, 15} = 20.51$, $p < 0.001$, Fig. 4.4B), where a higher expression was observed in the telencephalon of fish treated with 32.5 and 57.7 µg Se/g diets relative to the control fish ($p < 0.001$ and $p = 0.004$, respectively). The expression of the MAO gene did not change in fish fed with SeMet-spiked food when compared to the control group (one-way ANOVA; $F_{4, 15} = 0.62$, $p = 0.64$, Fig. 4.4C).

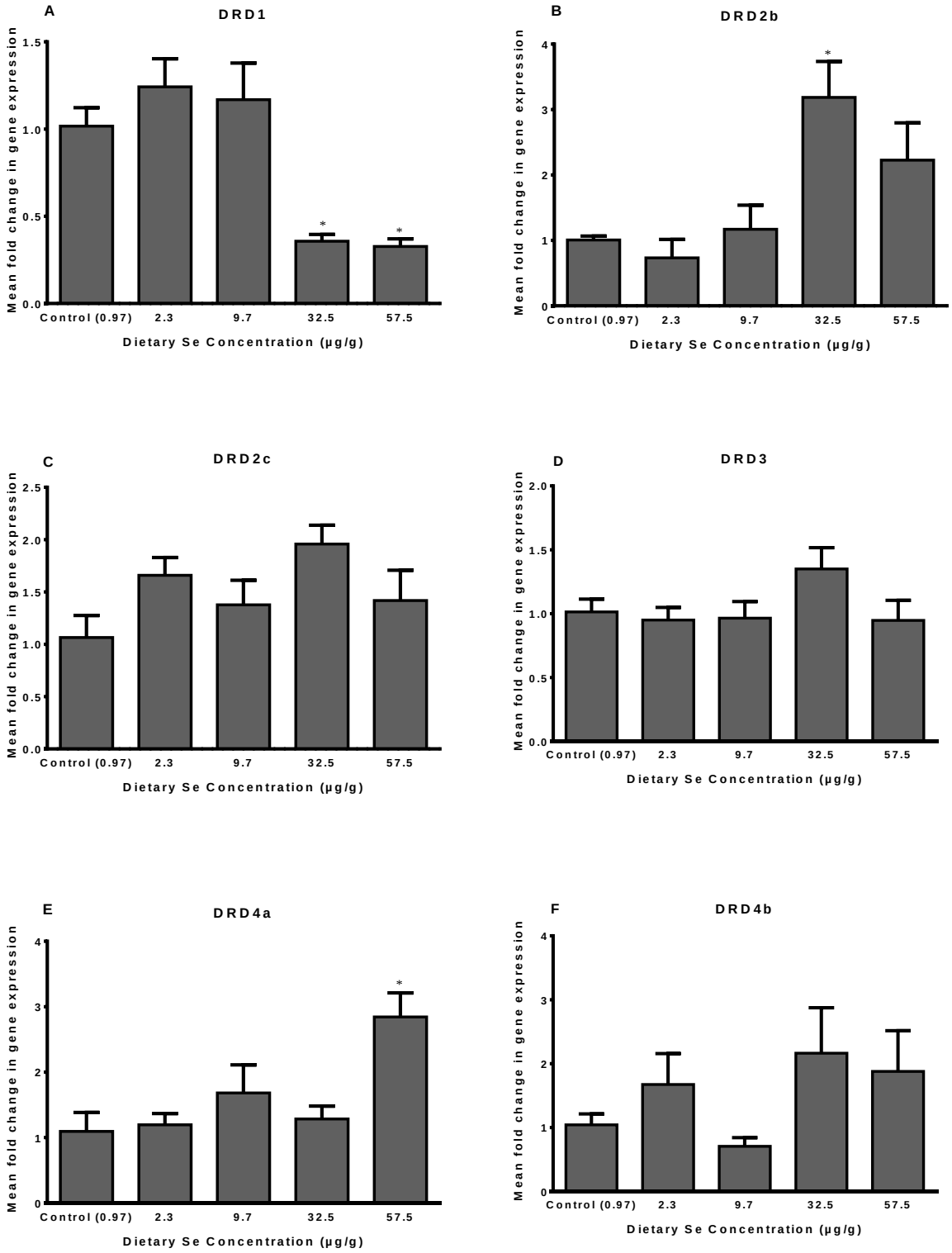

Figure 4.3. Mean fold change in expression of DRD1 (A), DRD2b (B), DRD2c (C), DRD3 (D), DRD4a (E), and DRD4b (F) in the zebrafish telencephalon. Asterisks above error bars denote a significant difference at $p < 0.05$. $n = 4$ per treatment.

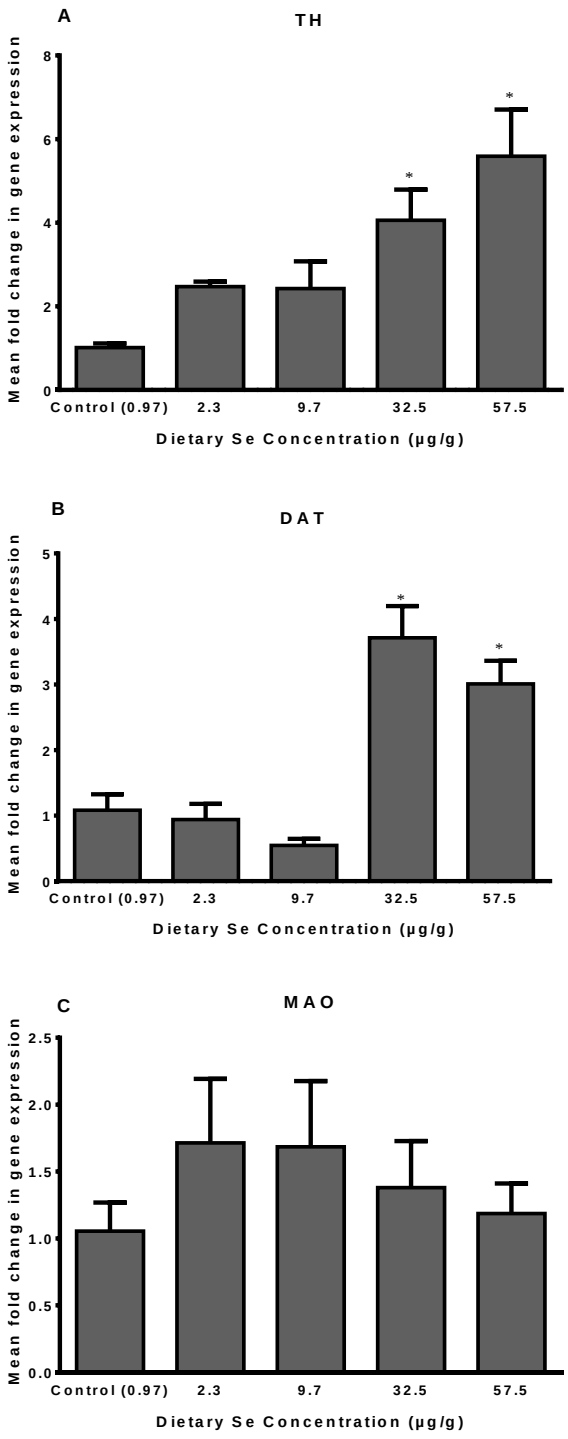

Figure 4.4. Mean fold change in expression of TH (A), DAT (B), and MAO (C) in the zebrafish telencephalon. Asterisks above error bars denote a significant difference at $p < 0.05$. $n = 4$ per treatment.

A dose-dependent change in the expression of immediate early genes (BDNF and EGR-1− markers of neuronal activity) was also observed in this study. A significant alteration was detected in the expression of the BDNF gene (the Welch test; $F_{4, 6.85} = 19.95$, $p = 0.001$), and post hoc analysis (Games-Howell post hoc test) showed a significant up-regulation in fish exposed to 2.3 µg Se/g diet ($p = 0.026$), and a significant down-regulation in fish fed with 32.5 µg Se/g diet, compared to the control group ($p = 0.011$, Fig. 4.5A). Similarly, the expression of the EGR-1 gene also exhibited a significant treatment effect (one way ANOVA; $F_{4, 15} = 21.11$, $p < 0.001$); a marked transcriptional up-regulation of the EGR-1 gene was recorded in fish fed with 2.3 µg Se/g diet relative to the control (Tukey's post hoc test; $p<0.001$, Fig. 4.5B).

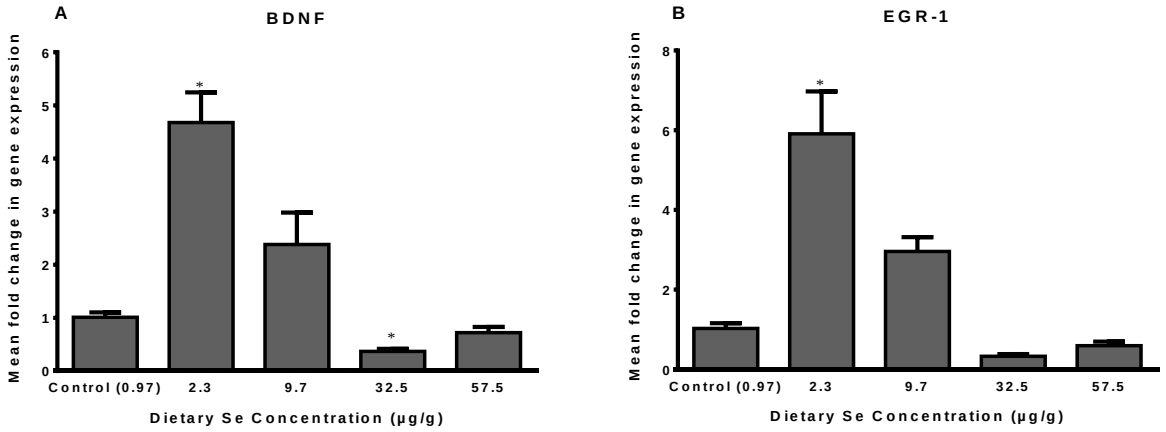

Figure 4.5. Mean fold change in expression of BDNF (A) and EGR-1 (B) in the zebrafish telencephalon. n = 4 per treatment.

4.4. Discussion

The present study is the first to investigate the effects of Se on learning and memory in adult zebrafish. Our results indicated that chronic dietary exposure to Se (as SeMet) leads to impaired latent learning performance, and causes oxidative stress and disruption of the dopaminergic transmission in the zebrafish brain. We examined Se neurotoxicity over a fairly broad range of Se exposure concentrations (2.3-57.5 µg/g), and the adverse effects on zebrafish learning and memory were only observed at the two highest exposure concentrations used (32.5 and 57.5 µg/g). These exposure concentrations, although high, are still environmentally relevant (Driedger et al., 2009; May et al., 2008; Muscatello and Janz, 2009; Schuler et al., 1990). Moreover, these exposure concentrations resulted in elevated whole-body Se concentrations in zebrafish (4.2-25.5 µg/g),

which were comparable to that reported in different wild fish species collected from the Se-impacted aquatic systems (fathead minnow (*Pimephales promelas*; 3-29 µg Se/g), white sucker (*Catostomus commersoni*; 3-42 µg Se/g), and burbot (*Lota lota*; 11 µg Se/g) (Driedger et al., 2009; Muscatello and Janz, 2009). The increased accumulation of Se at two highest exposure concentrations used in our study was also associated with significantly higher fish mortality, further indicating the toxicity of Se.

In a previous study, SeMet co-exposure was employed to ameliorate the deleterious effects of developmental exposure to methylmercury (MeHg) on spatial learning performance in zebrafish. Interestingly, SeMet co-exposure not only failed to prevent this deficit, but exposure to SeMet alone (in the absence of MeHg) was also associated with decreased learning success (Smith et al., 2010). In line with this study, the results of the present study clearly demonstrated the detrimental effects of Se on learning and memory in zebrafish. The prolonged latency to leave the start chamber, increased dwelling time in the incorrect tunnel, and increased latency to reach the reward chamber implicated latent learning impairment in fish exposed to dietary SeMet (32.5 and 57.5 µg Se/g). A marked decline in the shoaling tendency of fish treated with the two highest dietary Se concentrations was also observed in the current study, suggesting negative effects of this metalloid on social behaviour and/or reward processing in zebrafish. Since we observed significantly higher mortality in fish treated with the two highest Se-enriched diets, one may assume that the learning and memory functions in fish in these treatments were compromised by their apparent poor health. To exclude that possibility, we tested the learning performance using fish that demonstrated no apparent symptoms of poor health (e.g., erratic swimming, freezing or jumping) and actively explored the maze during the training sessions. It should also be noted here that although exposure to high dietary Se concentrations has been suggested to cause locomotor deficits and impaired swimming performance in fish (Tashjian et al., 2006; Thomas and Janz, 2011), we did not record any significant change in locomotion (total distance covered) of fish among different treatments compared with the control group. This suggests that the impaired learning and memory functions recorded in our study were not likely induced by any deficits in the locomotory activity of fish.

The collective evidence suggests that exposure to elevated SeMet induces oxidative stress in animals, particularly in fish. An increased lipid peroxidation and a decreased GSH:GSSG ratio has been reported in rainbow trout acutely exposed to SeMet (Schlenk et al., 2003). More recently, a

reduced concentration of GSH, and a reduced GSH:GSSG ratio have been found in zebrafish embryos following aqueous exposure to SeMet (Arnold et al., 2016). Consistent with these findings, results of the present study also demonstrated a marked decrease in GSH:GSSG ratio and a marked increase in LPO concentration following chronic dietary exposure to 32.5 and 57.5 µg Se/g, indicating the oxidative damage in the zebrafish brain. Interestingly, we also recorded a significant increase in GSH:GSSG ratio in the brain of fish fed with 2.3 µg Se/g diet. Mammalian studies indicate that an increase in the GSH:GSSG ratio can occur via a combination of two different mechanisms. Exposure to low Se concentrations has been reported to increase synthesis of GSH by up-regulating the rate-limiting biosynthetic enzyme, γ-glutamyl-cysteine ligase (Richie Jr et al., 2011). On the other hand, it has been suggested that Se can increase the rate of conversion of GSSG to GSH by up-regulating glutathione reductase activity (Chung and Maines, 1981). Collectively, our results indicate that the anti-oxidative capacity in zebrafish brain might have been stimulated by the low Se exposure concentration, while higher Se exposure concentrations overwhelmed the cellular antioxidant defense mechanisms, as suggested in previous studies with fish (Elia et al., 2014; Zee et al., 2016).

A consistent body of evidence indicates that elevated oxidative stress in the brain is one of the main contributors to the cognitive decline in animals (Dröge and Schipper, 2007; Huang et al., 2015). Enhanced oxidative stress in the zebrafish brain has been shown to impede cognitive functions in associative and spatial learning tasks (Ruhl et al., 2016). Thus, the increase in Se-induced oxidative stress might be attributed to the diminished latent learning performance in zebrafish observed in the present study. Oxidative stress may alter neurotransmission, cellular signalling, dendritic network, neuronal function, and overall brain activity, which could be possible mechanisms underlying the memory impairment (Bouayed et al., 2009).

It has been suggested that exposure to Se may alter the integrity of dopaminergic neurons in several brain regions and DA neurochemistry, including both increase and decrease in the DA levels of the brain (Khan, 2010; Qureshi et al., 2006; Romero-Ramos et al., 2000). In a study with male Sprague-Dawley rats, Se exposure (as sodium selenite) was found to increase synaptic DA in the striatum and nucleus accumbens via modulation of D2 receptors (Rasekh et al., 1997). A similar increase in DA level following exposure to Se-containing molecules has been reported in mice, where activation of both D1 and D2 receptors were involved (Oliveira et al., 2012). Our data showed that

dietary exposure to Se alters the mRNA expression of the D1 receptor and specific subtypes of D2 receptors. The change in the transcriptional activity of DA receptors was evident by the down-regulation of the DRD1 gene, which occurred in parallel with the up-regulation of DRD2b and DRD4a genes. We previously demonstrated that the activity of DA receptors (D2 receptors in particular) is crucially important for the regulation of latent learning of spatial information in zebrafish (Naderi et al., 2016b). Hence, it is plausible that latent learning impairment observed in zebrafish could be attributed to increased DRD2 and decreased DRD1 levels and/or any coordinated actions of these two DA receptors. DA receptors regulate different aspects of reward processing and socially motivated behaviours in vertebrates, including fish (Krach et al., 2010; Naderi et al., 2016b). Therefore, disruption of the dopaminergic system may underlie the reduced shoaling tendency in fish fed with the two highest concentrations of Se.

Besides the activity of DA receptors, we also found a change in the activity of both TH and DA transporter, as reflected by the up-regulation of TH and DAT genes, suggesting that the DA biosynthesis and re-uptake are other targets of Se neurotoxicity. The increase in DA synthesis and turn-over may alter the expression of DA receptors as indicated by our results. Monoamine oxidase is the enzyme principally responsible for the metabolism of monoamines, which regulates their intracellular concentrations in the brain. It has been claimed that both inorganic and organic Se supplements can reduce MAO activity in the rat brain (Tang et al., 2008). However, no significant change in MAO activity following treatment with organo-selenium compounds has also been reported in rodents by others (Da Rocha et al., 2012). In agreement with the latter report, our results showed that dietary exposure to SeMet did not alter the activity of MAO, thereby indicating that this enzyme does not directly contribute to the neurotoxic effects of Se in zebrafish.

The increase in intracellular levels of DA via autoxidation process may also induce oxidative stress in dopaminergic neurons and subsequently changes the expression of DA receptors (Martin and Teismann, 2009). It has frequently been reported that down-regulation of D1 receptors provides neuroprotection against psychostimulants via the reduction in DA content and turn-over, and regulate the redistribution of this neurotransmitter inside the neurons where sequestration into synaptic vesicles occur (Ares-Santos et al., 2013). Conversely, the stimulation of D2 receptors could also exert neuroprotection via a decrease in extracellular levels of DA (Bozzi and Borrelli, 2006). Previous research (Bozzi and Borrelli, 2006; Ishige et al., 2001) has shown that D2 and D4

subtypes play a more important role in neuroprotection, while the D3 subtype is not involved in this process, which is in agreement with our observations. Therefore, alterations in the mRNA expression of D1 and D2 receptors in this study might be linked to their neuroprotective roles to counteract DA-induced oxidative stress.

Immediate-early genes are a class of genes that are rapidly up-regulated following neural stimulation in the brain. These genes play fundamental roles in neural activation, neurogenesis, neuron survival, neuronal maturation, and/or synaptic plasticity (Pérez-Cadahía et al., 2011). In the present study, fish fed with 2.3 µg Se/g diet exhibited a higher mRNA expression of BDNF and EGR-1 compared to the control group. Contrastingly, the reduction in the mRNA expression of IEGs was apparent in fish treated with 32.5 and 57.5 µg Se/g diets, although this reduction was only statistically distinguishable for the BDNF gene in fish fed with 32.5 µg Se/g diets. Expression of IEGs is stimulated during the initial stages of oxidative stress, contributing to neural cell survival (Farooqui, 2014). However, elevated ROS generation may reduce and/or inhibit this neuroprotective response (Liu and Arora, 2002), which might have occurred at two highest Se exposure concentrations in our study. The suppressed expression of IEGs may consequently lead to an inability to form or maintain neural pathways related to learning the task, as reflected by the marked decline in latent learning performance in groups fed with the two highest concentrations of Se.

4.5. Conclusion

Overall, our results suggest that chronic exposure to dietary Se may lead to adverse neurobehavioural effects in zebrafish, mediated by Se-induced oxidative stress and disruption of dopaminergic neurotransmission in the brain. Moreover, alterations in DA homeostasis exacerbate the Se-induced oxidative stress in the brain, which appears to be the underlying mechanism of impaired latent learning in adult zebrafish.

Chapter 5: Dopaminergic dysregulation and impaired associative learning behaviour in zebrafish during chronic dietary exposure to selenium

Preface

The aim of this chapter is to address the fourth objective of my doctoral research work, which is to determine the effects of chronic exposure to dietary selenomethionine (SeMet) on associative learning in adult zebrafish. To this end, fish were exposed to environmentally relevant concentrations of dietary SeMet (1.2, 3.5, 27.4, 63.4 µg/g Se) for 60 days. The results show that chronic exposure to dietary SeMet (mainly the two highest concentrations of Se) impairs associative learning in adult zebrafish. Selenium exposure is also found to induce oxidative stress, alters dopaminergic signalling, and suppresses the expression of immediate early genes in the zebrafish brain. The findings of this chapter complement the findings of Chapter 3 and provide a mechanistic understanding of how SeMet impairs associative learning in adult zebrafish. Moreover, zebrafish embryos obtained from this study were used in experiment 5 (Chapter 6).

The content of Chapter 5 was reprinted (adapted) from Environmental Pollution (DOI: 10.1016/j.envpol.2018.02.033). Naderi, M., Salahinejad, A., Ferrari, M.C.O., Niyogi, S., Chivers, D.P. "Dopaminergic dysregulation and impaired associative learning behavior in zebrafish during chronic dietary exposure to selenium". Copyright 2018, with permission from Elsevier Inc.

Author contribution

Mohammad Naderi (University of Saskatchewan) designed and conducted the experiment, generated and analyzed the data, prepared all the figures and tables, and drafted and revised the manuscript. Arash Salahinejad (University of Saskatchewan) provided technical assistance with behavioural tests. Maud Ferrari (University of Saskatchewan) assisted in data analysis and also edited the manuscript.

Som Niyogi and Doug Chivers (University of Saskatchewan) provided inspiration, scientific input and guidance, commented on and edited the manuscript, and provided funding for the research.

5.1. Introduction

The rising incidence and prevalence of neurological and neurodegenerative diseases have become a significant public health concern worldwide. New insights into the etiology of neurological disorders suggest that less than 10% of them have a genetic origin, while a gene-environment interaction is a potential candidate for deciphering the other 90% of cases (Johnson and Atchison, 2009). Trace elements have emerged as one the major concerns of environmental neurotoxicity due to their broad range of applications in daily life (e.g., food, dietary supplements, cosmetics, glass, plastics, and paints) and their adverse health effect on animals.

In recent years, selenium (Se) has become a contaminant of considerable environmental importance due to its paradoxical role in nature. As an essential trace element, Se is an integral component of many selenoproteins, which have crucial roles in many biological functions, such as antioxidant defense, neurodevelopment, neuroprotection, synthesis of thyroid hormones, and reproduction (Chen and Berry, 2003; Mehdi et al., 2013). However, Se becomes extremely toxic at concentrations slightly above its bio-essential level (Janz et al., 2010). Although Se is naturally present in the environment at trace amounts (e.g., in rocks, shales, coal deposits, surface water, and vegetation), anthropogenic activities such as agriculture, coal combustion, and metal mining and refining contribute to Se contamination particularly in the aquatic ecosystems (Janz et al., 2010; Wang and Becker, 2013). Se contamination of the aquatic ecosystems has frequently been reported in many parts of the world, especially in North America. Se bioaccumulation occurs in different components of the food chain such as aquatic plants and aquatic invertebrates in Se-impacted ecosystems and subsequently affects predatory animals such as fish (Hamilton, 2004; Muscatello and Janz, 2009). Indeed, this metalloid is among the most toxic elements to fish (Janz et al., 2010). There is now a general consensus that Se toxicity stems from its ability to promote the generation of reactive oxygen species (ROS) and thereby inducing oxidative stress, which can bring about developmental abnormalities, reproductive failure, and also death (Janz et al., 2010; Letavayová et al., 2006). Oxidative stress has long been considered to be a key driving factor of neurological diseases and cognitive decline in animals (Dias et al., 2013; Praticò et al., 2002). Se by virtue of its pro-oxidant properties can also lead to the development of several neurological disorders in both humans and animals (Ellwanger et al., 2016; Estevez et al., 2014; Vinceti et al., 2010; Vinceti et al., 2013). Since Se can be a double-edged sword with both toxic and beneficial properties, there is a growing interest in the outcome of exposure to various chemical forms of Se on the central

nervous system (CNS) functions. Although low Se concentrations may have neuroprotective effects (mainly as inorganic Se), there is a growing body of evidence indicating its neurotoxic effects at a higher dose range (Vinceti et al., 2014). Disruption of several different neurotransmitter systems, such as cholinergic system, glutamatergic system, and the dopaminergic system has been suggested to be the main consequence of Se neurotoxicity (Ardais et al., 2009; Estevez et al., 2012; Naderi et al., 2017; Rasekh et al., 1997).

Dopamine (DA) is the principal monoamine neuromodulator in the CNS of all vertebrates and is implicated in the regulation of motor function, emotion, motivation, learning and memory. This neurotransmitter has also long been strongly linked to oxidative stress, neurodegenerative diseases, and cognitive disorders (Juárez Olguín et al., 2015; Meiser et al., 2013). The dopaminergic system is not only a favorable target for a variety environmental neurotoxicants, but is also a fertile source of free radicals and oxidative stress (Jones and Miller, 2008; Meiser et al., 2013). Both enzymatic and non-enzymatic oxidation of DA itself can generate ROS, and thereby inducing oxidative damage in DA neurons (Dias et al., 2013; Meiser et al., 2013). Therefore, a delicate equilibrium of antioxidant defense and oxidative stress is crucial for dopaminergic cell viability and function.

Zebrafish (*Danio rerio*) are a valuable model in neurobehavioural research, and are increasingly used in studies investigating different aspects of learning and memory (Bailey et al., 2015). The major dopaminergic pathways and homologous receptors of mammals have been identified in the zebrafish brain (Ek et al., 2016). We have recently demonstrated that DA receptors are differentially involved in two different forms of learning and memory in zebrafish. While D1 receptor plays an important role in the associative learning task, D2 receptors mediate the effects of DA on different aspects of latent learning in zebrafish (Naderi et al., 2016a; Naderi et al., 2016b). Thus, it is reasonable to assume that the dysfunction of the dopaminergic system may lead to different forms of cognitive deficit in zebrafish. In our recent study, we have reported that chronic exposure to Se can adversely affect the dopaminergic system and latent learning behaviour in zebrafish (Naderi et al., 2017). The present study was designed to further explore the neurobehavioural effects of dietary Se and investigated how chronic environmentally relevant exposure to this element affects associative learning performance in zebrafish. To this end, we used a plus-maze associative learning paradigm following chronic exposure of zebrafish to different concentrations of organic Se in the form of selenomethionine (SeMet) via diet. It is important to

note here that dietary SeMet is the predominant bioavailable form of Se for fish in the natural environment (Janz et al., 2014). Redox homeostasis, DA levels, and the mRNA expression of dopaminergic cell markers in the zebrafish brain were evaluated. In addition, transcript levels of neuronal activity-dependent immediate early genes (IEGs) and late expression genes involved in neural plasticity, neurogenesis, and memory formation were also assessed.

5.2. Materials and methods

5.2.1. Animals

Adult wild-type (AB) zebrafish (n = 400, 6 months old) were obtained from a colony present at the R.J.F. Smith Center for Aquatic Ecology of the University of Saskatchewan (see section 6.2.1, Chapter 6 for more detail). For SeMet exposure and behavioural assessment, a total of 320 fish (3.67 ± 0.06 cm and 0.75 ± 0.11 g) were selected and randomly distributed into twenty 30-l experimental tanks (16 fish/tank). Then, treatments were randomly allocated to tanks, with four replicate tanks per treatment. All tanks were filled with de-chlorinated tap water and maintained with continuous aeration at a target temperature of 28 ± 1°C. The illumination of experimental tanks was provided by overhead fluorescent light tubes (23 W) mounted above the tanks, on a 14:10 hr light: dark photoperiod. Fish were fed twice daily with flake food (Nutrafin Max flakes, Germany). Fish were allowed to acclimate to these conditions for at least 3 weeks before the start of the experiment.

5.2.2. Diet preparation and exposure

Diet preparation and exposure duration were based on previous research (Chernick et al., 2016; Thomas and Janz, 2011). Diets containing varying concentrations of Se (i.e. 3, 10, 30, and 60 µg/g dry weight (dw)) were prepared by adding appropriate amounts of SeMet (Seleno-L-methionine, purity >98%, Sigma-Aldrich, USA) to the flake food as described previously (Naderi et al., 2017). These concentrations were selected to cover a broad range of environmentally relevant Se concentrations that have previously been reported in aquatic invertebrates and small prey fish species collected from Se contaminated waters (Driedger et al., 2009; May et al., 2008; Muscatello and Janz, 2009). The control diet was made by adding deionized distilled water without SeMet to flake food. Fish were fed with either a control diet or SeMet-spiked diet at 5% body weight/day for 30 days. After 30 days, fish were fed equal portions (2.5%) of control or SeMet spiked foods and frozen brine shrimp (Sally's, San Francisco Bay Brand Inc., USA) for a further 30 days. Fish were

fed with frozen brine shrimp to improve egg production because in a companion study we also investigated the effects of the maternal SeMet transfer on the learning ability of F1-generation (see Chapter 6). Triplicate samples from each SeMet-spiked diet and three replicates from brine shrimp were collected for determination of total concentrations of Se. During the exposure, the excess food at the bottom of tanks was siphoned out, 1 hr after the last feeding time. A 75% water change was performed in each day daily. On 30^{th} and 60^{th} day of the exposure period, water samples (n = 3 per treatment) were collected, filtered (using 0.45 μm disposable filters), and stored at 4 ℃ for quantification of dissolved Se.

5.2.3. Associative learning

In order to evaluate learning performance of zebrafish after 60 days of exposure to dietary Se, a plus-maze associative learning paradigm was used: fish were required to develop a relationship between a conditioned visual stimulus (CS) and the presence of conspecifics as the reward or unconditioned stimulus (UC). The apparatus used in this study was a plus-shaped maze we employed previously (see Fig. 3.1; Chapter 3). The testing paradigm included 3 phases: (1) a 4-day habituation phase, to acclimate the fish to their novel environment. Fish were placed for 2 h in the maze. All fish in each exposure tank were introduced to the maze, but the number was gradually reduced each day (16, 8, 4, and 1), until a single fish was placed in the maze and allowed to freely swim. (2) A 4-day training trial, to allow the fish to make the correct association between a visual cue (a red card) and a social reward. In the plus maze, one arm was randomly selected as the target arm and contained a red-cue card at the end of it, while the other arms (the incorrect arms) had white cue cards. Four identical glass aquaria were placed at the end and adjacent to the left side of each arm of the maze. The aquarium adjacent to the target arm contained six fish originating from the same source as the focal fish (hereafter referred as stimulus group), while the other tanks were empty. Therefore, the red and white cure cards served as indicators of the presence or absence of the stimulus group, respectively. Zebrafish are highly social animals. Accordingly, the sight of stimulus group has been established as a potential reward in reward-related associative learning task (Al-Imari and Gerlai, 2008). During training trials, a single fish was placed in the center of the maze and released after 30 s. The fish was allowed to explore the maze for 5 min. Each focal fish experienced four daily training sessions for 4 days (16 training sessions in total). All fish received associative learning training. (3) The probe trial which was conducted the following day of the last training trial to evaluate the learning performance of the focal fish. During this phase, all stimulus

tanks were empty and only the red and white cue cards were presented at the end of the arms. Twenty fish from each treatment group were randomly selected and tested in a probe trial. Zebrafish behaviour during the probe trial was recorded and analyzed by MATLAB software (Academic version R2016). To determine the location of the fish in each frame, the motion detection technique was used. Each fish was detected as a group of black pixels in a white background. The mathematical average of these pixels was calculated, which resulted in a single pixel in the image that was interpreted as the fish central coordinate. We extracted the following data for each trial: percentage of time that the focal fish spent in the target arm, the number of times that focal fish visit the target arm, the number of incorrect arm entries, and locomotion (total distance moved). The average body length of fish was scored as an arm entry. Immediately after the probe trial, the focal fish was euthanized with an overdose of Aquacalm solution (Syndel Laboratories, Canada), and its brain removed using a dissecting microscope mounted with an Axiocam camera (Zeiss, Germany). Brain and whole-body samples were stored at −80 ℃ for further analysis.

5.2.4. Selenium analysis

Total Se concentrations in the water, food, and fish whole-body samples (n = 3) were quantified using a graphite furnace atomic absorption spectrometer (AAnalyst 800, Perkin Elmer, USA) as described elsewhere (see section 4.2.4; Chapter 4).

5.2.5. Biochemical assays

The ratio of reduced GSH to oxidized GSH is widely employed as a marker of oxidative stress and redox status (Owen and Butterfield, 2010). The concentration of the GSH and GSSG in the zebrafish whole brain (pools of three brains, n = 4) was measured using a fluorometric method as described previously (Jamwal et al., 2016). Lipid peroxidation (LPO) is another important manifestation of oxidative stress, which can alter membrane functions (Niki, 2008). LPO in zebrafish whole-brain (pools of three brains, n = 4) was analyzed using a lipid hydroperoxide assay kit following the manufacturer's protocol (Abcam, USA). This kit directly measures lipid hydroperoxides utilizing redox reaction with ferrous ions, rather than malondialdehyde degradation products. Homogenization of the brain tissues (pools of 3 brains) was conducted using ice-cold phosphate buffered saline (PBS) followed by centrifugation at 10,000 *g* for 10 min. Supernatants were carefully collected and transferred to new Eppendorf tubes. 500 µl of Extract R saturated

methanol solution was added to each tube and thoroughly vortexed. Then, 1 ml of cold chloroform was added to each tube followed by centrifuging at 1,500 *g* for 5 min at 0 °C. 500 µl of the bottom chloroform layer was collected and transferred to glass vials and mixed with 450 µl of chloroform-methanol (2:1) mixture. Then standard chromogen (50 µl, freshly prepared) was added to each vial and vortexed. The absorbance was read at 490 nm using a multimode microplate reader (Varioskan Flash, Thermo Fisher Scientific, Finland). LPO in the brain samples was expressed as µM.

Measurement of total DA was performed using a DA enzyme-linked immunosorbent assay kit following the manufacturer's instructions (ELISA, Biovison, USA). The brain tissues (pools of 2-3 brains, 50 mg) were homogenized in ice-cold PBS and centrifuged at 5,000 *g* for 5 min to retrieve the supernatant. 50 µl of supernatant was transferred to 96-well ELISA plates and incubated with Biotin-detection antibody for 45 min at 37 ℃. The solution was discarded and the plate washed 3 consecutive times using a wash buffer. A volume of 100 µl of HRP-Conjugated Streptavidin was added to each well and the plates were again incubated at 37 ℃. After 30 min the solution was discarded and the plate was washed 5 times using the wash buffer. Subsequently, 90 µl of TMP chromogenic substrate (3, 3', 5, 5'-Tetramethylbenzidine) was added to each well, the plate was incubated at 37 ℃ in the dark for 20 min prior to the addition of the stop solution. DA levels were determined in the microplate reader at an absorbance of 450 nm and expressed as pg/ml.

5.2.6. Quantitative real-time polymerase chain reaction

The mRNA expression of genes encoding for antioxidant enzymes, including manganese superoxide dismutase (Mn-SOD), copper/zinc superoxide dismutase (Cu/Zn-SOD), catalase (CAT), and glutathione peroxidase 1a (GPX1a) was quantified in the zebrafish whole-brain. Furthermore, the mRNA expression of genes associated with DA receptors (DA receptor D1b [DRD1] and D2 [DRD2b, DRD2c, DRD3, DRD4a, DRD4b], DA synthesis (tyrosine hydroxylase 1 [TH]), storage (vesicular monoamine transporter-2 [VMAT2]), transportation (dopamine transporter [DAT]), and metabolism (monoamine oxidase [MAO]) was assessed. Moreover, the mRNA expression of genes involved in neural plasticity was evaluated in the zebrafish brain. This included brain-derived neurotrophic factor ([BDNF], involved in the modification of synaptic strength), neuronal PAS domain protein 4a ([NPAS4], involved in activity-dependent synaptic modulation in both excitatory and inhibitory neurons), and neuronal differentiation 1(NEUROD 1, involved in adult neurogenesis) (Aimone et al., 2014; Leal et al., 2015; Lin et al., 2008). Total RNA

extraction from the zebrafish whole-brain (pools of 3 brains, n = 3) was performed using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The cDNA was produced using a QuantiTect Reverse Transcription® kit (Qiagen, Germany). Quantitative real-time in triplicates was conducted using SYBR Green PCR Master Mix (SensiFAST, Bioline, USA) on an iCycler Thermal Cycler (Bio-Rad, USA). Target gene mRNA abundance was quantified by normalizing to the expression of β-actin as the housekeeping gene. It is to be noted that the expression of β-actin was found to be unaffected in zebrafish brain during chronic exposure to similar dietary concentrations of Se in our previous study (Naderi et al., 2017). Likewise, in the present study, there was no significant change in the mRNA expression of β-actin in response to Se treatment (data not shown). The relative expression of target genes was calculated by the $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen, 2001). The sequences of primers are provided in Table 5.1.

Table 5.1. Primer sequences for quantitative PCR used in this study. Detailed information on the primers used can be read in previous reports (Filby et al., 2010; Oliveira et al., 2016; Pan et al., 2012; Sarkar et al., 2014; Teles et al., 2016; Yamamoto et al., 2011).

Target Gene	Forward primer (from 5' to 3')	Reverse primer (from 5' to 3')	GenBank accession no.
DRD1b	ACTGCATGGTTCCTTTTTGC	GGATTTGTGCTGTCCGTTTT	NM_001135976
DRD2b	GTCTCCATCTCCGTCCTCTC	TTACCGAACACCACACAGAAG	AY333791
DRD2c	ATGCTCCTGACTCTCCTC	ATCTGCCACCGCCAAG	AY333792
DRD3	TTCAGACCACCACCAACTACC	GCTCCGCCGACCACTTC	NM_183067.1
DRD4a	GGTCCCTCAATATGACCGTATG	GCGATGAACCTGTCTATGCT	AY750152.1
DRD4b	GAGCTCTGGTCGTAACCGCTCACA	ACAGGCAGCACCTTCATTGCCTTG	AY750153.1
TH1	GCCATACCAAGACCAGACTTAC	GCTTGATGCGGGTCACATA	NM_131149.1
DAT	ACTTTCTGCTGTCCGTCATC	GCTCCTCCGCCATTCTTG	NM_131755
MAO	GCAGTCAGAGCCCGAATC	CACACCCATAAACTTGAGGAATC	NM_212827
VMAT2	ATGGGGATGCTGGCAAGTGTTTAC	GAGCGGGTCTTCATTGAGCAGTTA	BC090766
CAT	CTCCTGATGTGGCCCGATAC	TCAGATGCCCGGCCATATTC	AF170069.1
Cu/Zn SOD	CAACACAAACGGCTGCATCA	TTTGCAACACCACTGGCATC	BC055516.1
Mn-SOD	AGCGTGACTTTGGCTCATTT	ATGAGACCTGTGGTCCCTTG	NM_199976.1
GPX1a	CCCTCTGTTTGCGTTCCTGA	TCTTGAATGGTTCCCCGTCC	BC164790.1
NEUROD 1	AAGTCAGATCCCTGCGTCAT	GGGAATTGTGCAACTCTGC	NM_130978
NPAS4	GACACGGGTTGAGAATGGTT	GCACCAAGCACCCTGTAAAT	NM_001045321.1
β-Actin	AGGTCATCACCATTGGCAAT	GATGTCCACGTCGCACTTCAT	AF057040

5.2.7. Statistical analysis

Data are expressed as mean ± the standard error of the mean (SEM), unless stated otherwise. All data were tested for normality and homogeneity of variance by use of the Kolmogorov–Smirnov one-sample test and Levene's test, respectively (SPSS software, version 23.0, IBM SPSS Inc., USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was employed to determine significant differences in different parameters among treatment groups. Data for the number of incorrect arm entries, Se concentrations in whole-body zebrafish, DA levels of the brain, and also the expression of BDNF did not meet the assumption of homoscedasticity and therefore were tested for significance using a Welch's test followed by Games-Howell post hoc test. Statistical significance was accepted when $p<0.05$.

5.3. Results

5.3.1. Selenium analysis

The concentrations of total Se in diets, water, and in whole-body zebrafish samples are presented in Table 5.2. The measured concentrations of Se in the control diet and brine shrimp were 1.2 ± 0.2 µg/g dry weight (dw) and 1.0 ± 0.1 µg/g wet weight, respectively. Se concentrations in SeMet-spiked foods (nominal concentrations of 3, 10, 30 and 60 µg/g Se) were 3.5 ± 0.3, 11.1 ± 0.9, 27.4 ± 0.4, and 63.4 ± 0.4 µg/g dw, respectively. These concentrations were significantly different (one-way ANOVA: $F_{4, 10} = 2351.34$, $p < 0.001$). At the exception of the diet contained 3.5 µg Se/g dw group ($p = 0.076$), the measured Se in other SeMet-spiked foods were significantly higher than the control group (Tukey post hoc tests: all $p < 0.001$). Dietary Se exposure significantly affected the whole-body Se burden of zebrafish (Welch's test: $F_{4, 4.74} = 52.79$, $p < 0.001$). Fish in groups receiving the two highest concentrations of Se had significantly higher Se concentrations in their bodies (Games-Howell post hoc test: both $p < 0.001$) than the control group. Our results also showed that dietary exposure to different concentrations of Se resulted in significantly higher dissolved Se concentrations in water (one-way ANOVA: $F_{4, 25} = 48.28$, $p < 0.001$), in exposure tanks treated with 27.4 and 63.4 µg Se/g diets (Tukey post hoc tests: both $p < 0.001$).

Table 5.2. Total Se concentrations in the exposure water (µg/l), food (µg/g dry weight), and brine shrimp and fish (µg/g wet weight).

Nominal concentrations of Se in diet (µg/g)	Food (µg/g dry weight)	Water (µg/l)	Whole body (µg/g wet weight)
Control	1.2 ± 0.2	0.4 ± 0.1	2.24 ± 0.3
3	3.5 ± 0.3	0.3 ± 0.1	2.66 ± 0.3
10	11.1 ± 0.9*	0.8 ± 0.2	5.91 ± 0.4
30	27.4 ± 0.4*	1.6 ± 0.1*	16.57 ± 2.0*
60	63.3 ± 0.4*	2.4 ± 0.2*	39.21 ± 2.6*
			Brine shrimp
			1.0 ± 0.1

*Significantly different from the control group at $p < 0.05$ (n = 3 per treatment except for dissolved Se (n = 6)).

5.3.2. Associative learning performance

Exposure to Se significantly affected the percent time that fish spent in the target arm (one-way ANOVA: $F_{4, 95} = 7.30$, $p < 0.001$, Fig. 5.1A). Fish fed with 11.1, 27.4, and 63.4 µg Se/g diets spent significantly lower time in the target arm compared to the control group (Tukey post hoc tests: all $p < 0.005$). Se exposure also altered the number of target arm entries among groups ($F_{4, 95} = 13.08$, $p < 0.001$, Fig. 5.1B). Fish fed with each of the four SeMet-spiked diets exhibited significantly fewer target arm entries compared to the control group (Tukey post hoc tests: all $p < 0.001$). Fig. 5.1C illustrates the number of incorrect arm entries made by fish in different groups during the probe trial. Dietary exposure to Se resulted in a significant difference in the number of incorrect arm entries among treatment groups (Welch's test: $F_{4, 42.06} = 10.75$, $p < 0.001$). This difference was attributed to the groups exposed to the two highest dietary concentrations of SeMet (27.4 and 63.4 µg Se/g) compared to the control group (Games-Howell post hoc test: $p = 0.005$ and $p = 0.001$, respectively). A significant difference in total distance traveled by fish was also found among different treatment groups (one-way ANOVA: $F_{4, 95} = 3.26$, $p = 0.015$, Fig. 5.1D). However, this difference was only attributed to the lower locomotor activity of fish fed with 3.5 µg Se/g diet compared to fish treated with 27.4 µg Se/g diet (Tukey post hoc tests: $p = 0.017$). Indeed, there were no significant differences in locomotor activity among Se treated groups when compared to the control group (Tukey post hoc tests: all $p > 0.05$), suggesting that fish in different treatment groups actively explored the maze.

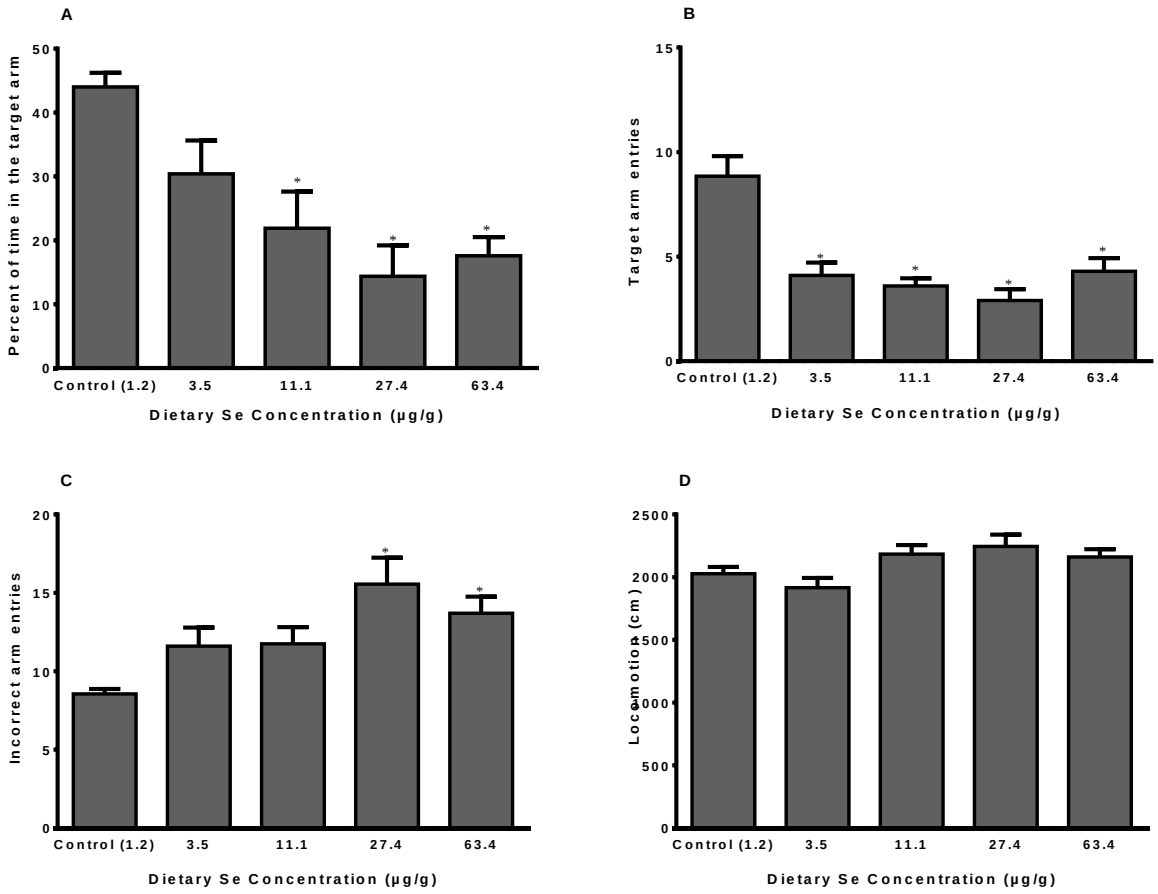

Figure 5.1. The effects of dietary Se exposure on associative learning performance of adult zebrafish indicated by: the percentage of time zebrafish spent in the target arm during the probe (A), target arm entries (B), incorrect arm entries (C), and locomotion (D). Asterisks above data bars denote a significant difference vs. control group at $p < 0.05$ ($n = 20$).

5.3.3. Estimation of oxidative stress in the zebrafish brain

GSH:GSSG ratio was measured to evaluate the extent of oxidative stress in the brain of zebrafish exposed to different dietary concentrations of Se (Fig. 5.2A). A significant effect of Se on this biomarker was found in zebrafish brain (one-way ANOVA: $F_{4, 15} = 11.75$, $p < 0.001$). A significant reduction in GSH:GSSG ratio was found in the fish fed with 27.4 and 63.4 µg Se/g diets (Tukey post hoc tests: $p = 0.046$ and $p = 0.028$, respectively), indicating a significant depletion in GSH levels in the brain of fish in these groups. Lipid peroxidation, another indicator of oxidative stress, also appeared to differ among the treatment groups (one-way ANOVA: $F_{4, 15} = 433.51$, $p < 0.001$, Fig. 5.2B). A significant induction in LPO levels was observed in fish treated with 27.4 and 63.4 µg Se/g diets (Tukey post hoc tests: both $p < 0.001$).

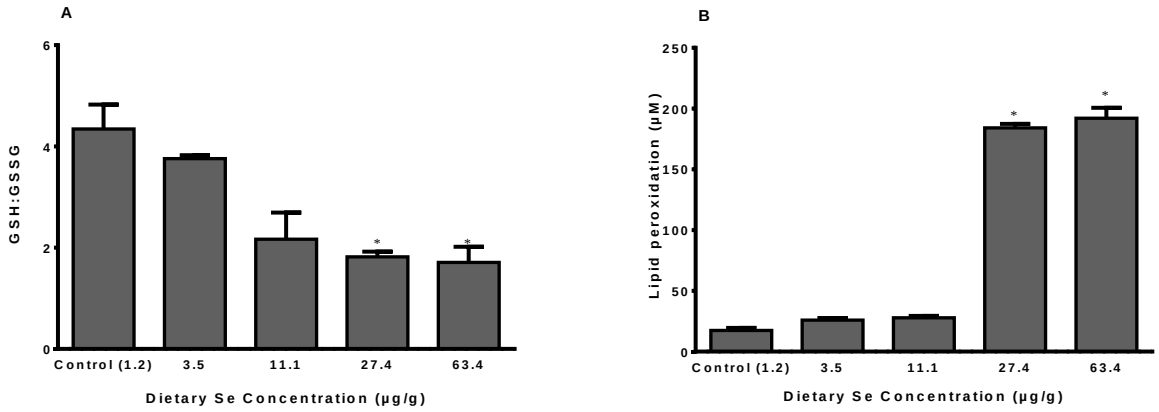

Figure 5.2. The GSH:GSSG ratio (A) and lipid peroxidation levels (B) of the zebrafish brain exposed to different concentrations of dietary Se. Asterisks above bars represent a significant difference vs. control group at $p < 0.05$ ($n = 4$).

A significant alteration was found in transcript level of Mn-SOD ($F_{4, 10} = 22.44$, $p < 0.001$, Fig. 5.3A) and Cu/Zn-SOD ($F_{4, 10} = 32.87$, $p < 0.001$, Fig. 5.3B). It was subsequently elucidated that these changes stemmed from the marked up-regulation of both Mn-SOD and Cu/Zn-SOD in fish treated with 27.4 and 63.5 µg Se/g diets (Tukey post hoc tests: all $p < 0.003$). A significant change in the mRNA expression of CAT was also found (one-way ANOVA: $F_{4, 10} = 79.46$, Fig. 5.3C). Exposure to 27.4 and 63.5 µg Se/g diets resulted in a significant up-regulation of CAT mRNA expression (Tukey post hoc tests: both $p < 0.001$). Likewise, the mRNA level of GPX1a was significantly affected by Se treatment (one-way ANOVA: $F_{4, 10} = 7.70$, $p = 0.004$, Fig. 5.3D). Although fish treated with the two highest concentrations of Se exhibited a relative increase in the mRNA level of GPX1a, only a significant increase in fish treated with 27.4 µg Se/g diet was confirmed compared to the control group (Tukey's post hoc tests: $p = 0.041$).

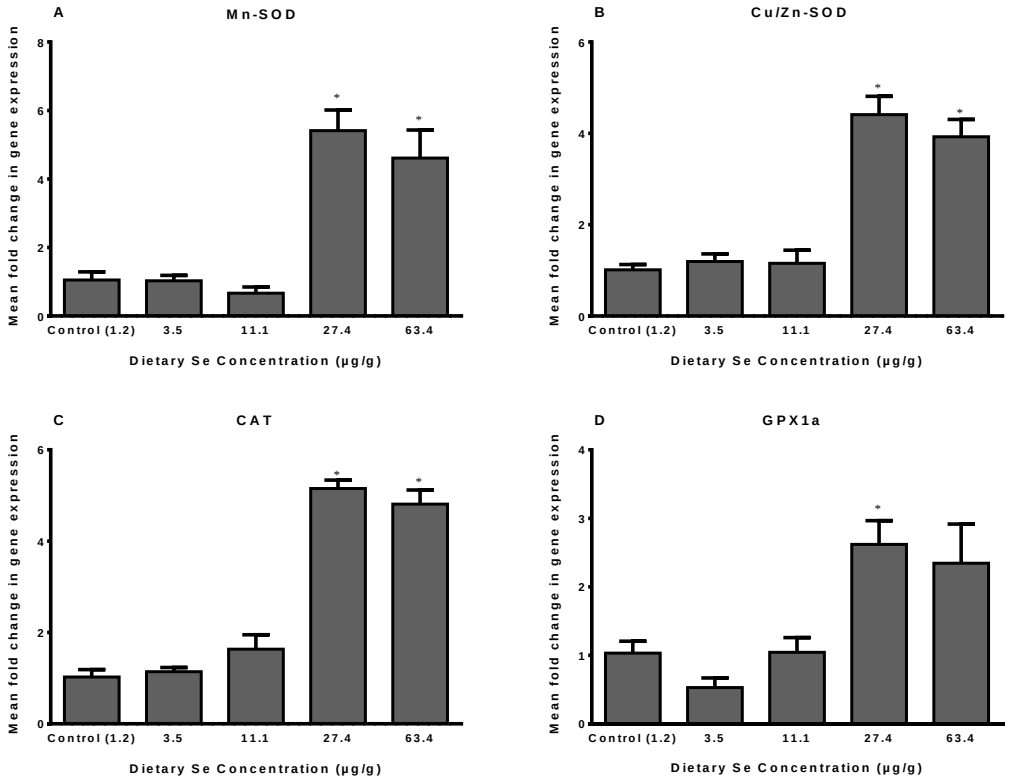

Figure 5.3. Mean fold change in the expression of antioxidant genes, including Mn-SOD (A), Cu/Zn-SOD (B), CAT (C), and GPX1a (D) in the zebrafish brain after 60 days of exposure to dietary Se. Asterisks above data bars denote a significant difference vs. control group at $p<0.05$ (n=3).

5.3.4. Dopaminergic cell markers in the brain

Dietary Se exposure induced a significant change in DA levels of fish among different groups (Welch's test: $F_{4, 6.04} = 12.19$, $p < 0.001$, Fig. 5.4A). DA levels in the groups treated with 11.1, 27.4, and 63.4 µg Se/g diets were significantly higher than fish fed with only control diet (Games-Howell post hoc test: all $p < 0.005$). Fig. 5.4 also depicts the mRNA expression profile of different genes involved in dopaminergic neurotransmission. As to the four genes associated with the DA biosynthesis, transport, storage, and degradation (TH, DAT, VMAT2, and MAO), one-way ANOVA declared a significant effect of Se exposure on TH ($F_{4, 10} = 6.50$, $p = 0.008$, Fig. 5.4B), DAT ($F_{4, 10} = 34.07$, $p < 0.001$, Fig. 5.4C), and VMAT2 ($F_{4, 10} = 12.99$, $p = 0.001$, Fig. 5.4D), but indicated no significant change in the transcription status of MAO ($F_{4, 10} = 0.911$, $p = 0.494$, Fig. 5.4E). A significant induction of TH mRNA expression was found in fish fed with 11.1, 27.4, and 63.4 µg Se/g diets (Tukey's post hoc tests: $p = 0.035$ and $p = 0.036$), and also a significant increase in the mRNA levels of DAT was found in fish treated with 27.4 and 63.4 µg Se/g (Tukey's post

hoc tests: both $p < 0.008$). However, a significant down-regulation in the transcript level of VMAT2 was observed in fish treated with 11.1, 27.4, and 63.4 µg Se/g diets (Tukey's post hoc tests: $p = 0.016$, $p = 0.039$, and $p = 0.033$, respectively).

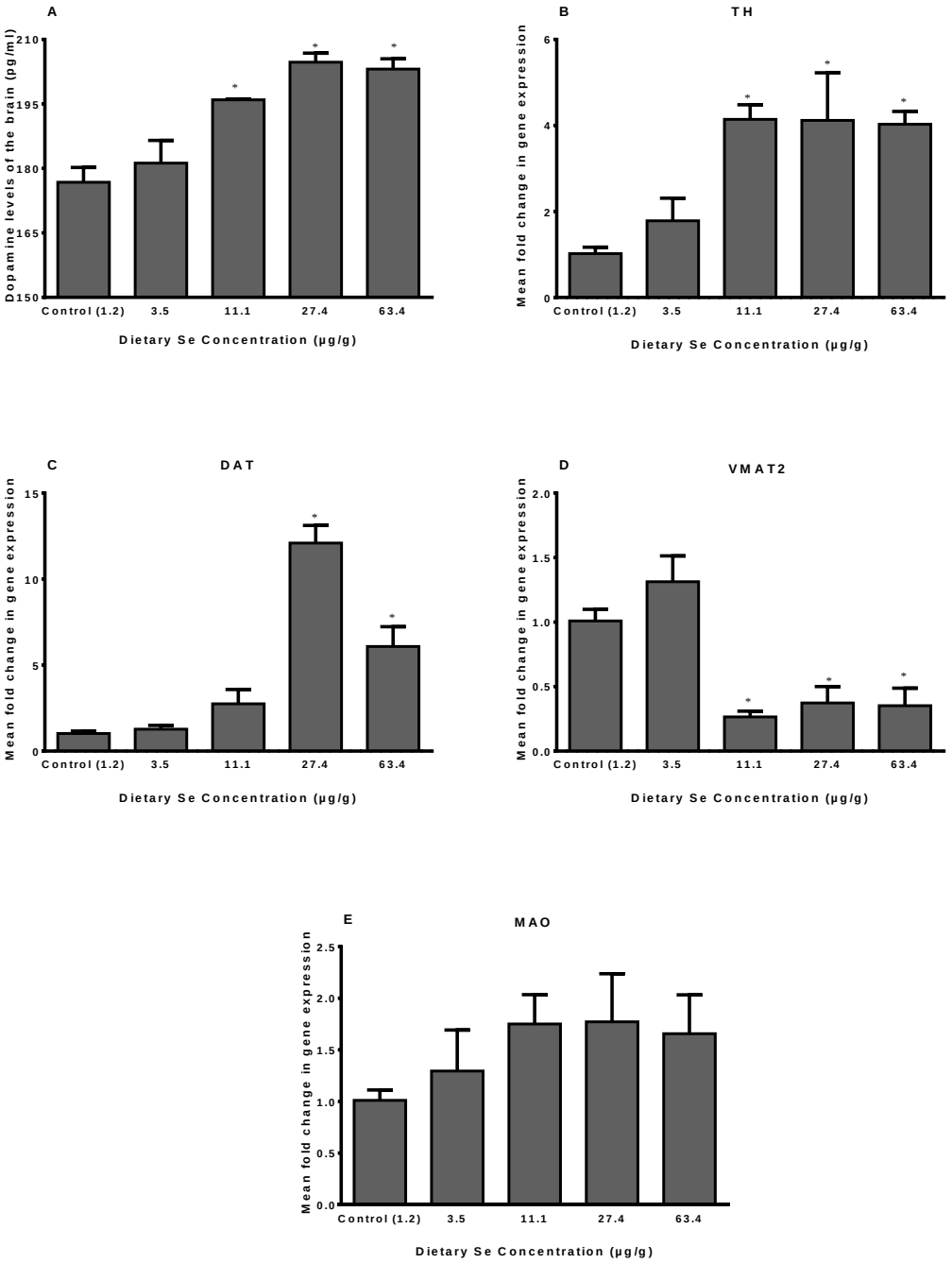

Figure 5.4. DA levels of the zebrafish brain exposed to different dietary concentrations of Se (A). Mean fold change in the expression of TH (B), DAT (C), VMAT2 (D), and MAO (E) in the zebrafish brain. Asterisks above data bars denote a significant difference vs. control group at $p < 0.05$ ($n = 4$ for DA and $n = 3$ for genes).

Dietary Se exposure significantly altered the mRNA expression of DRD1 (one-way ANOVA: $F_{4, 10} = 40.35$, $p < 0.001$, Fig. 5.5A). This gene was up-regulated in fish fed with 11.1, 27.4, and 63.4 µg Se/g diets in comparison to the control group (Tukey's post hoc tests: $p = 0.011$, $p = 0.006$, and $p < 0.001$, respectively). Moreover, there was a significant treatment effect on the mRNA expression levels of DRD2b (one-way ANOVA: $F_{4, 10} = 9.19$, $p = 0.002$, Fig. 5.4B) and DRD2c (one-way ANOVA: $F_{4, 10} = 6.86$, $p = 0.006$, Fig. 5.4C). However, there was no significant difference in the mRNA expression of the DRD3 gene among different treatment groups (one-way ANOVA: $F_{4, 10} = 2.31$, $p = 0.129$, Fig. 5.5D). A marked transcriptional up-regulation of these genes was found in fish treated with 11.1 (Tukey's post hoc tests: $p = 0.009$) and 3.5 µg Se/g diets (Tukey's post hoc tests: $p = 0.031$), respectively. There was also a significant difference in the mRNA abundance of DRD4a (one-way ANOVA: $F_{4, 10} = 46.65$, $p < 0.001$, Fig. 5.5E) and DRD4b among groups (one-way ANOVA: $F_{4, 10} = 10.94$, $p = 0.001$, Fig. 5.5F). For DRD4a, post hoc comparisons attributed this difference to the up-regulation of mRNA expressions in fish treated with 11.1, 27.4, and 63.4 µg Se/g diets (Tukey's post hoc tests: all $p < 0.001$). A significant increase in the mRNA expression of DRD4b was noted in fish receiving the two highest concentrations of Se (Tukey's post hoc tests: $p = 0.002$ and $p = 0.009$).

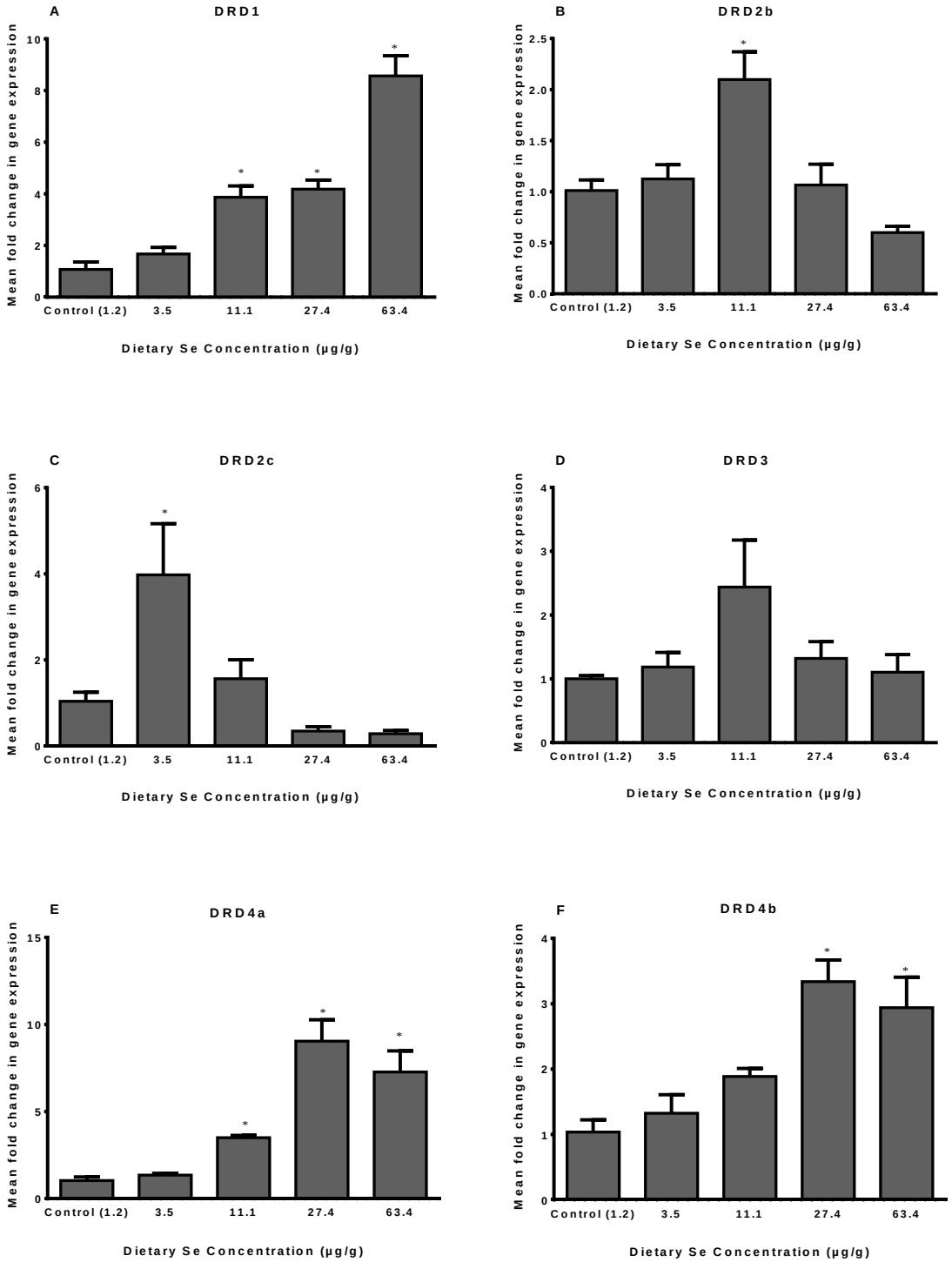

Figure 5.5. Mean fold change in the expression of DRD1 (A), DRD2b (B), DRD2c (C), DRD3 (D), DRD4a (E), and DRD4b (F) in the zebrafish brain. Asterisks above data bars denote a significant difference vs. control group at $p < 0.05$ ($n = 3$).

5.3.5. Expression of immediate early and late response genes

A significant change in the mRNA expression of the BDNF gene was observed (Welch's test: $F_{4, 4.60} = 34.60$, $p = 0.001$). Se treatment suppressed the expression of this gene in fish treated with 27.4 and 63.4 µg Se/g diets compared to the control group (Games-Howell post hoc test: $p = 0.024$ and $p = 0.002$, Fig. 5.6A). As represented in Fig. 5.6B, a biphasic change in the mRNA expression of NPAS4 was observed (one-way ANOVA, $F_{4, 10} = 49.80$, $p < 0.001$). A significant up-regulation was found in fish fed with the lowest concentration of dietary Se (Tukey's post hoc tests: $p < 0.001$) relative to the control, while a marked down-regulation in the expression of this gene was noted in fish that received the two highest dietary concentrations of Se (Tukey's post hoc tests: $p = 0.026$ and $p = 0.010$, respectively). Se treatment also significantly affected the mRNA expression of NEUROD 1 (one-way ANOVA: $F_{4, 10} = 6.90$, $p = 0.006$). A significant down-regulation in the NEUROD 1 expression was detected in fish treated with the highest concentration of dietary Se (Tukey's post hoc tests: $p = 0.004$, Fig. 5.6C).

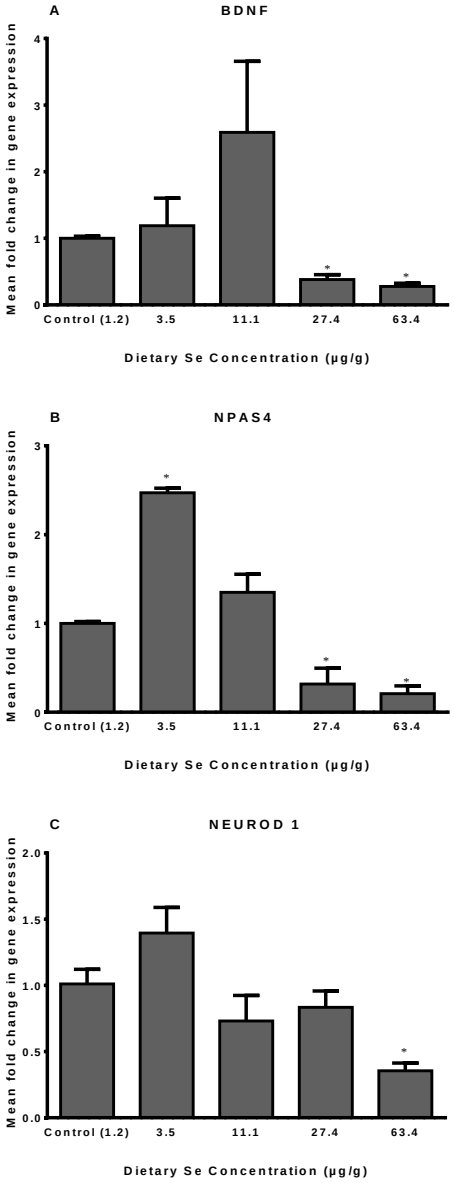

Figure 5.6. Mean fold change in the expression of BDNF (A), NPAS4 (B), and NEUROD 1 (C) in the zebrafish brain after 60 days of exposure to dietary Se. Asterisks above data bars denote a significant difference vs. control group at $p < 0.05$ ($n = 3$).

5.4. Discussion

The results of the present study suggest that chronic sublethal dietary SeMet exposure can impair associative learning in zebrafish. Whole-body Se concentrations found in this study ranged from 2 to 39 µg Se/g wet weight. These concentrations were similar to the Se body burden reported in different wild fish species collected from Se-contaminated natural waters (Driedger et al., 2009;

Muscatello and Janz, 2009). Therefore, it is possible that Se neurotoxicity affects the cognitive ability of fish inhibiting the contaminated waters. Despite the increasing evidence indicating neurotoxic effects of Se, few have addressed the negative effects of Se on learning and memory. For example, in a study using mice, exposure to selenite impaired active avoidance learning (Ajarem et al., 2011a). In another study, Smith et al. (2010) have reported that developmental exposure to SeMet compromised spatial learning in zebrafish. In our recent study, we have also found that 30 days of exposure to SeMet impaired latent learning performance in adult zebrafish (Naderi et al., 2017). The results of the present study add to the growing body of literature on the neurobehavioural effects of this trace element. Here, we reported that exposure to dietary Se diminished the target arm preference of zebrafish, as evidenced by a decline in the duration of time fish spent in the target arm, fewer correct arm entries, and more incorrect arm entries. These results imply that chronic exposure to environmentally relevant concentrations of Se may perturb the cognitive ability of fish to associate a conditioned visual stimulus with the location of the reward, suggesting an impaired associative learning. Our results showed that chronic Se exposure, even at low concentrations triggered a confusion with regards to locating the reward, as demonstrated by a significant decrease in target-arm entries. Reciprocally, we found an increase in incorrect arm entries in all Se treatment groups. However, this reached a significant level only in fish treated with the two highest concentrations of dietary Se (27.4 and 63.4 µg Se/g). It seems that fish in groups treated with the two highest concentrations of Se preferred to explore the maze by randomly entering into the arms to find the location of the reward, which resulted in higher incorrect arm entries. On the other hand, it is likely that fish treated with the two lowest concentrations of Se (3.5 and 11.1 µg Se/g) preferred to spend more time in each arm they entered in to find the reward. There was also no difference in total distance traveled between Se-treated fish in comparison to the control fish. Therefore, we conclude that exploratory activity was not an influencing factor in Se-induced learning impairment observed in this study.

The unique characteristics of the brain such as high oxygen demand, high content of polyunsaturated fatty acids, and low activity of antioxidant defenses make this organ highly liable to oxidative stress (Li et al., 2013). Induction of oxidative has been associated with several types of cognitive impairment in both humans and animals (Glade, 2010). In zebrafish, the induction of oxidative stress in the brain led to an impairment of associative and spatial learning (Ruhl et al., 2016). Se-induced oxidative stress has also been found to disrupt latent learning in zebrafish

(Naderi et al., 2017). Therefore, oxidative stress might be one of the major upstream pathways in the induction of learning deficit in zebrafish. In this study, we found a marked reduction in GSH:GSSG ratio in parallel with an induction in LPO levels in the zebrafish brain, clearly indicating the occurrence of oxidative damage. Cellular GSH depletion is closely related to the loss of detoxification capability. Decreased GSH content may also result in disruption of signalling pathways that are tightly related to alterations in the cellular redox status (Amado and Monserrat, 2010), which might be important in the cognitive processing of information. The induction of LPO is one of the most damaging effects of ROS. The peroxidation of membrane lipids, which subsequently results in the leaky membrane may disrupt the intracellular homeostasis of essential ions, specifically calcium (Ca^{2+}). Ca^{2+} plays a pivotal role in the regulation of neuronal plasticity underlying learning and memory and neuronal survival (Zündorf and Reiser, 2011). Thus, it is plausible that dysregulation of Ca^{2+} in the brain contributes to learning impairment observed in our study.

In the present study, we also measured the mRNA expression of four antioxidant enzyme genes (Mn-SOD, Cu/Zn-SOD, Cat, and GPX1a). Our results, for the first time, showed chronic exposure to dietary Se induced the transcriptional expression of the antioxidant genes in the zebrafish brain. However, this up-regulation was mainly observed in fish treated with the two highest concentrations of Se (27.4 and 63.4 µg Se/g). Increase in SOD activity is considered as a primary component of the intracellular anti-oxidative defense system against the oxidative stress damage (Wang et al., 2011). The primary role of SOD is to catalyze the dismutation reaction of superoxide anion which ultimately forms oxygen and hydrogen peroxide (H_2O_2). The results of our study showed an increase in the mRNA expression of Mn-SOD and Cu/Zn-SOD in the zebrafish brain treated with 27.4 and 63.4 µg Se/g diets. These up-regulations might be a cascading anti-oxidative mechanism to cope with the Se-induced accumulation of superoxide radicals in the zebrafish brain. The complete protective effect of SOD can only be achieved by subsequent actions of CAT and GPX1a enzymes to further decompose H_2O_2 generated into the water and oxygen (Wang et al., 2011). The increase in the transcriptional level of both CAT and GPX1a enzymes was evident in fish treated with the two highest dietary concentrations of Se. This indicates that the up-regulation of CAT and GPX1a genes might have occurred as an adaptive response to the accumulation of H_2O_2 and to protect the zebrafish brain against Se-induced oxidative stress.

It is widely believed that the dopaminergic system is the Achille's heel of the CNS in response to environmental neurotoxicants. There is a diverse collection of environmental neurotoxicants that preferentially target the dopaminergic system and disturb single or multiple aspects of DA neurotransmission to alter behaviour. Se has been shown to increase synaptic DA in the striatum and nucleus accumbens of male Sprague-Dawley rats (Rasekh et al., 1997). Likewise, elevated levels of this neurotransmitter in the mice brain have also been reported following exposure to Se (Oliveira et al., 2012). Our data showed that chronic exposure to dietary Se increased DA levels of the brain in the treatment groups received more than 3.5 µg/g of Se relative to the control group, further confirming the stimulatory effect of Se on the brain DA level reported in previous mammalian studies.

The DA homeostasis is regulated via several linked processes including synthesis, release, uptake, storage, degradation, and receptor activation. DA is synthesized from conversion of tyrosine to L-DOPA under the action of the key enzyme TH. Once synthesized, DA is sequestrated into the synaptic vesicles via VMAT2, released in response to the pre-synaptic action potential and then activates a variety of pre- and post-synaptic receptors associated with numerous signalling mechanisms. DA transmission is terminated by re-uptake of DA through DAT into the pre-synaptic neuron where it is partly reloaded back into the vesicles by VMAT2 for storage and subsequent release. The surplus DA in the cytoplasm is metabolized by MAO and catechol-O-methyl transferase enzymes (Meiser et al., 2013). Therefore, dopaminergic signalling is a heavily regulated and interconnected process. Hence, this is not unexpected that alteration even in a single process negatively affects other processes involved in DA transmission. Alterations in the activity of TH, DAT, VMAT2, and MAO have frequently been reported as common mechanisms underlying metal-induced neurotoxicity (Altenhofen et al., 2017; Jones and Miller, 2008; Kumar et al., 2010). We have also shown that dietary exposure to Se increased the mRNA expression of TH and DAT in adult zebrafish (Naderi et al., 2017). In agreement with our previous study, the results of the present study showed that 60 days of exposure to dietary Se induced the mRNA expression of TH in fish treated with 11.1, 27.4, and 63.4 µg Se/g – an observation that is consistent with the observed increase in the brain DA levels in these treatments. Presumably, this up-regulation of TH transcription contributed to the increase in DA levels and subsequently also led to the increase in DA turn-over in the zebrafish brain. The transcription of DAT was also up-regulated in fish fed with the two highest concentrations of dietary Se (27.4 and 63.4 µg Se/g). Elevated DAT

transcription may occur as a compensatory mechanism in response to the increased biosynthesis of DA, as indicated by the increased TH mRNA expression. In contrast, exposure to dietary Se reduced the transcription of VMAT2, indicating a reduction in loading of DA into the vesicles. It is also conceivable that the decrease in DA storage might have triggered an induction of TH mRNA expression in order to produce more DA, which subsequently resulted in increased re-uptake of DA. While Se exposure appeared to have altered DA synthesis, storage, and re-uptake pathways, the transcription of MAO was unaffected in all treatment groups. This is consistent with our previous observation that 30 days of dietary exposure to SeMet did not affect the mRNA expression of MAO in zebrafish brain (Naderi et al., 2017), further indicating that DA metabolism is not a target of Se neurotoxicity. Taken together, the changes in the mRNA expression documented in our study elucidate the increase of the cytoplasmic DA content of the zebrafish brain. Since cytosolic DA is notoriously prone to autoxidation, the excessive amount of this neurotransmitter and its improper sequestration could potentially lead to increased production of ROS and DA-quinones. In this situation, the depletion of cellular GSH content due to SeMet metabolism (Misra et al., 2012a) can exacerbate the oxidative stress induced by the autoxidation of DA.

The effects of DA are elicited through its interaction with D1 receptors, which are located in the post-synaptic neurons and functioning to increase the adenylyl cyclase (AC) activity and cyclic adenosine monophosphate (cAMP) levels. In addition, DA also acts via its interaction with D2 receptor family, which are located in both pre- and post-synaptic neurons to negatively regulate AC and cAMP. Following the synaptic release, DA via interaction with DA receptors mediates synaptic modifications underpinning associative learning and mnemonic processes (Schultz, 2002). There is convincing evidence indicating the adverse effects of a wide range of environmental compounds on DA receptors and their associated signalling pathways (Jones and Miller, 2008). The elevated activities of both D1 and D2 receptors have been demonstrated in mice treated with Se-containing compounds (Oliveira et al., 2012). However, we previously demonstrated that exposure to 32.5 and 57.5 µg/g of Se decreased the mRNA expression of D1 receptor, while up-regulated the transcription of D2 receptors (DRD2b and DRD4a subtypes) (Naderi et al., 2017). The results of the present study showed a marked increase in the mRNA expression of both D1 and D2 receptors (except DRD3 subtype) in the brain of zebrafish following dietary exposure to similar concentrations of Se. These findings indicate that Se affects the transcriptional activity of DA receptors in a task-specific manner. It seems that Se exposure stimulated DA production via

induction of TH. Subsequently, the increase in DA levels of the brain might have stimulated the activation of D1 receptors and led to the increase in abundance of post-synaptic D1 receptors. It is widely accepted that phasic DA release through the activation of D1 receptor pathway plays a critical role in the initiation and facilitation of reward-related behaviours (Beninger and Miller, 1998; Messias et al., 2016). Moreover, since D2 autoreceptors reduce the DA synthesis, release, and re-uptake, the increase in the expression of D2 receptors might have occurred in response to increased DA synthesis observed in our study. Post-synaptic D2 receptors also regulate DA transmission by increasing the activity of DAT (Schmitz et al., 2003). Therefore, the increase in the expression of DAT recorded in our study further confirms the regulatory role of D2 receptors.

We have previously shown that D1 and D2 receptors play important roles in associative learning performance in zebrafish with a more prominent role for the D1 receptor (Naderi et al., 2016a). However, since D1 and D2 receptors mediate opposing excitatory and inhibitory cellular responses, their simultaneous stimulation seems to be problematic in a psychological situation. We previously demonstrated that activation of both D1 and D2 receptors disrupted the latent learning of spatial information in adult zebrafish (Naderi et al., 2016b). Concomitant activation of these receptors has also been implicated in impairment of associative learning in healthy humans (Breitenstein et al., 2006). This might be due to opposite roles of DA receptors in regulating long-term potentiation (LTP; an increase in synaptic efficacy believed to underlie learning and memory mechanisms). While the activation of D1 receptors facilitates induction of LTP, stimulation of D2 receptors directly inhibits this process in the brain structures associated with learning (Gurden et al., 1999; Xu et al., 2009). Therefore, the elevated transcriptional activity of D2 and D4 receptors might have blocked the facilitating effect of D1 receptors on LTP, which resulted in impaired associative learning in adult zebrafish in the present study. Different subtypes of D2 receptors (i.e. D2 and D4 receptor subtypes) might have mediated this inhibitory effect. While the up-regulation of DRD2b and DRD2c might have affected D1 receptor's function at lower Se exposure concentrations, the increased transcription of DRD4a and DRD4b might have compromised the facilitating effect of DRD1 at higher Se exposure concentrations. It is also noteworthy that a delicate transition from tonic to phasic firing in dopaminergic neurons essentially regulates associative learning (Day et al., 2007). The phasic DA release and an optimal post-synaptic activation of D1 receptors enhance the discrimination of behaviourally relevant stimuli and promote attention to predictors for reward during associative learning (Fiorillo et al., 2005; Popescu et al., 2016). Overexpression of D2

receptors has been shown to alter the firing pattern of dopaminergic neurons and activation of the midbrain in response to reward stimuli in mice and humans (Breitenstein et al., 2006; Krabbe et al., 2015). It has also been shown that the overexpression of D2 receptor via imbalance in the activation of D1 receptors leads to the deficit in the working memory task in mice (Kellendonk et al., 2006). Therefore, it is possible that the increased expression of D2 receptors due to the imbalance between phasic and tonic DA signals or imbalance in the activation of D1 receptor led to associative learning impairment in zebrafish.

Interestingly, alterations in the mRNA abundance of different D2 receptor subtypes displayed a dose-specific effect. While the expression of DRD2b and DRD2c increased in the lower Se concentrations, the up-regulation of DRD4a and DRD4b was evident in fish treated with higher concentrations of dietary Se. However, no change in DRD3 expression was recorded among any treatments. Therefore, it seems that the increase in DA levels stimulated the expression of different D2 receptor subtypes in a dose-specific manner. In addition, Se may have changed the binding affinity and/or ligand sensitivity of these subtypes. Future studies are required to examine the precise neuronal basis of this effect. Although our results clearly show that chronic exposure to dietary Se affects the dopaminergic system in zebrafish, we cannot rule out the possibility that Se may act on multiple neurotransmitter systems, which might also contribute to learning impairment observed in this study. Further studies are required to elucidate the effects of Se on other neuromodulatory systems (e.g., norepinephrine and serotonin) which are also involved in learning and memory in zebrafish.

In order to further elucidate the molecular pathways underlying Se-induced cognitive deficit, we examined the expression of two IEGs (BDNF and NPAS4) and a late expression gene (NEUROD 1). These genes are implicated in neural plasticity, synaptic strength, and memory formation (Sun and Lin, 2016). We previously showed that chronic exposure to dietary Se led to a biphasic change in the mRNA expression of BDNF in the zebrafish brain. While the lowest concentration of Se (2.3 µg/g) increased the mRNA expression of BDNF, a down-regulation was observed in fish treated with the higher Se concentrations (32.5 and 57.5 µg/g) (Naderi et al., 2017). In the present study, we found a down-regulation of BDNF mRNA in fish treated with the two highest concentrations of Se (27.4 and 63.4 µg Se/g). BDNF is a key molecule which controls neuronal survival, differentiation, and synapse formation. Since it is known as a major regulator of activity-dependent

synaptic plasticity, BDNF alterations are implicated in memory acquisition and consolidation (Bekinschtein et al., 2014). Thus, the down-regulation of this gene concomitant with impaired associative learning in fish exposed to high concentrations of Se is not surprising, and might suggest that high levels of Se triggered synaptic changes in the zebrafish brain. NPAS4 is a novel neuronal activity-dependent transcription factor which regulates homeostatic excitatory/inhibitory balance in neurons and controls the expression of a wide variety of activity-dependent genes involved in synaptic plasticity and long-term memory formation (Sun and Lin, 2016). NPAS4 knockout mice displayed memory impairments in several different learning paradigms (Coutellier et al., 2012; Ramamoorthi et al., 2011). Our results showed an increase in the mRNA expression of NPAS4 in fish treated with 3.5 µg Se/g diet. This induction might be due to the neuroprotective role of NPAS4 against Se-induced oxidative stress at the lowest exposure dose, limiting progressive neurodegeneration. The down-regulation of NPAS4 in the higher Se exposure concentrations might be a consequence of intensive oxidative stress, which through dysregulation of the balance between neuronal excitation and inhibition contributed to the impairment of associative learning in zebrafish. Interestingly, NPAS4 can also directly control the expression of BDNF (Ramamoorthi et al., 2011). Therefore, it can be assumed that the down-regulation of NPAS4 gene expression may contribute to the decreased transcriptional activity of BDNF leading to the cognitive impairment in zebrafish. Our results also showed a marked reduction in NEUROD 1 transcript level in fish treated with the highest dietary concentration of Se (63.4 µg Se/g). NEUROD 1 is a key transcription factor which regulates neuronal differentiation, dendritic spine stability, and the maturation of neurons (Aimone et al., 2014). The decrease in the expression of this late neuronal marker in fish brain treated with the highest concentration of dietary Se probably reflects a loss of certain sub-populations of neurons or reduced neurogenesis in the zebrafish brain. Taken together, our results showed that the exposure to the two lower concentrations of dietary Se (3.5 and 11.1 µg Se/g) changed the expression of certain dopaminergic cell markers, and immediate-early and late response genes in the zebrafish brain. These changes may compromise learning ability or reward responsivity in zebrafish, as indicated by lower dwelling time in the target arm and less target arm entries. However, Se-induced oxidative stress was only observed in fish treated with the two highest concentrations of Se (27.4 and 63.4 µg Se/g). Enhanced oxidative stress may trigger a cascade of events that lead to cell dysfunction and death. This was indicated by the down-regulation of genes involved in neuronal growth and survival, neurogenesis, and synaptic plasticity in these

groups. Therefore, it seems that the induction of oxidative stress along with the alterations in gene expressions provide a reasonable explanation for more pronounced learning impairment observed in fish treated with two highest concentrations of dietary Se.

5.5. Conclusion

In conclusion, the present study is the first to demonstrate that exposure to environmentally relevant concentrations of dietary Se impaired associative learning in zebrafish. We have shown that chronic dietary Se exposure induced oxidative stress in the zebrafish brain, which subsequently altered the integrity of the dopaminergic system, including perturbations in the DA synthesis, release, uptake, and receptor activation. The normal functioning of the dopaminergic system is essential for reward-based stimulus association learning. Therefore, an imbalance in DA levels and altered expression of DA receptors in the brain may be the underlying contributors to the significant learning deficit induced by Se in adult zebrafish. Finally, our observation that chronic exposure to dietary Se led to the down-regulation of genes involved in neural plasticity and memory formation suggests the perturbation of potential downstream pathways leading to impaired memory in zebrafish. Although Se is an essential micronutrient, its multi-faceted effects at supranutritional doses on fish are still being discovered, and thus further investigations may provide more insights into the neurotoxic effects of this metalloid on the CNS.

Chapter 6: Maternal exposure to dietary selenium causes dopaminergic hyperfunction and cognitive impairment in zebrafish offspring

Preface

The aim of this chapter is to address the fifth objective of my doctoral research work, which is to investigate the effects of maternal exposure to dietary selenomethionine on latent learning in F1-generation adult zebrafish. To this end, zebrafish embryos obtained from experiment 4 (Chapter 5) were raised up to the age of six months in the clean water and fed a normal diet. F1-generation adult zebrafish were tested in a latent learning task. The results of this study show that maternal transfer of Se to embryos leads to a long-lasting learning impairment in offspring, which is associated with the hyperfunctional dopamine (DA) neurotransmission and up-regulation in the transcript levels of immediate early genes in the brain. Elevated DA levels are also accompanied by oxidative damage in the zebrafish brain. The findings of this chapter complement the findings of Chapter 2, 4, and 5 and provide a mechanistic explanation of how maternal exposure to SeMet influences latent learning performance in zebrafish offspring.

The content of Chapter 6 was adapted from our manuscript which has been published in Environmental Science and Technology (DOI: 10.1021/acs.est.8b04768). Naderi, M., Ferrari, M.C.O., Chivers, D.P., Niyogi, S. “Maternal exposure to dietary selenium causes dopaminergic hyperfunction and cognitive impairment in zebrafish offspring”. Copyright 2018, with permission from American Chemical Society Publications.

Author contribution

Mohammad Naderi (University of Saskatchewan) designed and conducted the experiment, generated and analyzed the data, prepared all the figures and the table, and drafted and revised the manuscript. Maud Ferrari (University of Saskatchewan) provided assistance with data analysis and also edited the manuscript. Doug Chivers and Som Niyogi (University of Saskatchewan) provided inspiration, scientific input and guidance, commented on and edited the manuscript, and provided funding for the research.

6.1. Introduction

The early development of the central nervous system (CNS) is a critical period in the ontogeny of vertebrates that requires a choreographed sequence of events. This involves gene transcription, neurogenesis, neuronal migration, differentiation, and synaptic connectivity that eventually leads to the development of a functional brain connectome (Stiles and Jernigan, 2010). Neurotransmitters and their receptors appear early during nervous system development and are thought to play important roles in these paramount processes (Nguyen et al., 2001). Development, structure, and functions of neurotransmitter systems are profoundly affected by maternal nutrition (Prado and Dewey, 2014). In addition, maternal exposure to environmental contaminants could also result in the transfer of toxic chemicals from the mother to the embryo/fetus, influencing the early development of the brain and predisposing offspring to neurodevelopmental disorders (Mendola et al., 2002). Over the last decade, a small but growing number of studies have begun to draw attention to the role of prenatal and postnatal exposure to excess amounts of essential trace elements in the pathogenesis of neurodevelopmental disorders (Yang et al., 2013; Yanik et al., 2004).

Selenium (Se) is an essential trace element, but also considered to be a priority aquatic contaminant (Janz et al., 2010). As a constituent of various selenoproteins, Se is involved in diverse biological functions including neurodevelopment and normal functions of the brain in animals (Chen and Berry, 2003; Mehdi et al., 2013). Despite its biological importance, excess Se can be extremely toxic to a wide range of organisms (Janz et al., 2010; Vinceti et al., 2014). Anthropogenic activities such as agriculture on seleniferous soils, coal and uranium mining, and fossil-fuel processing operations can release Se into the environment. Se eventually enters aquatic ecosystems where it is biotransformed to selenomethionine (SeMet) and transferred through trophic chains to higher trophic level organisms (Janz et al., 2010). This organic form of Se can be incorporated into egg-yolk protein precursor vitellogenin and maternally transferred to developing embryos. Particularly affected are oviparous vertebrates such as fish, which are at risk of ingesting Se-enriched prey (Hamilton, 2004; Janz et al., 2010). Maternal transfer of Se to eggs has been reported to occur in various fish species inhabiting Se-contaminated aquatic ecosystems (Brandt et al., 2017; Janz et al., 2014). Exposure to Se at this stage commonly manifests as a suite of developmental abnormalities that include spinal curvatures, and craniofacial and fin deformities (Thomas and Janz, 2014, 2015). However, to date very little is known about the neurobehavioural consequences of maternal exposure to Se in fish.

There is burgeoning evidence that oxidative stress is the main underlying cause of Se (including SeMet) toxicity (Kupsco and Schlenk, 2016; Palace et al., 2004). Oxidative stress is, in turn, a common pathogenic mechanism shared by etiological determinants of neurodevelopment disorders (Wells et al., 2009). Moreover, Se can interfere with and disrupt the normal functions of the dopaminergic system (Rasekh et al., 1997). Dopamine (DA) is one of the earliest neurotransmitters to emerge in the fish brain (Souza and Tropepe, 2011), and plays an important role in the development and functions of neural circuits involved in the movement, emotion, learning and memory, social, and reward-related behaviours (Buske and Gerlai, 2012; Lambert et al., 2012; Roberts et al., 2013). Importantly, the dopaminergic system is one of the most redox-sensitive transmitter systems in the vertebrate brain. Indeed, a plethora of reactive oxygen species (ROS) is produced by the dopaminergic system itself, which makes it more susceptible to external oxidative insult (Meiser et al., 2013). A wealth of mammalian research points to dysfunction of the dopaminergic system as a common denominator of neurodevelopmental disorders, including attention deficit-hyperactivity disorder, autism, and schizophrenia (Money and Stanwood, 2013). In zebrafish, early transient alterations in the dopaminergic system have also been associated with the persistent behavioural abnormalities in adult zebrafish (Formella et al., 2012). Moreover, it has been demonstrated that exposure to SeMet during early development (from 2 to 24 hours post fertilization; hpf) leads to a long-lasting spatial learning impairment in adult zebrafish (Smith et al., 2010), while the underlying mechanism(s) remains to be elucidated. We have previously demonstrated that exposure to dietary SeMet induces oxidative stress and impairs the dopaminergic signalling in the brain, ultimately leading to learning impairment in adult zebrafish (Naderi et al., 2018; Naderi et al., 2017). Therefore, it is logical to hypothesize that embryonic exposure to SeMet via maternal transfer can lead to long-lasting behavioural abnormalities in adult fish, due to the induction of oxidative stress and dysfunction of the DA neurotransmission in the brain.

Learning and memory directly or indirectly modulate a broad spectrum of fish behaviour in their natural environment. For instance, learning and memory play a decisive role in foraging activities, mate choice strategies, anti-predatory behaviours, agonistic interactions, shoaling behaviour, and group joining decision of fish (Brown et al., 2008). All these behaviours are strongly tied to fish fitness and survival, and thus learning impairment may bring about a wide array of negative consequences for fish. This study was aimed to investigate the effects of maternal exposure to dietary SeMet on the learning and memory in zebrafish offspring using a latent learning paradigm.

Latent learning is defined as a form of learning that occurs in the absence of any environmental reward and does not immediately manifest itself (Brown et al., 2008). This form of learning plays a crucial role in the spatial navigation of fish within their environment. Moreover, we have previously shown that DA is critically involved in the regulation of this type of learning in zebrafish (Naderi et al., 2016b).

6.2. Materials and methods

6.2.1. Fish maintenance and exposure

Mature adult zebrafish (n = 400) were sourced from the R.J.F. Smith Center for Aquatic Ecology of the University of Saskatchewan. Fish were housed in 30-l glass tanks supplied with aerated de-chlorinated tap water at 28 ± 1 ℃ on a 14/10 hr, light/dark cycle. Fish were fed twice daily with flake food (Nutrafin Max flakes, Germany) and allowed to acclimate to these conditions for at least 3 weeks before the start of the experiment. This acclimation period consisted of a 14-day pre-exposure period to ascertain that unexposed fish were reproductively active (Ankley et al., 2001). A total of 320 fish (3.67 ± 0.06 cm and 0.75 ± 0.11 g) were then selected and randomly allocated into twenty 30-l experimental tanks (12 females and 4 males per tank), with four replicate tanks per treatment. Fish were exposed to different nominal concentrations of dietary Se (3, 10, 30, and 60 µg/g; as SeMet) as described previously, for 60 days (see section 5.2.1, Chapter 5). These concentrations reflect the range of dietary Se concentration that fish are exposed to in Se contaminated natural waters (Driedger et al., 2009; May et al., 2008; Muscatello and Janz, 2009). For the first 30 days of exposure, fish were fed either the control diet or SeMet-spiked food at 5% body weight/day ration. After the first 30 days, fish received equal portions (2.5%) of the control or SeMet-spiked foods and frozen brine shrimp (Sally's, San Francisco Bay Brand Inc., USA) for a further 30 days. This exposure regime was based on a previous study, which resulted in the improved egg production by fish (Thomas and Janz, 2011). Triplicate samples from each SeMet-spiked diet and frozen brine shrimp were taken for the measurement of total concentrations of Se. Moreover, water samples (n = 3) were collected from each experimental treatment on 30^{th} and 60^{th} days of the exposure period, and filtered using 0.45 µm disposable nylon filters for measurement of dissolved Se.

Zebrafish have asynchronous ovaries, containing follicles at all stages of development and eggs are spawned throughout the year under laboratory conditions (Selman et al., 1993). However,

maximal embryo viability is observed when adult females are isolated and allowed to breed in 10-day intervals (Niimi and LaHam, 1974). Moreover, zebrafish are estimated to complete vitellogenesis and oocyte maturation within 10 days of oviposition (Wang and Ge, 2004a, b). During this period, dietary consumption of Se results in the substantial deposition of newly acquired Se into eggs (Conley et al., 2014). It is also noteworthy that previous studies have suggested that in asynchronous spawners such as zebrafish and medaka, the concentrations of maternally exposed Se might differ among spawning events (Chernick et al., 2016; Thomas and Janz, 2014). Therefore, in this study, we used a modified mixed exposure-breeding regime to minimize the variability of maternal transfer of Se to eggs during the spawning event and possible subsequent effects on developing fish. After 50 days of exposure, adult male fish were removed from each exposure tank while female fish were still feeding on SeMet-spiked diets. In the evening of day 60, female fish (n = 8) from each exposure tank were netted and placed in breeding tanks. Moreover, 4 sexually mature untreated male fish originating from the same source as the exposed fish were added to each breeding tank to make breeding colonies of 4 untreated males and 8 Se-treated females (4 replicates per treatment). Fish were then left to settle overnight. The following morning, spawned eggs were collected from the bottom of the breeding tanks 2 hrs after the light was turned on. Collected eggs were inspected under a dissecting microscope and scored for viability. Embryos were then transferred to deep Petri dishes containing E3 embryo medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, and 0.33 mM MgSO4) and incubated at 28°C. The percent egg hatchability, larval survival, and larval deformities were determined in F1- generation larval fish as described previously (Thomas and Janz, 2014). To ascertain maternal transfer of Se from female fish to eggs, Se concentrations in whole-body female fish (n = 3, see section 5.2.4, Chapter 5) and their eggs (n = 4 replicates of 80-100 pooled eggs) were quantified. A sub-group of surviving larval zebrafish with no apparent symptoms of skeletal deformities from each treatment group was reared to 180 days post fertilization (dpf) to evaluate the persistent adverse effects of maternal exposure to SeMet on learning performance of adults. It is of note that embryos obtained from fish treated with 60 µg/g Se diet were excluded from this study due to high rates of mortality and deformity.

6.2.2. Latent learning performance

In this study, we employed a latent learning paradigm to evaluate the learning performance of F1-generation adult zebrafish maternally exposed to dietary Se. Latent learning task was conducted in

a maze (see Fig. 2.1, Chapter 2) comprising a start chamber, a reward chamber, and two tunnels that linked these two chambers to each other. The learning procedure has been described in detail elsewhere (see section 2.2.3, Chapter 2). Briefly, the learning paradigm consisted of two phases: a training phase followed by a probe phase. During the training phase, only one of the two tunnels leading to the reward chamber was open. Fish in groups of 15 were trained with the maze for 30 min each day while either the right or left tunnel was open. Moreover, no reward was presented in the maze. After 16 consecutive days of training, a probe trial was conducted to test the leaning performance of fish. During this phase, a single fish was located in the start chamber while both right and left tunnels were open and the reward chamber contained a group of fish (n = 6). Since zebrafish are highly social animals, the sight of conspecifics is used as a powerful rewarding stimulus for such studies (Al-Imari and Gerlai, 2008). During the probe trial, zebrafish performance was recorded for 10 min using an over-head HD camera (Logitech c310, USA). The latency to leave the start chamber, the time spent in the correct versus incorrect tunnel, the latency to enter the reward chamber, the time spent in the reward chamber, and locomotion (total distance traveled by the fish) were quantified by MATLAB (Academic version R2015a). At the conclusion of probe trials, fish were euthanized by of Aquacalm (Syndel Laboratories, Canada) and the whole-brain was removed under a dissecting microscope that was equipped with an Axiocam camera (Zeiss, Germany). In addition, the telencephalon was separated from a subset of brains as described previously (see section 4.2.3, Chapter 4). Brain tissues were stored at −80°C until further analysis.

6.2.3. Measurement of selenium

Selenium concentrations in water samples (dissolved Se), food (SeMet-spiked food and brine shrimp), whole-body female fish, and eggs were measured using a graphite furnace atomic absorption spectrometer (AAnalyst 800, Perkin Elmer, USA) as described previously (Naderi et al., 2017). Briefly, Se concentrations were directly measured in water samples treated with 0.2% (v/v) of concentrated nitric acid. Food, fish body, and eggs samples were digested with 1N nitric acid (1 to 5 mass (g): volume (ml) ratio) at 60 °C for 48 hrs. Subsequently, the digested samples were centrifuged at 15,000 *g* for 4 min and supernatants were collected for Se analysis. The concentrations of Se in eggs were measured on wet weight basis and converted to dry weight based on a moisture content of 90% as previously suggested (Thomas and Janz, 2014). Reagent blank and certified reference material (Dolt-4 dogfish liver; National Research Council of Canada) were

processed simultaneously to validate the Se measurement procedure used. The recovery rate of Se was found to be 96%.

6.2.4. Biochemical assays

Quantification of DA levels in the zebrafish brain (pools of 2-3 brains) was performed using an enzyme-linked immunosorbent assay kit following the manufacturer's instructions (Biovison, USA; detail in section 5.2.5, Chapter 5). To evaluate oxidative stress and antioxidant balance in the brain of zebrafish maternally exposed to Se, we measured the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) and the lipid peroxidation (LPO). For these measurements, 2-3 whole-brains were pooled together. The GSH:GSSG ratio was determined using a fluorometric method in the presence of *o*-phthalaldehyde as described previously (Naderi et al., 2018; Naderi et al., 2017). LPO was quantified using a commercially available kit following the manufacturer's protocol (Abcam, USA; see section 5.2.5, Chapter 5).

6.2.5. Quantitative real-time polymerase chain reaction

The mRNA expression of genes associated with DA receptors (DA receptor D1b [DRD1] and D2 [DRD2b, DRD2c, DRD3, DRD4a, DRD4b]) as well as genes involved in the DA synthesis (tyrosine hydroxylase1 [TH]), storage (vesicular monoamine transporter-2 [VMAT2]), re-uptake (dopamine transporter [DAT]), and metabolism (monoamine oxidase [MAO]) was evaluated. We also quantified the mRNA expression of brain-derived neurotrophic factor (BDNF) and early growth response 1 (EGR-1), which are involved in the modulation of neuronal growth, maturation, and plasticity. In addition, the transcription level of neuronal differentiation 1 (NEUROD 1) was also examined as an indicator of early neurogenesis in the zebrafish brain (Close et al., 2002; De Felice et al., 2014; Korzh et al., 1998). As described in our previous study (section 4.1, Chapter 4), the expression of dopaminergic cell markers and genes involved in synaptic plasticity and neurogenesis was assessed in the telencephalon of the brain (pools of 3, n = 5) (Naderi et al., 2017). This brain region was specifically chosen for its involvement in various forms of learning and memory in fish, including latent learning (Saito and Watanabe, 2006). Moreover, the aforementioned genes are mainly localized in the telencephalon (Maximino et al., 2016). Thus, measuring the expression of these genes in the telencephalon specifically provides a higher resolution relative to the whole-brain analysis. Moreover, to further evaluate the induction of oxidative stress in the zebrafish brain, we measured the expression level of genes encoding for

antioxidant enzymes, including copper/zinc superoxide dismutase (Cu/Zn-SOD), manganese superoxide dismutase (Mn-SOD), catalase (CAT), and glutathione peroxidase 1a (GPX1a) in the zebrafish whole-brain (pools of 2 brains, n = 5).

Total RNA was extracted from the telencephalon (pools of 3) or the whole-brain tissues (pools of 2) using the RNeasy Mini Kit (Qiagen, Germany), followed by a DNase treatment and verification by Nanodrop (NanoDrop, Thermo Scientific, USA). Subsequently, the cDNA was produced using a QuantiTect Reverse Transcription® kit (Qiagen, Germany). The gene encoding β-actin was used as the housekeeping gene. For each sample (n = 5), transcript levels of candidate genes and the reference gene were measured in triplicate in 20 µl reaction volumes on an iCycler Thermal Cycler (Bio-Rad, USA) using SYBR Green PCR Master Mix (SensiFAST, SYBR No-ROX Kit, Bioline, USA) as described previously (Naderi et al., 2017). The relative expression of target genes was calculated by the $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen, 2001). The sequences of primers have been reported elsewhere (see Table 5.1, Chapter 5; Naderi et al., 2018).

6.2.6. Statistical analysis

Data are expressed as mean ± the standard error of the mean (SEM), unless stated otherwise. The data were checked for normality and homogeneity of variance using the Kolmogorov−Smirnov one-sample test and Levene's test, respectively. Once data met the normal distribution and showed homogeneity of variance, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was employed to determine significant differences among treatment groups. In the case of heteroscedasticity, the Welch's test with the Games−Howell post hoc test was performed. If the data did not have equal variance and normal distribution, Kruskal-Wallis test with Dunn's posthoc test was performed. When data were percentages (percent viability, hatchability, and total deformities) they were normalized using an arcsine square root transformation. A Kaplan–Meier survival analysis with the log-rank test was carried out to determine differences in cumulative survival rates among different treatment groups. The alpha level was set at 0.05. However, the Bonferroni's correction was applied to minimize Type I error rate resulting from multiple comparisons, when appropriate.

6.3. Results

6.3.1. Selenium concentrations

The concentrations of total Se in water samples, diets (both in flake food and brine shrimp), and whole-body adult zebrafish are shown in Table 5.2, Chapter 5. Our results showed that maternal transfer of Se to eggs were proportional to the Se concentrations in diets fed to the female fish (Table 6.1). In other words, Se concentrations of 2.3 ± 0.6, 3.9 ± 0.7, 9.2 ± 1.4, and 24.2 ± 3.2 µg/g dry weight were found in eggs of adult zebrafish fed with diets containing 1.2 ± 0.2 (control food), 3.5 ± 0.3, 11.1 ± 0.9, and 27.4 ± 0.4 µg Se/g dw, respectively. The Se concentrations in eggs collected from adult female zebrafish fed with SeMet-spiked diets were significantly different relative to the control (Welch's test: $F_{3,\ 6.25} = 16.03$, $p < 0.001$). A significantly higher Se accumulation was observed in eggs collected from adult female fish treated with 11.1 ± 0.9 and 27.4 ± 0.4 µg Se/g dw diets (Games-Howell post hoc test: p=0.037 and p = 0.017).

6.3.2. Embryo viability, hatchability, larval deformities, and survival rate

Maternal exposure to different concentrations of dietary Se did not significantly affect percent egg viability (one-way ANOVA: $F_{3,\ 12} = 1.10$, $p = 0.385$; Table 6.1). However, the hatching rate significantly differed among treatment groups (one-way ANOVA: $F_{3,\ 12} = 7.08$, $p = 0.005$; Table 6.1). The percent hatch of eggs laid by fish treated with the highest concentration of Se was significantly less than that of controls (Tukey post hoc tests: $p = 0.045$). Maternal transfer of Se to eggs also resulted in larval deformities evaluated at 6 dpf (one-way ANOVA: $F_{3,\ 12} = 68.68$, $p < 0.001$; Table 6.1). The occurrence of larval deformities was higher in groups maternally exposed to 11.1 and 27.4 µg Se/g diets compared to the control (Tukey post hoc tests: both $p < 0.001$). The survival distributions of embryos/larvae (2-6 dpf) were also significantly different among treatment groups (Kaplan–Meier survival analysis: $X^2_3 = 143.55$, $p < 0.001$; Fig. 6.1). A higher mortality rate was observed in embryo/larval fish maternally exposed to 11.1 and 27.4 µg Se/g diet compared to the control (both $p < 0.001$).

Table 6.1. Total Se concentrations in eggs and percent egg viability (at 2 hpf), embryo hatchability (at 72 hpf), and total deformities (at 6 dpf) in F1-generation larval fish maternally exposed to increasing concentrations of SeMet via diet [a].

Nominal Se concentrations (µg/g dry weight)	Egg Se (µg/g dry weight)	Percent egg viability	Percent embryo hatchability	Percent total deformities [b]
Control	2.3 ± 0.6	85 ± 2.1	90.5 ± 1.1	3.1 ± 0.6
3	3.9 ± 0.7	84.7 ± 4.1	93.4 ± 1.7	5.8 ± 0.8
10	9.2 ± 1.4*	83.7 ± 1.6	88.3 ± 1.5	13.3 ± 0.9*
30	24.2 ± 3.2*	79.2 ± 1.1	82.5 ± 2.1*	23.8 ± 1.5*

[a] Data are mean ± SEM (n = 4 replicates of 80-100 pooled eggs for quantification of Se in eggs; n = 4 replicates of 100 eggs/larvae for quantification of mortalities and developmental toxicities in offspring).

[b] Larval deformities included skeletal, craniofacial and fin deformities, and edema.

* Significantly different from the control group using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

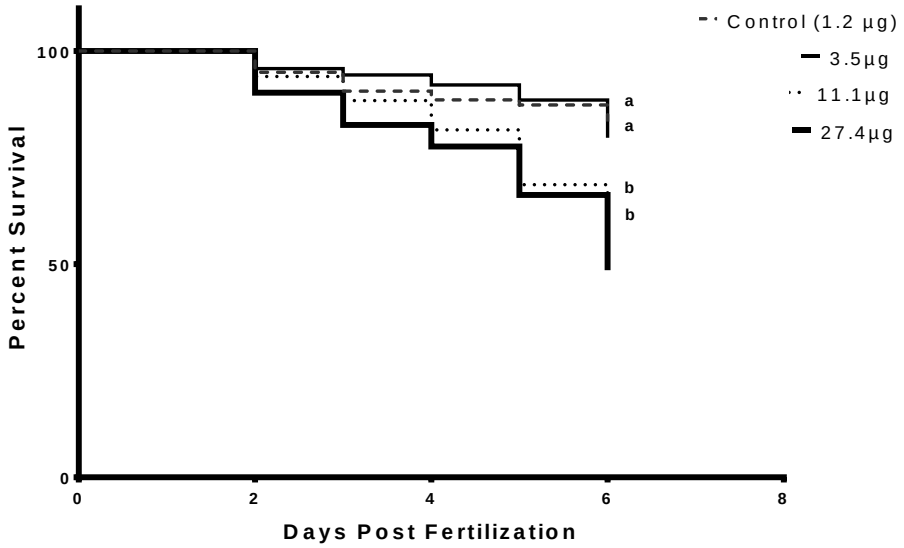

Figure 6.1. Kaplan-Meier survival curves of zebrafish larvae (2-6 dpf) maternally exposed to different concentrations of dietary Se. The survival curve of groups treated with 11.1 and 27.4 was significantly different compared to that of the control group (both $p < 0.001$). Letters on the graph denote significant differences from controls ($p < 0.008$). n = 4 replicates of 100 embryos.

6.3.3. Latent learning performance

Fig. 6.2 illustrates changes in parameters related to latent learning performance in F1-generation adult zebrafish. The latency to leave the start chamber differed significantly among treatment groups (Kruskal-Wallis test: $X^2_3 = 36.21$, $p < 0.001$). A prolonged latency to leave the start chamber

was observed in all Se-treated groups compared to controls (Dunn's post hoc tests: all $p < 0.001$; Fig. 6.2A). The amount of time that fish spent in the correct versus incorrect tunnel was also significantly different among groups (one-way ANOVA: $F_{3, 185} = 14.01$, $p < 0.001$; Fig. 6.2B). Fish in groups maternally treated with 3.5, 11.1, and 27.4 µg Se/g diets spent significantly less time in their training tunnel (Tukey post hoc tests: all $p < 0.001$). Moreover, the latency to enter the reward chamber was significantly affected in F1-generation adult zebrafish (Kruskal-Wallis test: $X^2_3 = 37.57$, $p < 0.001$; Fig. 6.2C). A prolonged latency to reach and enter the reward chamber was found in all groups maternally exposed to dietary Se compared to controls (Dunn's post hoc tests: all $p < 0.001$). A significant difference in the amount of time fish spent in the reward chamber was found among treatment groups (one-way ANOVA: $F_{3, 185} = 7.20$, $p < 0.001$). As represented in Fig. 6.2D, maternal exposure to all SeMet-spiked diets reduced the amount of time that fish spent in the reward chamber, which contained their conspecifics (Tukey post hoc tests: all $p < 0.009$). The locomotion (total distance traveled by the fish) was another parameter altered significantly among treatment groups (Kruskal-Wallis test: $X^2_3 = 17.45$, $p = 0.001$). However, as shown in Fig. 6.2E, the locomotor activity decreased in fish maternally exposed to 3.5 µg Se/g diet compared to fish maternally treated with 27.4 µg Se/g diet (Dunn's post hoc tests: $p < 0.001$). However, no statistically significant differences were found in Se treated groups when compared with the control group (Dunn's post hoc tests: all $p > 0.05$).

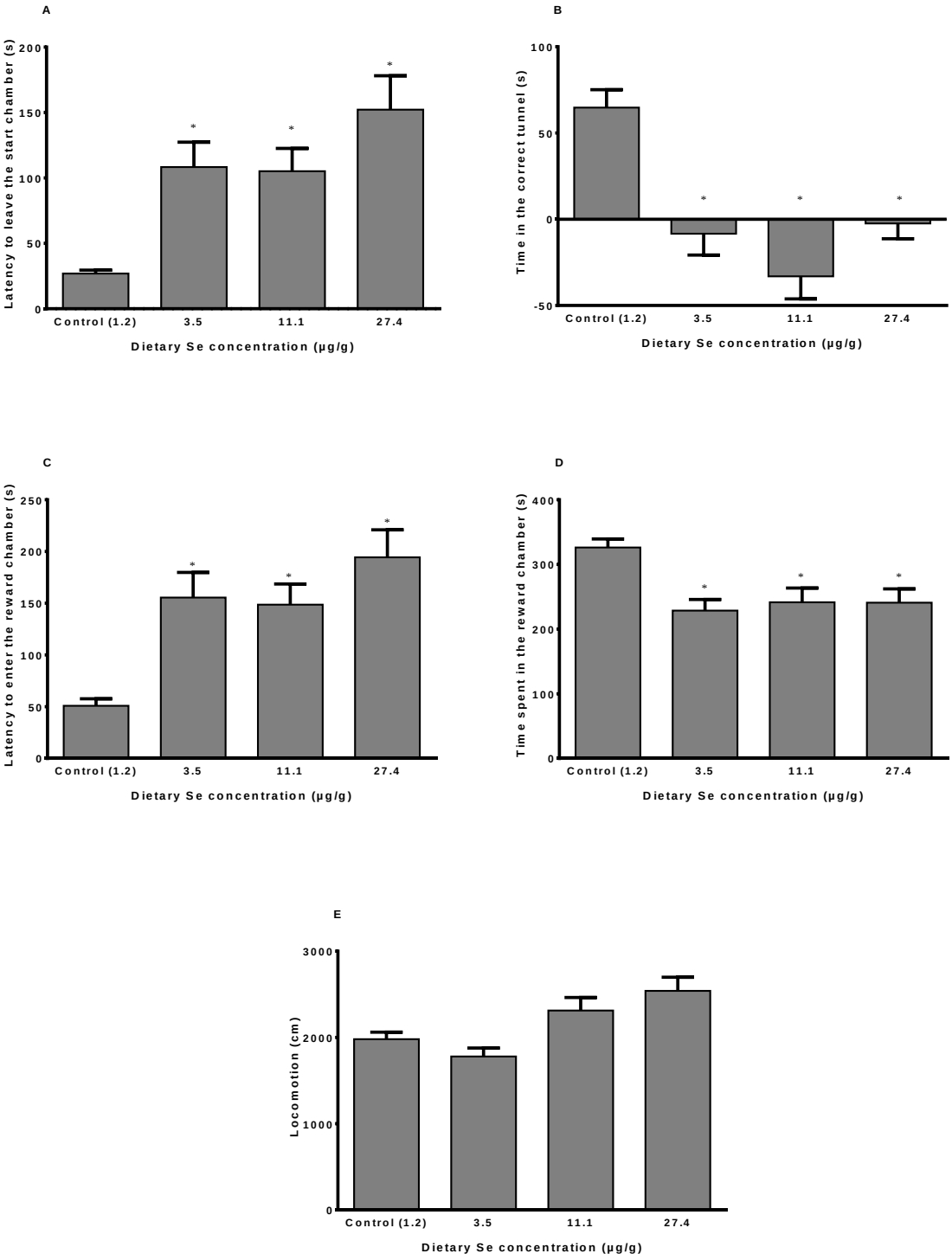

Figure 6.2. Latent learning performance of adult fish maternally exposed to different concentrations of dietary Se indicated by: the latency to leave the start chamber (A), difference between the time spent in the correct vs. incorrect tunnel, according to the training condition (B), the latency to enter the reward chamber (C), the time the fish spent in the reward chamber (D), and locomotion (E). Asterisks above data bars denote a significant difference relative to the control group at $p < 0.05$ ($n = 4$ replicates of 36-56 fish).

6.3.4. Dopaminergic cell markers in the brain

Maternal exposure to dietary Se induced a significant change in DA levels of the brain (one-way ANOVA: $F_{3, 12} = 33.18$, $p < 0.001$; Fig. 6.3A). An elevated level of DA was found in groups maternally exposed to 11.1 and 27.4 μg Se/g diets (Tukey post hoc tests: $p = 0.014$ and $p < 0.001$, respectively). Our results showed a significant change in the expression of TH gene (Kruskal-Wallis test: $X^2_3 = 14.95$, $p = 0.002$; Fig. 6.3B). This change was due to an up-regulation in the expression of this gene in groups maternally treated with the two highest concentrations of dietary Se (Dunn's post hoc tests: $p = 0.015$ and $p = 0.011$, respectively). Maternal exposure to Se also induced significant alterations in the transcription of VMAT2 (Welch's test: $F_{3, 7.95} = 14.91$, $p = 0.002$; Fig. 6.3C) and DAT (one-way ANOVA: $F_{3, 16} = 17.04$, $p = 0.002$; Fig. 6.3D). The elevated expression level of these genes was found in fish maternally exposed to the two highest concentrations of dietary Se (for VMAT, Games-Howell post hoc tests: $p = 0.019$ and $p < 0.001$; for DAT, Tukey post hoc tests: both $p < 0.001$). A notable change in the relative expression of MAO was also detected (one-way ANOVA: $F_{3, 16} = 30.76$, $p = 0.001$; Fig. 6.3E). The expression of MAO exhibited a marked up-regulation in all Se treated groups (Tukey post hoc tests: all $p < 0.007$).

Our results also showed a significant change in the expression level of DRD1 (Welch's test: $F_{3, 6.67} = 19.30$, $p < 0.001$; Fig. 6.4A). The expression of this gene increased significantly in groups that were maternally exposed to the two highest concentrations of dietary Se (Games-Howell post hoc tests: both $p < 0.006$). Similarly, the expression level of D2 receptor subtypes differed significantly among treatments. A significant alteration in the expression of DRD2b (one-way ANOVA: $F_{3, 16} = 4.62$, $p = 0.016$; Fig. 6.4B) and DRD2c (Welch's test: $F_{3, 7.05} = 50.17$, $p < 0.001$; Fig. 6.4C) was detected. The mRNA expression of DRD2b was up-regulated in fish maternally exposed to 11.1 and 27.4 μg Se/g diets (Tukey post hoc tests: both $p \leq 0.037$). For DRD2c, this up-regulation was observed in all Se treatment groups (Games-Howell post hoc tests: all $p < 0.038$). Moreover, the mRNA expression of the DRD3 gene also differed significantly among treatments (one-way ANOVA: $F_{3, 16} = 10.85$, $p < 0.001$; Fig. 6.4D), which resulted from the up-regulation of this gene in fish maternally fed with 11.1 and 27.4 μg Se/g diets (Tukey post hoc tests: $p = 0.002$ and $p = 0.01$, respectively). A significant alteration in the transcript level of DRD4a (Welch's test: $F_{3, 7.66} = 11.81$, $p = 0.003$; Fig. 6.4E) and DRD4b (one-way ANOVA: $F_{3, 16} = 7.87$, $p = 0.002$; Fig. 6.4F) was also found. The expression of DRD4a was up-regulated in groups maternally treated with the

two highest concentrations of dietary Se (Games-Howell post hoc tests: both $p < 0.028$). For DRD4b, however, this increase was found only in the group maternally exposed to 27.4 μg Se/g diet (Tukey post hoc test: $p = 0.003$).

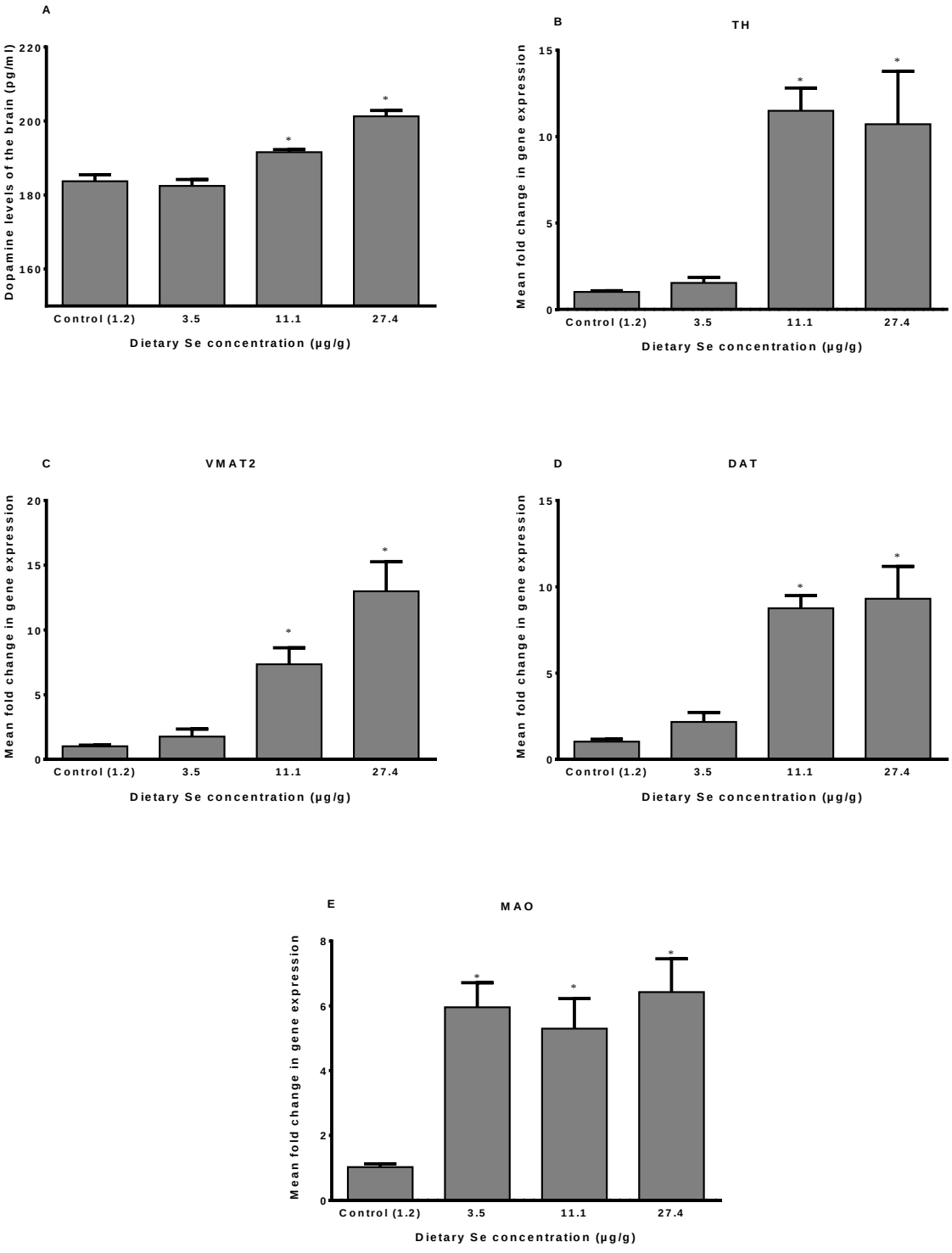

Figure 6.3. The effects of maternal exposure to dietary Se on DA levels of the brain (A), and the mRNA expression of TH (B), VMAT2 (C), DAT (D), and MAO (E) in the zebrafish telencephalon. Asterisks above data bars denote a significant difference relative to the control group at $p < 0.05$ ($n = 4$ for DA and $n = 5$ for genes).

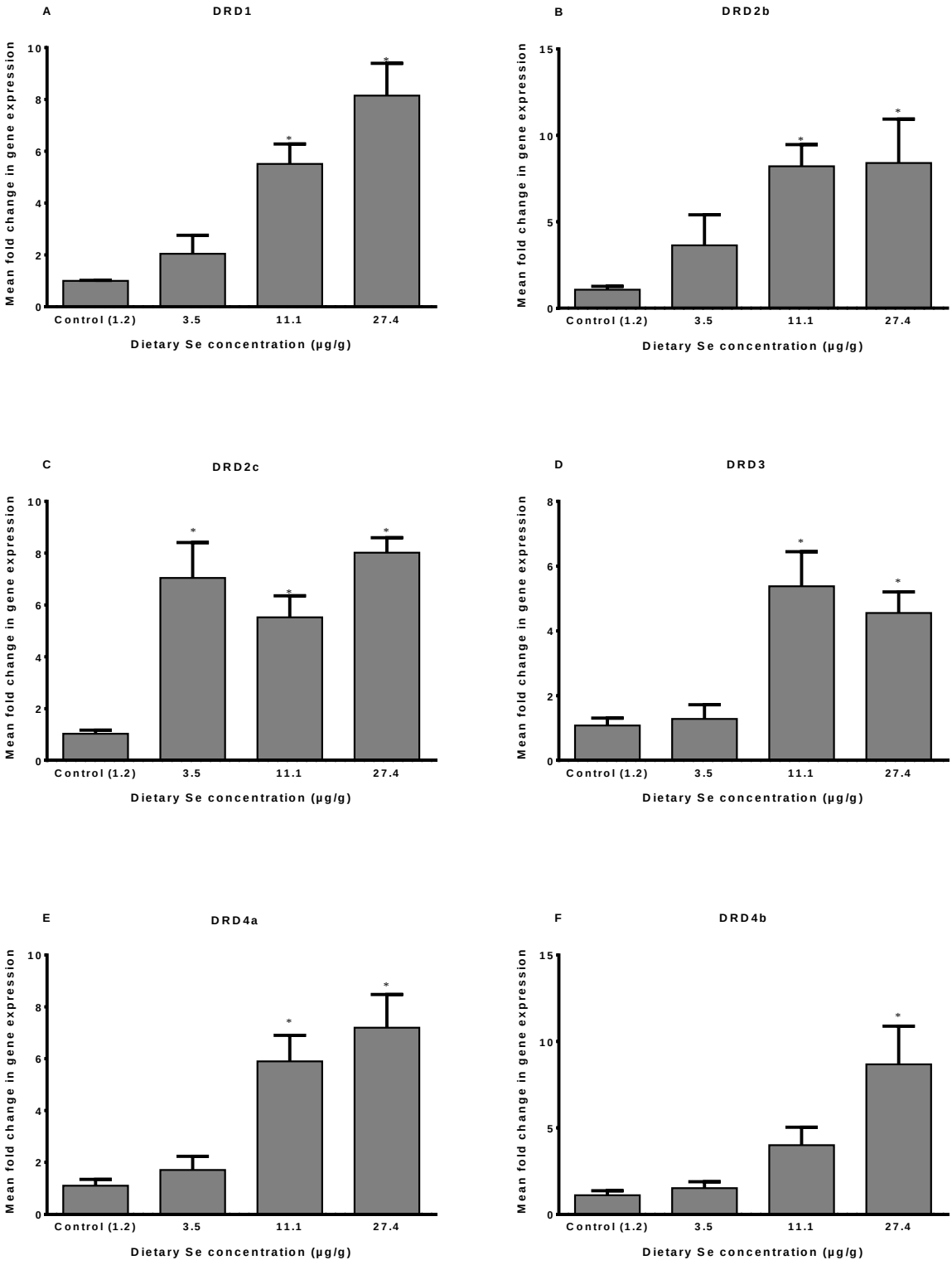

Figure 6.4. The mRNA abundance of DRD1 (A), DRD2b (B), DRD2c (C), DRD3 (D), DRD4a (E), and DRD4b (F) in the zebrafish telencephalon maternally exposed to dietary Se. Asterisks above data bars denote a significant difference relative to the control group at $p < 0.05$ ($n = 5$).

6.3.5. Markers of neural activity and development of the brain

Maternal exposure to dietary Se also changed the mRNA abundance of IEGs in F1-generation adult zebrafish. The BDNF gene showed a different level of expression among treatment groups (one-way ANOVA: $F_{3, 16} = 10.21$, $p = 0.001$; Fig. 6.5A). The enhanced mRNA expression of this gene was found in groups maternally exposed to 11.1 and 27.4 μg Se/g diets (Tukey post hoc tests: $p = 0.001$ and $p = 0.012$, respectively). Likewise, our results showed a significant change in the expression of EGR-1 (one-way ANOVA: $F_{3, 16} = 20.19$, $p < 0.001$; Fig. 6.5B). This gene was up-regulated in groups that were maternally treated with the two highest dietary Se concentrations (Tukey post hoc tests: both $p < 0.001$). Similarly, a significant difference in the mRNA abundance of NEUROD 1 (one-way ANOVA: $F_{3, 16} = 7.27$, $p = 0.003$; Fig. 6.5C) was recorded, due to the up-regulation of this gene in fish maternally treated with 11.1 and 27.4 μg Se/g diets (Tukey post hoc tests: both $p \leq 0.031$).

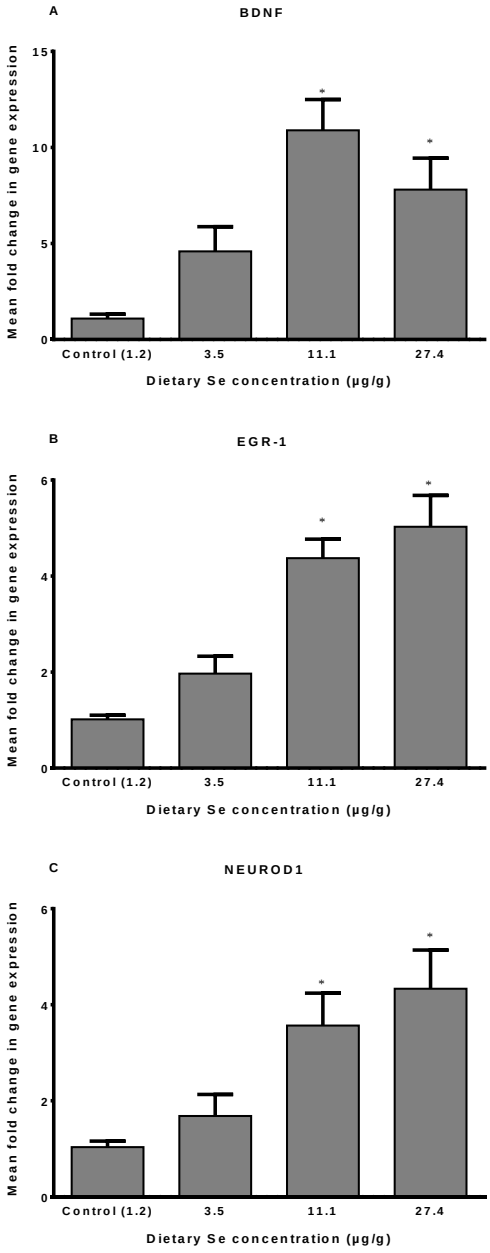

Figure 6.5. The mRNA abundance of BDNF (A), EGR-1 (B), and NEUROD 1 (C) in the zebrafish telencephalon maternally exposed to dietary Se. Asterisks above data bars denote a significant difference relative to the control group at $p < 0.05$ ($n = 5$).

6.3.6. Antioxidative responses in the brain

As shown in Fig. 6.6A, GSH:GSSG ratio in the zebrafish brain differed significantly among treatment groups (one-way ANOVA: $F_{3, 16} = 6.95$, $p = 0.006$). This ratio was significantly diminished in groups maternally exposed to 11.1 and 27.4 µg Se/g diets (Tukey post hoc tests: $p = 0.029$ and $p = 0.005$, respectively). Moreover, LPO content of the brain varied significantly among

treatment groups (one-way ANOVA: $F_{3, 16} = 62.04$, $p < 0.001$; Fig. 6.6B). This marker of oxidative damage showed a significant increase in groups maternally treated with the two highest dietary Se concentrations (Tukey post hoc tests: $p = 0.014$ and $p < 0.001$, respectively). In line with these biochemical alterations, the maternal exposure to Se also induced changes in the mRNA expression of several antioxidant genes. As Fig. 6.6 further depicts, a significant change in the expression of Cu/Zn-SOD (Kruskal-Wallis test: $X^2_3 = 14.29$, $p = 0.003$; Fig. 6.6C), Mn-SOD (Welch's test: $F_{3, 7.25} = 10.85$, $p < 0.001$; Fig. 6.6D), CAT (Welch's test: $F_{3, 7.45} = 11.24$, $p = 0.004$; Fig. 6.6E), and GPX1a (Welch's test: $F_{3, 6.86} = 35.93$, $p < 0.001$; Fig. 6.6F) was detected. The expression level of Cu/Zn-SOD, CAT, and GPX1a genes was higher in fish maternally exposed to 11.1 and 27.4 μg Se/g diets (all $p < 0.048$). The transcription level of Mn-SOD up-regulated only in the group maternally exposed to 27.4 μg Se/g diet (Games-Howell post hoc test: $p = 0.032$).

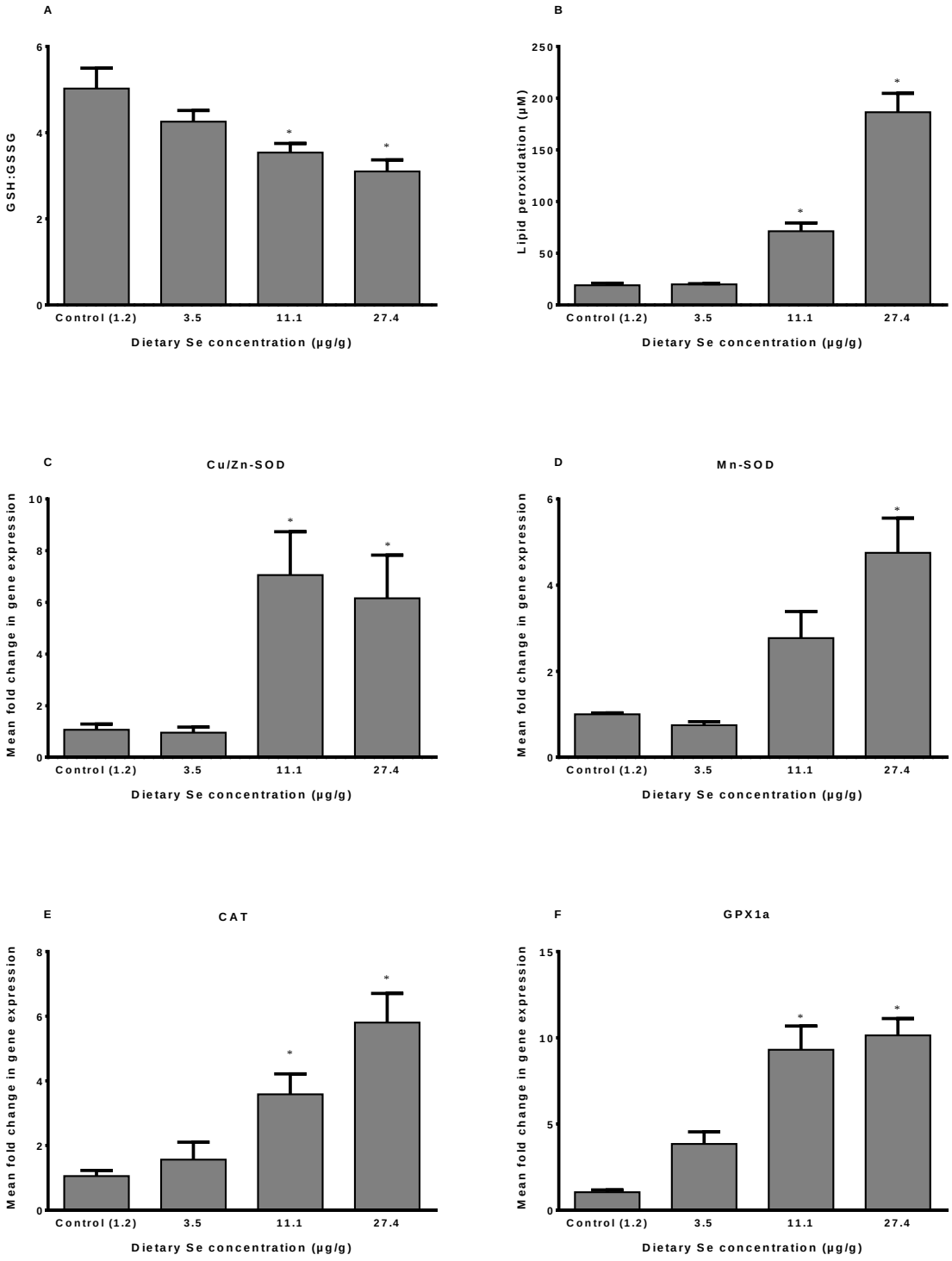

Figure 6.6. The GSH:GSSG ratio (A), LPO content of the brain (B), and the mRNA levels of Cu/Zn-SOD (C), Mn-SOD (D), CAT (E), and GPX1a (F) in the zebrafish brain maternally treated with different concentrations of dietary Se. Asterisks above data bars denote a significant difference relative to the control group at $p < 0.05$ ($n = 4$ for GSH:GSSG ratio and LPO levels; $n = 5$ for genes).

6.4. Discussion

The early maternal environment can be challenged by exposure to environmental contaminants or abnormal nutritional state. This is subsequently transmitted to the embryo/fetus and may lead to developmental abnormalities in the offspring that persist later into adulthood. In aquatic ecosystems, dietary intake and maternal transfer are two major exposure routes of Se to adult fish and their embryos, respectively (Janz et al., 2010). In this study, maternal exposure to dietary Se led to the accumulation of this element in zebrafish eggs, which consequently brought about developmental toxicities and neurobehavioural abnormalities in zebrafish offspring.

The concentrations of Se in zebrafish eggs recorded in our study were comparable to Se concentrations in fish eggs collected from Se contaminated natural waters (McDonald et al., 2010; Muscatello et al., 2006; Rudolph et al., 2008), suggesting that the dietary Se exposure concentrations and the exposure regime used in this study were environmentally relevant. As our results showed, the maternal transfer of Se to eggs did not affect the egg viability. However, there was a marked reduction in the number of hatching eggs in fish that were maternally exposed to the highest concentration of dietary Se. Furthermore, an increase in larval deformities (including skeletal, craniofacial, and fin deformities as well as edema) and larval mortality rates were observed in groups maternally exposed to 11.1 and 27.4 µg Se/g diets. Previous studies showed that maternal exposure to SeMet did not affect the viability of zebrafish embryos (Thomas and Janz, 2014, 2015). The alterations in hatchability, deformity, and survival rate were also consistent with previous studies in zebrafish and medaka (*Oryzias latipes*) exposed to SeMet (Chernick et al., 2016; Lavado et al., 2012; Thomas and Janz, 2016; Thomas and Janz, 2014). Free SeMet can be incorporated into eggs in place of methionine, which can consequently disrupt protein synthesis or functions, influencing embryo hatchability (Thomas and Janz, 2016). Metabolism of free SeMet for energy production may also result in the generation of ROS (Palace et al., 2004), causing a subsequent embryo mortality or reduced hatchability. SeMet can also impair skeletogenesis process in fish embryos. It has been established that embryonic exposure to SeMet via induction of oxidative stress and the unfolded protein response altered skeletal gene expression in medaka (Kupsco and Schlenk, 2016). Taken together, the elevated oxidative stress might account for reduced hatchability and increased larval deformities and mortalities in fish maternally treated with SeMet.

Learning plays a crucial role in fish for adapting to environmental change (Brown et al., 2008). Therefore, maternal exposure to Se in fish may compromise the ability of the offspring to survive and thrive in a challenging environment, by inducing learning impairment. Our results, for the first time, revealed that maternal exposure to SeMet impaired latent learning performance in F1-generation of adult zebrafish. It is important to note here that there were no apparent morphological differences (skeletal deformities) in fish among different treatments used in the learning trials of our study. Almost every fish, regardless of treatment group, left the start chamber and actively browsed the maze. However, fish that were maternally treated with SeMet showed a prolonged latency to leave the start chamber indicating a lower motivation to browse the maze or even less familiarity to the maze environment. These fish also spent markedly less time in their corresponding tunnels (according to their past training experience) indicating disturbed acquisition and/or consolidation of memory in F1-generation adult fish. The increased latency to enter the reward chamber may stand for impaired spatial memory in maternally treated fish. The length of time that fish spent in the reward chamber reduced markedly, further indicating the inability of fish to locate the reward chamber. It might also implicate lower social preference (grouping) in fish. In this study, fish with abnormal swimming performance or physical condition were excluded from training and probe phases of the experiment. Moreover, locomotion (distance traveled in the maze during probe) in fish maternally exposed to SeMet was unchanged in comparison to the control group. Thus, we argue that learning deficit observed in this study is not simply due to the physical condition of fish. In accordance with our findings, a previous study has highlighted that embryonic exposure (2-24 hpf) to SeMet resulted in spatial learning deficits in adult zebrafish (Smith et al., 2010). Collectively, it seems that developmental exposure to Se produces long-lasting effects on cognitive and social functioning in zebrafish.

DA innervation and its corresponding receptors are present early in development even prior to synaptogenesis, assembling and sculpting neural circuits involved in motor function, social behaviour, and cognition (Money and Stanwood, 2013). Mammalian studies have clearly demonstrated that abnormalities in the structure and function of the dopaminergic circuitry during early development contribute to a broad range of neurobehavioural disorders later in life (Robinson and Gradinaru, 2018). It has also been reported that early transient alterations in the dopaminergic system produced persistent alterations in brain function and behavioural abnormalities in adult zebrafish (Formella et al., 2012). The first appearance of dopaminergic neurons in the zebrafish

brain occurs between 15 and 18 hpf. Moreover, all DA receptors are strongly expressed in the zebrafish telencephalon by 5 dpf indicating the significant role of dopaminergic signalling in the development of this brain region (Boehmler et al., 2004; Souza and Tropepe, 2011). Notably, this period overlaps with yolk resorption in zebrafish (Jardine and Litvak, 2003). Therefore, resorption of Se-rich yolk during this window might have disrupted primary dopaminergic neurotransmission and alter brain structure and connectivity with enduring behavioural effects through adulthood.

Excessive subcortical dopaminergic activity with the increased DA release, synthesis, and storage are the characteristic features of neurodevelopmental disorders such as schizophrenia (Eyles et al., 2012). In this study, we also found an elevated level of DA in F1-generation of zebrafish maternally exposed to SeMet. This increase was accompanied by the up-regulation of TH, VMAT2, and DAT genes in the zebrafish telencephalon. TH is a critical enzyme in the synthesis of DA. Therefore, the elevated levels of DA might have occurred due to the up-regulation of this enzyme. The enhancement of DA synthesis requires increased vesicular storage capacity, which is aided by the enhanced VMAT2 expression. Accordingly, our data showed an increase in the transcription of VMAT2. In monoaminergic neurons, VMAT2 plays a neuroprotective role by providing a slightly acidic environment to stabilize oxidation-prone DA (Meiser et al., 2013). Besides mitigating DA toxicity, VMAT2 determines peak DA release from pre-synaptic neurons and hence regulates neurochemical output (Meiser et al., 2013). This means that the increased vesicular packaging can translate to the elevation of DA release and subsequently change DA-related functions, including learning and memory as proposed previously (Zubieta et al., 2000). Alternatively, the up-regulation of VMAT2 could also be due to an increase in DA re-uptake by DAT. Our results showed a marked increase in the expression of DAT in groups maternally treated with 11.1 and 27.4 μg Se/g diets, lending support to this notion. Indeed, the elevated mRNA levels of DAT may be a response to increased extracellular DA levels. Consistent with these findings, we have previously found that dietary exposure to SeMet led to a concomitant increase in DA levels of the brain and the mRNA expression of TH and DAT in adult zebrafish (Naderi et al., 2018; Naderi et al., 2017). However, in contrast to the findings of the present study, we previously observed that Se reduced transcript abundance of VMAT2 in the adult zebrafish brain (Naderi et al., 2018). The reason for this apparent discrepancy may lie in differences in exposure regimes (adult vs maternal exposure) between these studies. In fact, the up-regulation of VMAT2 might be a compensatory mechanism to minimize DA-induced oxidative stress during development. MAO is a mitochondrial enzyme that catalyzes

the oxidative deamination of monoamine neurotransmitters, including DA, norepinephrine, and serotonin, producing hydrogen peroxide (H_2O_2) as a by-product (Meiser et al., 2013). In the present study, a substantial increase in the transcription of MAO was found in all maternally-transferred Se treatment groups. This is in contradiction with our previous studies in which dietary Se did not affect the mRNA abundance of MAO in the adult zebrafish brain (Naderi et al., 2018; Naderi et al., 2017). A possible explanation for this disparity may derive from life cycle-dependent response of the dopaminergic system to Se neurotoxicity. On the other hand, this increase might be in response to the elevated levels of DA in the zebrafish brain. However, it should be noted that MAO in zebrafish plays a more pronounced role in the catabolism of other monoamines such as serotonin than DA metabolism (Anichtchik et al., 2006). Therefore, we speculate that maternal exposure to dietary Se may provoke alterations in other monoaminergic neurotransmitter systems in the zebrafish brain. This is quite conceivable since the expression of MAO was one of the few genes examined in this study that changed in the group maternally treated with the lowest concentration of Se (3.5 μg Se/g), the same fish that showed a marked learning impairment. Further studies are needed to elucidate the maternal effects of dietary Se on other neurotransmitter systems.

The increase in DA synthesis and turn-over may alter the transcription of DA receptors as indicated by our results. There was a marked increase in the expression of DRD1 and all DRD2 subtypes, mostly attributable to the groups maternally fed with 11.1 and 27.4 μg Se/g diets. Similar Se concentrations have been shown to cause an up-regulation of both DRD1 and DRD2 receptors in adult zebrafish (Naderi et al., 2018). However, we have previously reported that 30 days exposure to dietary Se (32.5 and 57.5 μg/g) in adult zebrafish decreased the telencephalic expression of DRD1 receptors, while increasing the expression level of distinct D2 receptors (DRD2b and DRD4a) (Naderi et al., 2017). Our present study reinforces our previous findings that alteration in the expression of DA receptors is an inevitable consequence of exposure to dietary SeMet. We have previously established that D2 receptors play a leading role in latent learning in zebrafish. We demonstrated that D2 receptor agonist quinpirole impaired latent learning in zebrafish, while antagonism of this receptor family improved their learning performance (Naderi et al., 2016b). Hence, the abnormal increase in the transcriptional activity of D2 receptors might be a plausible explanation for the latent learning deficit found in F1-generation adult zebrafish maternally exposed to SeMet. Rodent studies have repeatedly shown that developmental up-regulation of D2 receptors can cause persistent cognitive deficits in adults (reviewed in Kellendonk, 2009).

Moreover, overexpression of D2 receptors through the change in efficiency of the cortico-striatal synapses involved in learning and memory, as well as via an increase in the activity of D1 receptors led to cognitive impairment in mice (Kellendonk et al., 2006). It is also noteworthy that DRD2c is the only altered DA receptor in fish maternally treated with the lowest concentration of Se (3.5 μg Se/g). Similarly, we previously found that this receptor subtype was up-regulated in adult fish exposed to the similar concentration of Se (Naderi et al., 2018). This may imply that DRD2c as an autoreceptor is activated in lower concentrations of DA to control DA release. D2 receptors can also modulate social and reward-seeking behaviours. Overexpression of this receptor family has been implicated in social deficits and reduced effortful behaviour to obtain rewards in mice (Kabitzke et al., 2015; Ward et al., 2012). Our previous studies also showed that dietary exposure to Se reduced shoaling preference used as the reward in associative and latent learning tasks. The common thread between these studies was the up-regulation of D2 receptors (Naderi et al., 2018; Naderi et al., 2017). Consequently, the telencephalic up-regulation of D2 receptors might account for learning impairment and social deficit in zebrafish maternally exposed to Se.

Our results revealed an elevated level of mRNA transcripts encoding BDNF and EGR-1 in the zebrafish telencephalon maternally treated with the two highest concentrations of dietary Se (11.1 and 27.4 μg Se/g). IEGs are a heterogeneous group of genes in the CNS that respond rapidly to a spectrum of extracellular stimuli and participate in diverse functions (Bahrami and Drabløs, 2016). BDNF and EGR-1 are well-known IEGs that act as transcription factors to coordinate the activation and repression of target genes in the developing CNS leading to long-term functional changes in neurons (Close et al., 2002; De Felice et al., 2014). Primary alterations in the activity of these genes can bring about inappropriate changes in cortical circuitry and synaptic transmission in the developing brain, which subsequently translates into the neural dysfunction (Gallo et al., 2018). The increase in BDNF levels has been detected in the cortical areas and hippocampus of schizophrenic humans (Durany et al., 2001; Takahashi et al., 2000). Interestingly, there is a positive relationship between the functions of DA and BDNF (Iwakura et al., 2008). A previous study has also shown that stimulation of DA receptors increased the production of BDNF, which in turn enhanced morphological maturation and differentiation of striatal neurons in the mice brain (Hasbi et al., 2009). Therefore, maternal exposure to SeMet, via perturbation of this pathway, may undermine neuronal morphology and network development and cause learning impairment in zebrafish. In addition to IEGs, we found an increase in the telencephalic mRNA expression of

NEUROD1. This gene is a member of the basic helix-loop-helix transcription factors involved in primary neurogenesis in the zebrafish brain (Korzh et al., 1998). Primary neurogenesis is a highly regulated process in zebrafish (Schmidt et al., 2013). Therefore, any interference with this process may result in an atypical architecture of the brain and abnormal neural patterning and wiring lasting throughout the life. As a result, we propose that maternal exposure to Se may cause an aberrant increase in the expression of neurogenic and synaptogenic factors and thus impairs the process of shaping and fine-tuning of neural circuits involved in learning and memory in zebrafish.

The high oxidative load is a signature of dopaminergic neurons. It is well known that both enzymatic and non-enzymatic oxidation of DA generates ROS such as hydroxyl radical (•OH) and superoxide anion ($O2^{\bullet-}$) (Meiser et al., 2013). In this study, we found an elevated level of oxidative stress in F1-generation zebrafish brain maternally treated with 11.1 and 27.4 μg Se/g diets. This increase was evident by a reduction in GSH:GSSG ratio. In the brain, GSH is the main intracellular antioxidant that plays a central role in the protection against DA-induced toxicity (Grima et al., 2003). DA oxidation combined with a depletion of GSH can lead to increased LPO with concomitant changes in membrane permeability and cell damage (Grima et al., 2003). In line with this, we found an increase in LPO content of the brain in groups that also showed low GSH:GSSG ratio. Besides its antioxidative activity, GSH regulates a myriad of redox-sensitive molecules implicated in neurotransmission and synaptic plasticity (Esposito et al., 2004). Specifically, GSH deficit in the brain alters intracellular pathways implicated in DA signalling (Steullet et al., 2008). Thus, it is possible that a reduction in GSH levels altered DA signalling in the developing brain of zebrafish maternally exposed to dietary SeMet. The enhanced oxidative stress in this study was further confirmed by the up-regulation of antioxidant enzyme genes, including Mn-SOD, Cu/Zn-SOD, CAT, and GPX1a in fish maternally treated with 11.1 and 27.4 μg Se/g diets. Since SOD is directly related to dismutation of $O2^{\bullet-}$ to H_2O_2 (Halliwell and Gutteridge, 2015), the up-regulation of SOD expression in this study may reflect an adaptive mechanism against DA-induced superoxide radicals. The other antioxidative enzymes, GPX1a and CAT, work in concert with SOD to decompose H_2O_2 into the water (Halliwell and Gutteridge, 2015). The up-regulation of GPX1a and CAT may also represent a compensatory mechanism to detoxify H_2O_2 generated by DA oxidation. ROS can interfere with signal transduction pathways, cause DNA damage, and bring about the modification of gene expression. The over-production of free radicals can also alter neuronal functions and brain morphology in fish (Halliwell, 2006; Hensley et al., 2000). Moreover,

enhanced oxidative stress in the brain can impair different forms of learning and memory in zebrafish (Ruhl et al., 2016). Therefore, it is possible that DA-induced oxidative stress via disturbances of the brain structures involved in learning and memory impaired the ability to generate a latent memory in zebrafish maternally exposed to Se. It should also be noted that induced oxidative stress and lipid peroxidation in the zebrafish brain cannot be solely attributed to Se-induced increase in DA levels. Exposure to Se may cause alterations in the homeostasis of other neurotransmitters systems, which can produce ROS. This possibility warrants further examination in future studies.

6.5. Conclusion

In summary, our findings are the first to demonstrate that maternal exposure to environmentally relevant concentrations of dietary Se can impair latent learning in F1-generation adult fish. The incidental learning about the important landmarks/stimuli of the environment enables animals to demonstrate an efficient, goal-directed, and error-less navigation in their natural environment. Therefore, the transgenerational impairment of latent learning in fish inhibiting Se-contaminated ecosystems may alter the spatial orientation and navigation of individuals and consequently compromise their ability to appropriately respond to their environmental needs.

Chapter 7: General discussion

7.1. Introduction

The overall objective of this study was to investigate the direct and transgenerational effects of dietary SeMet on learning and memory in zebrafish as the model organism with a focus on alterations in DA neurotransmission in the brain. While DA is well-known to play an important role in social behaviour and motor control in zebrafish, its role in the regulation of learning and memory is yet to be determined. Since mammalian studies have shown that DA regulates both reinforced and unreinforced learning tasks (Seel, 2011), two different learning paradigms were used to determine the role of DA in learning and memory in zebrafish. Using dopaminergic drugs in experiments 1 and 2 (Chapter 2 and 3), I examined the role of DA receptors in the regulation of latent learning and associative learning (unreinforced and reinforced learning tasks, respectively) in adult zebrafish. In experiments 3 and 4 (Chapter 4 and 5), I investigated the effects of dietary exposure to SeMet on the dopaminergic system and its consequences in these two forms of learning in zebrafish. Since DA plays a fundamental role in the brain development in zebrafish and due to the fact that the maternal transfer is the major exposure pathway of Se to larval and juvenile fish, in experiment 5 (Chapter 6), I also investigated the effects of maternal exposure to dietary SeMet on the dopaminergic system and latent learning in F1-generation of zebrafish.

7.2. Differential roles of dopamine receptors in the regulation of latent learning and associative learning in adult zebrafish

The purpose of these experiments was to investigate the role of DA in two different forms of learning in zebrafish by pharmacological manipulation of DA receptors. The results of these two studies demonstrate a significant and task-dependant role of DA receptors in learning and memory in zebrafish. In experiment 1 (Chapter 2), the activation of both D1 and D2 receptors impaired the acquisition and consolidation of latent learning. However, antagonism of D2 receptors, but not D1 receptors improved both phases of learning in zebrafish. These results indicate that D2 receptors contribute significantly to the dopaminergic modulation of latent learning in zebrafish. This might

be due to the prominent role of D2 receptors in the regulation of selective attention, a process which is known to modulate the latent learning process by optimizing or enhancing the selection of information needed for further processing. Although latent learning occurs in the absence of the reward, the novelty of the maze environment *per se* can act as a reinforcer. DA is considered as a primary neurotransmitter modulator of novelty-seeking (Costa et al., 2014) and high novelty responsive animals possess higher DA levels in the nucleus accumbens and striatum (correspond to sub-pallium in zebrafish) than low novelty responders (Tournier et al., 2013; Zald et al., 2008). Therefore, memory enhancing effects of D2 receptor blockade might be due to the decreased inhibitory autoreceptor control of DA producing neurons in the zebrafish brain.

While D2 receptors are critically involved in the acquisition and consolidation of latent learning, the results of experiment 2 (Chapter 3) show that D1 receptors play a greater role in associative learning in zebrafish. In fact, the stimulation of D1 receptors improved both acquisition and consolidation of associative learning. The phasic DA release and subsequent activation of D1 receptors are necessary to encode the acquisition of cue-reward association (Ford, 2014; Grace, 1991). Therefore, in the present study, it seems that the stimulation of D1 receptors has potentiated the phasic DA signal during associative learning. D2 receptors can also be involved in these phases of associative learning. However, the results suggest that the effects of D2 receptors might be mediated through their interaction with D1 receptors. Indeed, it is possible that the activation or inactivation of D2 receptors via the change in tonic DA availability affects the phasic DA signal required for associative learning. However, D2 receptors appear to be more critically involved in the regulation of memory retrieval in zebrafish. This is due to the fact that that activation and inhibition of this receptor family improved and impaired, respectively, associative learning performance in zebrafish.

7.3. Effects of chronic exposure to dietary selenomethionine on dopamine neurotransmission and learning and memory in zebrafish

There is a growing body of evidence that oxidative stress is the main mechanism of Se (including SeMet) toxicity. This factor can be particularly detrimental to the dopaminergic system. Therefore, experiments 3 and 4 sought to uncover whether exposure to dietary SeMet via induction of oxidative stress disrupts the normal functions of the dopaminergic system leading to learning impairment in zebrafish. For this purpose, fish were exposed to either a control diet or SeMet-

spiked diets (3, 10, 30, and 60 μg/g dw; nominal concentrations). The results of these studies showed that chronic exposure to dietary SeMet impairs both latent learning and associative learning in adult zebrafish. In both experiments, an increased oxidative stress was observed in the zebrafish brain treated with the two highest Se exposure concentrations used indicating Se in excess can generate ROS. The induced oxidative stress through a myriad of pathways may lead to learning impairment (Dröge and Schipper, 2007; Praticò et al., 2002). The results of these experiments revealed that the dysfunction of the dopaminergic system is one of the main pathological characteristics of Se-induced oxidative stress in zebrafish. The up-regulated expression of TH and DAT genes involved in DA synthesis and re-uptake was the common manifestation of dopaminergic dysfunction in Se-treated fish exhibiting impaired latent learning and associative learning performance. As confirmed in experiment 4 (Chapter 5), the up-regulated expression of these genes was associated with elevated DA levels in the zebrafish brain. This indicates that Se primarily perturbs the homeostasis of the dopaminergic system via stimulation of DA synthesis and turn-over. Transcriptional, translational, and post-translational levels of TH are finely tuned by several regulatory systems. For instance, TH gene expression and subsequent translation are strictly regulated by Ca^{2+}, neuronal activity, oxygen levels, and stress. Phosphorylation and dephosphorylation of TH are important post-translational regulatory mechanisms of the enzymatic activity which determine the amount of catecholamine synthesized in the dopaminergic system (Tekin et al., 2014). Substantial evidence exists supporting that oxidative stress can alter the TH gene expression and activity via interference with these processes (Di Giovanni et al., 2012). Therefore, it is possible that SeMet via induction of oxidative stress increases the TH expression and activity in the zebrafish brain. Moreover, GSH is involved in the cytosolic storage of DA (Drukarch et al., 1996). Since the results of experiment 4 showed, dietary exposure to SeMet reduces the mRNA expression of VMAT2 indicating a reduction in cytosolic storage of DA in adult zebrafish. The reduced DA storage can increase both TH and DAT mRNA levels in order to maintain the intracellular pool of DA (Romero-Ramos et al., 2000). Therefore, Se may lead to a decrease in DA storage through depletion of GSH, which could bring a plausible answer for the subsequent increase in DA synthesis and turn-over in the adult zebrafish brain. The accumulation of DA in the cytosol is itself known as a source of oxidative stress and neurotoxicity (Meiser et al., 2013). Since in both studies the mRNA levels of MAO remained unchanged, the non-enzymatic

oxidation (autoxidation) of cytosolic DA may lead to the generation of ROS further exacerbating Se-induced oxidative stress in the adult zebrafish brain.

Changes in DA levels of the brain were also associated with alterations in the mRNA expression of DA receptors. However, the results were to some extent different in latent learning and associative learning experiments. An increase and decrease in the transcript levels of D1 and D2 receptors, respectively, were detected in fish treated with high concentrations of Se (32.5 and 57.5 µg/g Se). Nonetheless, an up-regulation in the expression of both DA receptor families was found in the zebrafish brain with associative learning impairment. This discrepancy may stem from different exposure regimes and learning paradigms used in these experiments. As it has been established in experiment 1 (Chapter 2), the activation of D2 receptors has disruptive effects on latent learning. Therefore, it seems that exposure to dietary SeMet leads to latent learning impairment in zebrafish through an increase in the transcriptional activity of D2 receptors. Moreover, the results of experiment 2 (Chapter 3) indicate that D1 receptors are primarily involved in the regulation of associative learning (the acquisition and consolidation phases), while the effects of D2 receptors are mainly mediated through their interaction with D1 receptors. Therefore, it is possible that the up-regulation of D2 receptors leads to associative learning impairment in zebrafish via an imbalance in the activation of D1 receptors. Indeed, the increased expression of D2 receptors indicates high levels of tonic DA at post-synaptic receptors (D1 in particular), which consequently reduces the phasic DA signalling required for coding of the rewarding stimulus. Furthermore, the opposite roles of DA receptors in synaptic plasticity cannot be ruled out. D1 receptors facilitate the formation of LTP while the activation of D2 receptors inhibits the expression of this process in learning-critical brain structures. Alterations in the mRNA expression of IEGs further confirmed a change in synaptic plasticity in the zebrafish. Interestingly, a bi-phasic pattern of response was found. In both experiments 3 and 4, the exposure to low concentrations of dietary SeMet (2.3 and 3.5 µg/g Se) increased the mRNA abundance of IEGs (including BDNF, EGR-1, and NPAS4) in the zebrafish brain. However, higher concentrations of Se (experiment 3: 32.5 and 57.5 µg/g Se; experiment 4: 27.4 and 63.4 µg/g Se) suppressed the expression of these genes as well as down-regulated the transcript levels of NEUROD 1, a gene which is involved in neurogenesis in the zebrafish brain. This might be due to the disruptive effects of Se-induced oxidative stress on neuronal structures and functions and intracellular signalling pathways involved in synaptic plasticity. These findings shed more light on different facets of Se, from essentiality to

neurotoxicity. Overall, the results of experiment 3 and 4 revealed that chronic exposure to dietary SeMet impairs two different forms of learning and memory in zebrafish. Induction of oxidative stress and dysfunction of the dopaminergic system are two mechanisms by which Se exerts its neurotoxic effects. However, the effects of this toxic trace element on other neurotransmitter systems cannot be ruled out.

7.4. Transgenerational effects of dietary exposure to SeMet on learning and memory in zebrafish offspring

The maternal transfer is the main pathway of Se exposure to fish embryo/larvae which is commonly manifested as a suit of developmental toxicities in early life stages of F1-generation of fishes (Janz, 2012). It has also been documented that early life exposure to SeMet (from 2 to 24 hpf) causes enduring learning deficits in adult zebrafish (Smith et al., 2010). DA and its corresponding receptors appear early during brain development in zebrafish and are believed to play an important role in the neuronal development (Formella et al., 2012; Souza and Tropepe, 2011). Therefore, alterations in DA innervation patterns and receptor expression during early development may produce long-lasting changes in DA-related neurological functions including learning and memory. Experiment 5 sought to assess whether maternal exposure to dietary SeMet via changes in dopaminergic signalling would result in a learning deficit in F1-generation adult zebrafish. The results showed that adult fish maternally treated with low, medium, and high concentrations of dietary SeMet exhibit a marked latent learning impairment. The elevated levels of DA were found in the fish brain maternally treated with 11.1 and 27.4 µg/g Se diets, which was accompanied by the up-regulation of TH and DAT genes. These results, in addition to the findings of experiments 3 and 4, suggest that hyperfunction of the dopaminergic system is an inevitable consequence of zebrafish exposure to excess dietary SeMet. However, maternal exposure to SeMet caused an up-regulation in the expression of VMAT2 and MAO genes, which was in contrast with the findings of experiments 3 and 4 where a down-regulation in the expression of VMAT2 (experiment 4) and an unchanged mRNA expression pattern of MAO (experiments 3 and 4) were observed. The reason for these discrepancies may reside in differences in exposure regimes (adult vs maternal exposure) between these studies. In addition to DA, VMAT2 and MAO are also involved in the sequestration and metabolism of other monoamine neurotransmitters such as serotonin (Maximino et al., 2016). Notably, the up-regulation in the expression of MAO was detected in fish maternally treated with the lowest concentrations of dietary SeMet (3.5 µg/g Se). This was one of two genes (along with

DRD2c) that changed in this treatment group, which was associated with a marked learning impairment. Thus, it is feasible to suggest that maternal exposure to Se triggers changes in neurotransmitter systems other than DA resulting in long-lasting cognitive impairment in zebrafish offspring. An up-regulation in the expression of different DA receptor subtypes was found in all treatment groups. As shown in experiment 1 (Chapter 2), a concomitant activation of D1 and D2 receptors using apomorphine impairs latent learning performance in zebrafish. This disruptive effect was in large part due to the activation of D2 receptors. Therefore, the impaired latent learning observed in fish maternally exposed to dietary SeMet could be attributed to the increased expression of DA receptors, D2 receptors in particular. In addition to changes in the expression of DA receptors, hyperfunctioning of the dopaminergic system in fish maternally treated with 11.1 and 27.4 µg/g Se diets led to elevated levels of oxidative stress in the zebrafish brain, which was evident by a decreased GSH:GSSG ratio, increased LPO levels, and increased mRNA expression of antioxidant enzymes. The increased oxidative stress may have resulted from the generation of ROS by enzymatic and/or non-enzymatic degradation of DA further resulting in learning impairment in zebrafish. Maternal exposure to 11.1 and 27.4 µg/g Se diets also led to an up-regulation in the expression of IEGs including BDNF and EGR-1 as well as the expression of NEUROD 1 gene in the zebrafish brain. These results are in sharp contrast with those of experiments 3 and 4 indicating that developmental exposure to dietary SeMet hampers synaptic refinement and the normal brain development in zebrafish via stimulatory effects on synaptogenic and neurogenic factors. The mechanism(s) underlying transgenerational effects of Se remains to be fully understood. However, it is possible that Se-induced oxidative stress during early life stages of zebrafish by dysregulation of signal transduction and/or macromolecular damage (e.g. lipids, proteins, and DNA) contributes to permanent neurobehavioural abnormalities. This is highly likely given that oxidative stress can alter DA signalling in the CNS (Steullet et al., 2008). Further studies are required to elucidate the neurodevelopmental effects of dietary exposure to SeMet.

7.5. Conclusion and environmental implications

The research presented in this thesis suggests that environmentally relevant dietary SeMet exposure can lead to learning deficits in adult fishes and their offspring. Latent learning and associative learning are two main components of fish cognition in the natural environment, which strongly linked to a wide array of fish behaviours including foraging activities, mate choice strategies, anti-predatory behaviours, and social interactions. Therefore, the learning impairment in fish inhibiting

Se-contaminated ecosystems may alter fish responses to a broad range of environmental stimuli and subsequently compromises fish health and survival.

Learning and memory reflect multiple neurological, physiological, and behavioural changes and links individual- to population-level processes, thereby representing favorable endpoints for assessing organismal and ecological effects of environmental contaminants. Moreover, the results presented in my thesis indicate that these cognitive functions are susceptible even to relatively low exposure concentrations of dietary Se, and therefore warrant further attention as tools for assessing the neurotoxicological effects of environmental contaminants. Moreover, the dysfunction of the dopaminergic system, enhanced oxidative stress, and impaired learning performance are the main characteristics of several neuropsychiatric and neurodevelopmental disorders. Therefore, my research offers new insights into the potential of the zebrafish model to explore the possible role of environmental contaminants such as Se in the development of brain disorders.

7.6. Future research perspectives and recommendations

Se is a priority aquatic contaminant in aquatic ecosystems, which can be extremely toxic to aquatic organisms including fish. However, most of our knowledge about Se toxicity comes from its reproductive and developmental effects. My research has provided novel and important mechanistic information on the neurobehavioural effects of this toxic trace element. Nonetheless, some aspects of my research could be expanded to provide a more comprehensive understanding of SeMet neurotoxicity in fish. Areas that require future research attention are listed below.

- In experiments 1 and 2, I demonstrated that activation and/or inactivation of DA receptors affect different phases of latent learning and associative learning in zebrafish. Future studies should investigate intracellular pathways underlying DA receptor-mediated effects on the acquisition, consolidation, and recall of these forms of learning and memory in zebrafish. Moreover, the role of other neurotransmitter pathways (e.g., serotonergic system and GABAergic system) in the modulation of these cognitive functions can be investigated.
- In experiments 3 and 4, I demonstrated that excess dietary SeMet exposure can cause latent and associative learning impairment in adult zebrafish mainly via induction of oxidative stress and dysfunction of the dopaminergic system. Future investigations can further explore the effects of this trace element on other neurotransmitter pathways relevant to learning and memory. For example, the glutamatergic system is known to be involved in

learning and memory in zebrafish (Sison and Gerlai, 2011). I found that SeMet changes the expression of BDNF in the zebrafish brain. BDNF enhances excitatory synaptic transmission through the regulation of glutamate release (Martin and Finsterwald, 2011). Therefore, it can be addressed whether SeMet would interfere with this neurotransmitter system and lead to learning deficits in the zebrafish. Moreover, the results of experiment 3 and 5 revealed that dietary exposure to SeMet may reduce the social preference in zebrafish. This was evident by the lower time that SeMet-treated fish spent in the reward chamber. DA plays a critical role in the regulation of social behaviours including social preference, shoaling, and aggressive behaviour (Scerbina et al., 2012; Shams et al., 2018; Teles et al., 2013). It will be interesting to examine the effects of dietary SeMet on different social behaviours such as social preference, shoaling, and aggressive interaction in adult zebrafish using specific behavioural paradigms.

- In experiment 5, I established that maternal exposure to dietary SeMet impairs the dopaminergic system and consequently leads to learning impairment in F1-generation of zebrafish. Future studies can investigate how DA intervention and activation of its receptors influence the development of brain structures and/or neural circuits involved in learning and memory in zebrafish. Moreover, while a dopaminergic hyperfunction was found in zebrafish offspring maternally exposed to SeMet, the mechanism(s) underlying this phenomenon is not clear. Therefore, more research is needed to uncover factors that might be responsible for neurodevelopmental effects of SeMet. Maternal exposure to dietary SeMet also altered the mRNA expression of MAO and VMAT2 which are involved in the degradation and storage of other monoamine neurotransmitters such as serotonin. Therefore, it can be interesting to examine whether maternal exposure to SeMet induces changes in other neurotransmitter systems relevant to learning and memory in zebrafish.
- The common trend among the effects of SeMet on the dopaminergic system in the experiments 3, 4, and 5 is an up-regulation in the expression of TH and DAT as well as an elevation in DA levels of the brain. However, the mechanisms underlying this stimulatory effect of Se remain to be elucidated. Hence, future studies should investigate how Se induces DA synthesis and turn-over. In addition to transcriptional levels, these studies can further focus on translational and post-translational levels of enzymes involved in DA synthesis and re-uptake.

References

Abbott, L.F., Nelson, S.B., 2000. Synaptic plasticity: taming the beast. Nature Neuroscience 3, 1178-1183.

Abraham, A.D., Neve, K.A., Lattal, K.M., 2016. Activation of D1/5 dopamine receptors: a common mechanism for enhancing extinction of fear and reward-seeking behaviors. Neuropsychopharmacology 41, 2072-2081.

Adinoff, B., 2004. Neurobiologic processes in drug reward and addiction. Harvard Review of Psychiatry 12, 305-320.

Adkins–Regan, E., MacKillop, E.A., 2003. Japanese quail (*Coturnix japonica*) inseminations are more likely to fertilize eggs in a context predicting mating opportunities. Proceedings of the Royal Society of London B: Biological Sciences 270, 1685-1689.

Ahlenius, S., Engel, J., Zöller, M., 1977. Effects of apomorphine and haloperidol on exploratory behavior and latent learning in mice. Physiological Psychology 5, 290-294.

Aimone, J.B., Li, Y., Lee, S.W., Clemenson, G.D., Deng, W., Gage, F.H., 2014. Regulation and function of adult neurogenesis: from genes to cognition. Physiological Reviews 94, 991-1026.

Aizenberg, M., Schuman, E.M., 2011. Cerebellar-dependent learning in larval zebrafish. Journal of Neuroscience 31, 8708-8712.

Ajarem, J., Basher, G., Ebaid, H., 2011a. Neurobehavioral changes in mice offspring induced by prenatal exposure to acute toxicity of sodium selenite. Biologia 66, 357-364.

Ajarem, J.S., Al Basher, G.I., Ebaid, H., 2011b. Neurobehavioral changes in mice offspring induced by prenatal exposure to acute toxicity of sodium selenite. Biologia 66, 357-364.

Akbaraly, T.N., Hininger-Favier, I., Carriere, I., Arnaud, J., Gourlet, V., Roussel, A. M., Berr, C., 2007. Plasma selenium over time and cognitive decline in the elderly. Epidemiology, 52-58.

Al Basher, G., Ebaid, H., Ajarem, J., Abu-Taweel, G., 2011. Impairment of active avoidance learning and sensory motor reflexes in mice offspring induced by perinatal acute toxic exposure to selenium. African Journal of Pharmacy and Pharmacology 5, 1389-1397.

Al-Imari, L., Gerlai, R., 2008. Sight of conspecifics as reward in associative learning in zebrafish (*Danio rerio*). Behavioural Brain Research 189, 216-219.

Altenhofen, S., Wiprich, M.T., Nery, L.R., Leite, C.E., Vianna, M.R.M.R., Bonan, C.D., 2017. Manganese (II) chloride alters behavioral and neurochemical parameters in larvae and adult zebrafish. Aquatic Toxicology 182, 172-183.

Amado, L.L., Monserrat, J.M., 2010. Oxidative stress generation by microcystins in aquatic animals: why and how. Environment International 36, 226-235.

Andersen, P.H., Nielsen, E.B., 1986. The Benzazepine, SCH 23390, inhibits 3H-NPA binding in mouse brain in vivo. Acta Pharmacologica et Toxicologica 59, 315-318.

Anichtchik, O., Sallinen, V., Peitsaro, N., Panula, P., 2006. Distinct structure and activity of monoamine oxidase in the brain of zebrafish (*Danio rerio*). Journal of Comparative Neurology 498, 593-610.

Anichtchik, O.V., Kaslin, J., Peitsaro, N., Scheinin, M., Panula, P., 2004. Neurochemical and behavioural changes in zebrafish *Danio rerio* after systemic administration of 6-hydroxydopamine and 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine. Journal of Neurochemistry 88, 443-453.

Ankley, G.T., Jensen, K.M., Kahl, M.D., Korte, J.J., Makynen, E.A., 2001. Description and evaluation of a short-term reproduction test with the fathead minnow (*Pimephales promelas*). Environmental Toxicology and Chemistry 20, 1276-1290.

Anwyl, R., 2006. Induction and expression mechanisms of postsynaptic NMDA receptor-independent homosynaptic long-term depression. Progress in Neurobiology 78, 17-37.

Aoki, R., Tsuboi, T., Okamoto, H., 2015. Y-maze avoidance: an automated and rapid associative learning paradigm in zebrafish. Neuroscience Research 91, 69-72.

Aragona, B.J., Wang, Z., 2009. Dopamine regulation of social choice in a monogamous rodent species. Frontiers in Behavioral Neuroscience 3, 1-11.

Archer, T., Palomo, T., Fredriksson, A., 2002. Functional deficits following neonatal dopamine depletion and isolation housing: circular water maze acquisition under pre-exposure conditions and motor activity. Neurotoxicity Research 4, 503-522.

Ardais, A.P., Viola, G.G., Costa, M.S., Nunes, F., Behr, G.A., Klamt, F., Moreira, J.C., Souza, D.O., Rocha, J.B., Porciúncula, L.O., 2009. Acute treatment with diphenyl diselenide inhibits glutamate uptake into rat hippocampal slices and modifies glutamate transporters, SNAP-25, and GFAP immunocontent. Toxicological Sciences 113, 434-443.

Ares-Santos, S., Granado, N., Moratalla, R., 2013. The role of dopamine receptors in the neurotoxicity of methamphetamine. Journal of Internal Medicine 273, 437-453.

Armando, I., Villar, V., Jose, P.A., 2011. Dopamine and renal function and blood pressure regulation. Comprehensive Physiology 1, 1075-1117.

Arnold, M., Forte, J., Osterberg, J., Di Giulio, R., 2016. Antioxidant rescue of selenomethionine-induced teratogenesis in zebrafish embryos. Archives of Environmental Contamination and Toxicology 70, 311-320.

Bach, M.E., Barad, M., Son, H., Zhuo, M., Lu, Y.-F., Shih, R., Mansuy, I., Hawkins, R.D., Kandel, E.R., 1999. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. Proceedings of the National Academy of Sciences 96, 5280-5285.

Baddeley, A.D., 2004. The psychology of memory, in: Baddeley, A.D., Kopelman, M.D., Wilson, B.A. (Eds.), The Essential Handbook of Memory Disorders for Clinicians. John Wiley and Sons, Chichester, pp. 1-13.

Bahrami, S., Drabløs, F., 2016. Gene regulation in the immediate-early response process. Advances in Biological Regulation 62, 37-49.

Bailey, J.M., Oliveri, A.N., Levin, E.D., 2015. Pharmacological analyses of learning and memory in zebrafish (*Danio rerio*). Pharmacology Biochemistry and Behavior 139, 103-111.

Balderas, I., Rodriguez-Ortiz, C.J., Bermudez-Rattoni, F., 2015. Consolidation and reconsolidation of object recognition memory. Behavioural Brain Research 285, 213-222.

Baldwin, A.E., Sadeghian, K., Kelley, A.E., 2002. Appetitive instrumental learning requires coincident activation of NMDA and dopamine D1 receptors within the medial prefrontal cortex. Journal of Neuroscience 22, 1063-1071.

Balleine, B.W., Delgado, M.R., Hikosaka, O., 2007. The role of the dorsal striatum in reward and decision-making. The Journal of Neuroscience 27, 8161-8165.

Beaulieu, J. M., Gainetdinov, R.R., 2011. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacological Reviews. 63, 182-217.

Bekinschtein, P., Cammarota, M., Medina, J.H., 2014. BDNF and memory processing. Neuropharmacology 76, 677-683.

Belinsky, G.S., Sirois, C.L., Rich, M.T., Short, S.M., Moore, A.R., Gilbert, S.E., Antic, S.D., 2013. Dopamine receptors in human embryonic stem cell neurodifferentiation. Stem Cells and Development 22, 1522-1540.

Benaliouad, F., Kapur, S., Natesan, S., Rompré, P. P., 2009. Effects of the dopamine stabilizer, OSU-6162, on brain stimulation reward and on quinpirole-induced changes in reward and locomotion. European Neuropsychopharmacology 19, 416-430.

Benedetto, A., Au, C., Avila, D.S., Milatovic, D., Aschner, M., 2010. Extracellular dopamine potentiates mn-induced oxidative stress, lifespan reduction, and dopaminergic neurodegeneration in a BLI-3–dependent manner in *Caenorhabditis elegans*. PLoS Genetics 6, e1001084.

Beninger, R.J., Miller, R., 1998. Dopamine D1-like receptors and reward-related incentive learning. Neuroscience and Biobehavioral Reviews 22, 335-345.

Berglind, W., Case, J., Parker, M., Fuchs, R., See, R., 2006. Dopamine D1 or D2 receptor antagonism within the basolateral amygdala differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. Neuroscience 137, 699-706.

Bergstrom, D., Carlson, J., Chase, T., Braun, A., 1987. D1 dopamine receptor activation required for postsynaptic expression of D2 agonist effects. Science 236, 719-722.

Bermúdez-Rattoni, F., 2007. Neural Plasticity and Memory: From Genes to Brain Imaging. CRC Press, Boca Raton, FL, USA.

Bernabeu, R., Bevilaqua, L., Ardenghi, P., Bromberg, E., Schmitz, P., Bianchin, M., Izquierdo, I., Medina, J.H., 1997. Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling

pathways in a late memory consolidation phase of aversively motivated learning in rats. Proceedings of the National Academy of Sciences 94, 7041-7046.

Bethus, I., Tse, D., Morris, R.G., 2010. Dopamine and memory: modulation of the persistence of memory for novel hippocampal NMDA receptor-dependent paired associates. Journal of Neuroscience 30, 1610-1618.

Biedenkapp, J.C., Rudy, J.W., 2009. Hippocampal and extrahippocampal systems compete for control of contextual fear: role of ventral subiculum and amygdala. Learning and Memory 16, 38-45.

Bilotta, J., Risner, M.L., Davis, E.C., Haggbloom, S.J., 2005. Assessing appetitive choice discrimination learning in zebrafish. Zebrafish 2, 259-268.

Bischoff, S., Heinrich, M., Krauss, J., Sills, M., Williams, M., Vassout, A., 1988. Interaction of the D1 Receptor Antagonist Sch 23390 with the Central 5-HT System: Radioligang Binding Studies, Measurements of Biochemical Parameters and Effects on L-5-HTP Syndrome. Journal of Receptor Research 8, 107-120.

Blaschko, H., 1939. The specific action of L-dopa decarboxylase. The Journal of Physiology 96, 50-61.

Blaschko, H., 1942. The activity of L-dopa decarboxylase. The Journal of Physiology 101, 337-349.

Blaser, R., Vira, D., 2014. Experiments on learning in zebrafish (*Danio rerio*): A promising model of neurocognitive function. Neuroscience and Biobehavioral Reviews 42, 224-231.

Bliss, T.V., Lømo, T., 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. The Journal of Physiology 232, 331-356.

Blodgett, H.C., 1929. The effect of the introduction of reward upon the maze performance of rats. University of California publications in psychology 4, 113-134.

Bodnar, M., Konieczka, P., Namiesnik, J., 2012. The properties, functions, and use of selenium compounds in living organisms. Journal of Environmental Science and Health, Part C 30, 225-252.

Boehmler, W., Carr, T., Thisse, C., Thisse, B., Canfield, V., Levenson, R., 2007. D4 Dopamine receptor genes of zebrafish and effects of the antipsychotic clozapine on larval swimming behaviour. Genes, Brain and Behavior 6, 155-166.

Boehmler, W., Obrecht-Pflumio, S., Canfield, V., Thisse, C., Thisse, B., Levenson, R., 2004. Evolution and expression of D2 and D3 dopamine receptor genes in zebrafish. Developmental Dynamics 230, 481-493.

Bortolotto, J.W., Cognato, G.P., Christoff, R.R., Roesler, L.N., Leite, C.E., Kist, L.W., Bogo, M.R., Vianna, M.R., Bonan, C.D., 2014. Long-term exposure to paraquat alters behavioral parameters and dopamine levels in adult zebrafish (*Danio rerio*). Zebrafish 11, 142-153.

Bouayed, J., Rammal, H., Soulimani, R., 2009. Oxidative stress and anxiety: relationship and cellular pathways. Oxidative Medicine and Cellular Longevity 2, 63-67.

Bouldin, J.L., Ingle, T.M., Sengupta, A., Alexander, R., Hannigan, R.E., Buchanan, R.A., 2008. Aqueous toxicity and food chain transfer of quantum dots™ in freshwater algae and *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry 27, 1958-1963.

Bowman, T.V., Zon, L.I., 2010. Swimming into the future of drug discovery: in vivo chemical screens in zebrafish. ACS Chemical Biology 5, 159-161.

Bowton, E., Saunders, C., Erreger, K., Sakrikar, D., Matthies, H.J., Sen, N., Jessen, T., Colbran, R.J., Caron, M.G., Javitch, J.A., 2010. Dysregulation of dopamine transporters via dopamine D2 autoreceptors triggers anomalous dopamine efflux associated with attention-deficit hyperactivity disorder. Journal of Neuroscience 30, 6048-6057.

Bozzi, Y., Borrelli, E., 2006. Dopamine in neurotoxicity and neuroprotection: what do D2 receptors have to do with it? Trends in Neurosciences 29, 167-174.

Bracs, P., Gregory, P., Jackson, D., 1984. Passive avoidance in rats: disruption by dopamine applied to the nucleus accumbens. Psychopharmacology 83, 70-75.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-254.

Brady, A.M., O'Donnell, P., 2004. Dopaminergic modulation of prefrontal cortical input to nucleus accumbens neurons in vivo. Journal of Neuroscience 24, 1040-1049.

Brandt, J.E., Bernhardt, E.S., Dwyer, G.S., Di Giulio, R.T., 2017. Selenium ecotoxicology in freshwater lakes receiving coal combustion residual effluents: A North Carolina example. Environmental Science and Technology 51, 2418-2426.

Breitenstein, C., Korsukewitz, C., Flöel, A., Kretzschmar, T., Diederich, K., Knecht, S., 2006. Tonic dopaminergic stimulation impairs associative learning in healthy subjects. Neuropsychopharmacology 31, 2552-2564.

Bretaud, S., Lee, S., Guo, S., 2004. Sensitivity of zebrafish to environmental toxins implicated in Parkinson's disease. Neurotoxicology and Teratology 26, 857-864.

Brouillet, E., Shinobu, L., McGarvey, U., Hochberg, F., Beal, M., 1993. Manganese injection into the rat striatum produces excitotoxic lesions by impairing energy metabolism. Experimental Neurology 120, 89-94.

Brown, C., Laland, K., Krause, J., 2008. Fish Cognition and Behavior. Blackwell Publishing Ltd, Oxford, UK.

Brown, J.H., Makman, M.H., 1972. Stimulation by dopamine of adenylate cyclase in retinal homogenates and of adenosine-3′: 5′-cyclic monophosphate formation in intact retina. Proceedings of the National Academy of Sciences 69, 539-543.

Brown, R.W., Bardo, M.T., Mace, D.D., Phillips, S.B., Kraemer, P.J., 2000. D-amphetamine facilitation of Morris water task performance is blocked by eticlopride and correlated with increased dopamine synthesis in the prefrontal cortex. Behavioural Brain Research 114, 135-143.

Buchanan, T.W., 2007. Retrieval of emotional memories. Psychological Bulletin 133, 761-779.

Buse, J., Schoenefeld, K., Münchau, A., Roessner, V., 2013. Neuromodulation in Tourette syndrome: dopamine and beyond. Neuroscience and Biobehavioral Reviews 37, 1069-1084.

Buske, C., Gerlai, R., 2011. Shoaling develops with age in Zebrafish (*Danio rerio*). Progress in Neuro-Psychopharmacology and Biological Psychiatry 35, 1409-1415.

Buske, C., Gerlai, R., 2012. Maturation of shoaling behavior is accompanied by changes in the dopaminergic and serotoninergic systems in zebrafish. Developmental Psychobiology 54, 28-35.

Byrne, J.H., 2010. Concise Learning and Memory: The Editor's Selection. Academic Press, Elsevier, Amsterdam, NL.

Byrnes, E.M., Abrams, D., Bruno, J.P., 1994. Co-activation of D1-and D2-like receptors is unnecessary for stimulated motor behavior in rats depleted of dopamine during development. Behavioural Brain Research 61, 205-214.

Cagiano, R., De Salvia, M., Renna, G., Tortella, E., Braghiroli, D., Parenti, C., Zanoli, P., Baraldi, M., Annau, Z., Cuomo, V., 1990. Evidence that exposure to methyl mercury during gestation induces behavioral and neurochemical changes in offspring of rats. Neurotoxicology and Teratology 12, 23-28.

Caito, S.W., Milatovic, D., Hill, K.E., Aschner, M., Burk, R.F., Valentine, W.M., 2011. Progression of neurodegeneration and morphologic changes in the brains of juvenile mice with selenoprotein P deleted. Brain Research 1398, 1-12.

Calabresi, P., Picconi, B., Tozzi, A., Di Filippo, M., 2007. Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends in Neurosciences 30, 211-219.

Calabresi, P., Saiardi, A., Pisani, A., Baik, J. H., Centonze, D., Mercuri, N.B., Bernardi, G., Borrelli, E., 1997. Abnormal synaptic plasticity in the striatum of mice lacking dopamine D2 receptors. Journal of Neuroscience 17, 4536-4544.

Carlsson, A., Lindqvist, M., Magnusson, T., 1957. 3, 4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180, 1200.

Carrera, M.P., Carey, R.J., Dias, F.R.C., de Matos, L.W., 2011. Reversal of apomorphine locomotor sensitization by a single post-conditioning trial treatment with a low autoreceptor dose of apomorphine: a memory re-consolidation approach. Pharmacology Biochemistry and Behavior 99, 29-34.

Castaño, A., Ayala, A., Rodríguez-Gómez, J.A., Herrera, A.J., Cano, J., Machado, A., 1997. Low selenium diet increases the dopamine turnover in prefrontal cortex of the rat. Neurochemistry International 30, 549-555.

Castellano, C., Cestari, V., Cabib, S., Puglisi-Allegra, S., 1991. Post-training dopamine receptor agonists and antagonists affect memory storage in mice irrespective of their selectivity for D1 or D2 receptors. Behavioral and Neural Biology 56, 283-291.

CCME, 2007. Canadian water quality guidelines for the protection of aquatic life: Summary table. In: Canadian Environmental Quality Guidelines, 1999, Canadian Council of Ministers of Environment, Task Force on Water Quality Guidelines, Winnipeg, MB, CA.

Centonze, D., Gubellini, P., Picconi, B., Saulle, E., Tolu, M., Bonsi, P., Giacomini, P., Calabresi, P., 2001. An abnormal striatal synaptic plasticity may account for the selective neuronal vulnerability in Huntington's disease. Neurological Sciences 22, 61-62.

Chacon, D.M., Luchiari, A.C., 2014. A dose for the wiser is enough: The alcohol benefits for associative learning in zebrafish. Progress in Neuro-Psychopharmacology and Biological Psychiatry 53, 109-115.

Chang, J., Kueon, C., Kim, J., 2014. Influence of lead on repetitive behavior and dopamine metabolism in a mouse model of iron overload. Toxicological Research 30, 267-276.

Chatterjee, D., Gerlai, R., 2009. High precision liquid chromatography analysis of dopaminergic and serotoninergic responses to acute alcohol exposure in zebrafish. Behavioural Brain Research 200, 208-213.

Chen, J., Berry, M.J., 2003. Selenium and selenoproteins in the brain and brain diseases. Journal of Neurochemistry 86, 1-12.

Cheng, R. K., Jesuthasan, S.J., Penney, T.B., 2014. Zebrafish forebrain and temporal conditioning. Philosophical Transactions of the Royal Society B: Biological Sciences 369, 20120462.

Chernick, M., Ware, M., Albright, E., Kwok, K.W., Dong, W., Zheng, N., Hinton, D.E., 2016. Parental dietary seleno-L-methionine exposure and resultant offspring developmental toxicity. Aquatic Toxicology 170, 187-198.

Chudasama, Y., Robbins, T.W., 2004. Dopaminergic modulation of visual attention and working memory in the rodent prefrontal cortex. Neuropsychopharmacology 29, 1628-1636.

Chung, A. S., Maines, M.D., 1981. Effect of selenium on glutathione metabolism: induction of γ-glutamylcysteine synthetase and glutathione reductase in the rat liver. Biochemical Pharmacology 30, 3217-3223.

Cibrian-Llanderal, T., Rosas-Aguilar, V., Triana-Del Rio, R., Perez, C.A., Manzo, J., Garcia, L.I., Coria-Avila, G.A., 2012. Enhaced D2-type receptor activity facilitates the development of conditioned same-sex partner preference in male rats. Pharmacology Biochemistry and Behavior 102, 177-183.

Citri, A., Malenka, R.C., 2008. Synaptic plasticity: multiple forms, functions, and mechanisms. Neuropsychopharmacology 33, 18-41.

Clayton, D.F., 2000. The genomic action potential. Neurobiology of Learning and Memory 74, 185-216.

Clopath, C., 2012. Synaptic consolidation: an approach to long-term learning. Cognitive Neurodynamics 6, 251-257.

Close, R., Toro, S., Martial, J.A., Muller, M., 2002. Expression of the zinc finger Egr1 gene during zebrafish embryonic development. Mechanisms of Development 118, 269-272.

Coelho, G.C., Galvanho, J.P., Carey, R.J., Carrera, M.P., 2011. Apomorphine locomotor sensitization can be potentiated by environmental change: Evidence for a non-Pavlovian associative behavioral contrast factor in sensitization expression. Behavioural Brain Research 220, 146-151.

Colwill, R.M., Raymond, M.P., Ferreira, L., Escudero, H., 2005. Visual discrimination learning in zebrafish (*Danio rerio*). Behavioural Processes 70, 19-31.

Conley, J.M., Watson, A.T., Xie, L., Buchwalter, D.B., 2014. Dynamic selenium assimilation, distribution, efflux, and maternal transfer in Japanese medaka fed a diet of Se-enriched mayflies. Environmental Science and Technology 48, 2971-2978.

Cory-Slechta, D., 1995. Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter system functions. Annual Review of Pharmacology and Toxicology 35, 391-415.

Cory-Slechta, D.A., Widzowski, D.V., 1991. Low level lead exposure increases sensitivity to the stimulus properties of dopamine D1 and D2 agonists. Brain Research 553, 65-74.

Costa, V.D., Tran, V.L., Turchi, J., Averbeck, B.B., 2014. Dopamine modulates novelty seeking behavior during decision making. Behavioral Neuroscience 128, 556.

Coutellier, L., Beraki, S., Ardestani, P.M., Saw, N.L., Shamloo, M., 2012. Npas4: a neuronal transcription factor with a key role in social and cognitive functions relevant to developmental disorders. PloS One 7, e46604.

Cropley, V.L., Fujita, M., Innis, R.B., Nathan, P.J., 2006. Molecular imaging of the dopaminergic system and its association with human cognitive function. Biological Psychiatry 59, 898-907.

Da Rocha, J.T., Gai, B.M., Pinton, S., Sampaio, T.B., Nogueira, C.W., Zeni, G., 2012. Effects of diphenyl diselenide on depressive-like behavior in ovariectomized mice submitted to subchronic stress: involvement of the serotonergic system. Psychopharmacology 222, 709-719.

da Silva, W.C., Köhler, C.C., Radiske, A., Cammarota, M., 2012. D1/D5 dopamine receptors modulate spatial memory formation. Neurobiology of Learning and Memory 97, 271-275.

Dahlbom, S.J., Backström, T., Lundstedt-Enkel, K., Winberg, S., 2012. Aggression and monoamines: effects of sex and social rank in zebrafish (*Danio rerio*). Behavioural Brain Research 228, 333-338.

Dalley, J.W., Everitt, B.J., 2009. Dopamine receptors in the learning, memory and drug reward circuitry. Seminars in Cell and Developmental Biology 20, 403-410.

Danby, F.W., Maddin, W.S., Margesson, L.J., Rosenthal, D., 1993. A randomized, double-blind, placebo-controlled trial of ketoconazole 2% shampoo versus selenium sulfide 2.5% shampoo in the treatment of moderate to severe dandruff. Journal of the American Academy of Dermatology 29, 1008-1012.

Darland, T., Mauch, J.T., Meier, E.M., Hagan, S.J., Dowling, J.E., Darland, D.C., 2012. Sulpiride, but not SCH23390, modifies cocaine-induced conditioned place preference and expression of tyrosine hydroxylase and elongation factor 1α in zebrafish. Pharmacology Biochemistry and Behavior 103, 157-167.

Day, J.J., Roitman, M.F., Wightman, R.M., Carelli, R.M., 2007. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nature Neuroscience 10, 1020-1028.

de Castro, M.R., Lima, J.V., de Freitas, D.P.S., de Souza Valente, R., Dummer, N.S., de Aguiar, R.B., dos Santos, L.C., Marins, L.F., Geracitano, L.A., Monserrat, J.M., 2009. Behavioral and neurotoxic effects of arsenic exposure in zebrafish (*Danio rerio*, Teleostei: Cyprinidae). Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 150, 337-342.

De Felice, E., Porreca, I., Alleva, E., De Girolamo, P., Ambrosino, C., Ciriaco, E., Germanà, A., Sordino, P., 2014. Localization of BDNF expression in the developing brain of zebrafish. Journal of Anatomy 224, 564-574.

de Lima, M.N.M., Presti-Torres, J., Dornelles, A., Scalco, F.S., Roesler, R., Garcia, V.A., Schröder, N., 2011. Modulatory influence of dopamine receptors on consolidation of object recognition memory. Neurobiology of Learning and Memory 95, 305-310.

Delcambre, S., Nonnenmacher, Y., Hiller, K., 2016. Dopamine metabolism and reactive oxygen species production, in: Buhlman, L.M. (Ed.), Mitochondrial Mechanisms of Degeneration and Repair in Parkinson's Disease. Springer, Cham, CH, pp. 25-47.

Di Ciano, P., Cardinal, R.N., Cowell, R.A., Little, S.J., Everitt, B.J., 2001. Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. Journal of Neuroscience 21, 9471-9477.

Di Giovanni, G., Pessia, M., Di Maio, R., 2012. Redox sensitivity of tyrosine hydroxylase activity and expression in dopaminergic dysfunction. CNS and Neurological Disorders-Drug Targets 11, 419-429.

Dias, V., Junn, E., Mouradian, M.M., 2013. The role of oxidative stress in Parkinson's disease. Journal of Parkinson's Disease 3, 461-491.

Diwadkar, V.A., Flaugher, B., Jones, T., Zalányi, L., Ujfalussy, B., Keshavan, M.S., Erdi, P., 2008. Impaired associative learning in schizophrenia: behavioral and computational studies. Cognitive Neurodynamics 2, 207-219.

Dreosti, E., Lopes, G., Kampff, A.R., Wilson, S.W., 2015. Development of social behavior in young zebrafish. Frontiers in Neural Circuits 9, 9.

Dreyer, J.K., Herrik, K.F., Berg, R.W., Hounsgaard, J.D., 2010. Influence of phasic and tonic dopamine release on receptor activation. Journal of Neuroscience 30, 14273-14283.

Driedger, K., Weber, L.P., Rickwood, C.J., Dubé, M.G., Janz, D.M., 2009. Overwinter alterations in energy stores and growth in juvenile fishes inhabiting areas receiving metal mining and municipal wastewater effluents. Environmental Toxicology and Chemistry 28, 296-304.

Driscoll, D.M., Copeland, P.R., 2003. Mechanism and regulation of selenoprotein synthesis. Annual Review of Nutrition 23, 17-40.

Dröge, W., Schipper, H.M., 2007. Oxidative stress and aberrant signaling in aging and cognitive decline. Aging Cell 6, 361-370.

Drukarch, B., Jongenelen, C.A., Schepens, E., Langeveld, C.H., Stoof, J.C., 1996. Glutathione is involved in the granular storage of dopamine in rat PC12 pheochromocytoma cells: Implications for the pathogenesis of Parkinson's disease. Journal of Neuroscience 16, 6038-6045.

Dudai, Y., 2012. The restless engram: consolidations never end. Annual Review of Neuroscience 35, 227-247.

Dufour, S., Weltzien, F.A., Sebert, M.E., Le Belle, N., Vidal, B., Vernier, P., Pasqualini, C., 2005. Dopaminergic inhibition of reproduction in teleost fishes: ecophysiological and evolutionary implications. Annals of the New York Academy of Sciences 1040, 9-21.

Durany, N., Michel, T., Zöchling, R., Boissl, K.W., Cruz-Sánchez, F.F., Riederer, P., Thome, J., 2001. Brain-derived neurotrophic factor and neurotrophin 3 in schizophrenic psychoses. Schizophrenia Research 52, 79-86.

ECCC/HC, 2017. Screening Assessment - Selenium and Its Compounds. Environment and Climate Change Canada/Health Canada, Minister of Environment and Minister of Health, Ottawa, ON, CA.

Eddins, D., Petro, A., Williams, P., Cerutti, D.T., Levin, E.D., 2009b. Nicotine effects on learning in zebrafish: the role of dopaminergic systems. Psychopharmacology 202, 103-109.

Egerer-Sieber, C., Herl, V., Müller-Uri, F., Kreis, W., Muller, Y.A., 2006. Crystallization and preliminary crystallographic analysis of selenomethionine-labelled progesterone 5β-reductase from *Digitalis lanata* Ehrh. Acta Crystallographica Section F 62, 186-188.

Ek, F., Malo, M., Åberg Andersson, M., Wedding, C., Kronborg, J., Svensson, P., Waters, S., Petersson, P., Olsson, R., 2016. Behavioral analysis of dopaminergic activation in zebrafish and rats reveals similar phenotypes. ACS Chemical Neuroscience 7, 633-646.

El-Ghundi, M., George, S.R., Drago, J., Fletcher, P.J., Fan, T., Nguyen, T., Liu, C., Sibley, D.R., Westphal, H., O'Dowd, B.F., 1998. Disruption of dopamine D 1 receptor gene expression attenuates alcohol-seeking behavior. European Journal of Pharmacology 353, 149-158.

El-Ghundi, M., O'Dowd, B.F., George, S.R., 2007. Insights into the role of dopamine receptor systems in learning and memory. Reviews in the Neurosciences 18, 37-66.

Elia, A.C., Abete, M.C., Pacini, N., Dörr, A.J.M., Scanzio, T., Prearo, M., 2014. Antioxidant biomarker survey ensuing long-term selenium withdrawal in *Acipenser baeri* fed Se-cysteine diets. Environmental Toxicology and Pharmacology 37, 1131-1139.

Ellwanger, J.H., Franke, S.I., Bordin, D.L., Pra, D., Henriques, J.A., 2016. Biological functions of selenium and its potential influence on Parkinson's disease. Anais da Academia Brasileira de Ciências 88, 1655-1674.

Esposito, F., Ammendola, R., Faraonio, R., Russo, T., Cimino, F., 2004. Redox control of signal transduction, gene expression and cellular senescence. Neurochemical Research 29, 617-628.

Estevez, A.O., Morgan, K.L., Szewczyk, N.J., Gems, D., Estevez, M., 2014. The neurodegenerative effects of selenium are inhibited by FOXO and PINK1/PTEN regulation of insulin/insulin-like growth factor signaling in *Caenorhabditis elegans*. Neurotoxicology 41, 28-43.

Estevez, A.O., Mueller, C.L., Morgan, K.L., Szewczyk, N.J., Teece, L., Miranda-Vizuete, A., Estevez, M., 2012. Selenium induces cholinergic motor neuron degeneration in *Caenorhabditis elegans*. Neurotoxicology 33, 1021-1032.

Eyles, D., Feldon, J., Meyer, U., 2012. Schizophrenia: do all roads lead to dopamine or is this where they start? Evidence from two epidemiologically informed developmental rodent models. Translational Psychiatry 2, e81.

Fan, T.W. M., Teh, S.J., Hinton, D.E., Higashi, R.M., 2002. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Aquatic Toxicology 57, 65-84.

Farooqui, A.A., 2014. Inflammation and Oxidative stress in Neurological Disorders: Effect of Lifestyle, Genes, and Age. Springer, New York, USA.

Fernandes, Y., Tran, S., Abraham, E., Gerlai, R., 2014. Embryonic alcohol exposure impairs associative learning performance in adult zebrafish. Behavioural Brain Research 265, 181-187.

Ferrari, M.C., Wisenden, B.D., Chivers, D.P., 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. Canadian Journal of Zoology 88, 698-724.

Filby, A.L., Paull, G.C., Hickmore, T.F., Tyler, C.R., 2010. Unravelling the neurophysiological basis of aggression in a fish model. BMC Genomics 11, 498.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239-247.

Fiorillo, C.D., Tobler, P.N., Schultz, W., 2005. Evidence that the delay-period activity of dopamine neurons corresponds to reward uncertainty rather than backpropagating TD errors. Behavioral and Brain Functions 1, 1-7.

Floresco, S.B., West, A.R., Ash, B., Moore, H., Grace, A.A., 2003. Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nature Neuroscience 6, 968-973.

Ford, C.P., 2014. The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. Neuroscience 282, 13-22.

Formella, I., Scott, E.K., Burne, T.H., Harms, L.R., Liu, P.-Y., Turner, K.M., Cui, X., Eyles, D.W., 2012. Transient knockdown of tyrosine hydroxylase during development has persistent effects on behaviour in adult zebrafish (*Danio rerio*). PloS One 7, e42482.

França, A.S., do Nascimento, G.C., Lopes-dos-Santos, V., Muratori, L., Ribeiro, S., Lobão-Soares, B., Tort, A.B., 2014. Beta2 oscillations (23–30 Hz) in the mouse hippocampus during novel object recognition. European Journal of Neuroscience 40, 3693-3703.

Freire, C., Koifman, S., 2012. Pesticide exposure and Parkinson's disease: epidemiological evidence of association. Neurotoxicology 33, 947-971.

Frey, U., Matthies, H., Reymann, K.G., Matthies, H., 1991. The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region in vitro. Neuroscience Letters 129, 111-114.

Friedrich, R.W., Jacobson, G.A., Zhu, P., 2010. Circuit neuroscience in zebrafish. Current Biology 20, 371-381.

Furini, C., Myskiw, J., Schmidt, B., Marcondes, L., Izquierdo, I., 2014. D1 and D5 dopamine receptors participate on the consolidation of two different memories. Behavioural Brain Research 271, 212-217.

Gallo, F.T., Katche, C., Morici, J.F., Medina, J.H., Weisstaub, N.V., 2018. Immediate early genes, memory and psychiatric disorders: Focus on c-fos, Egr1 and Arc. Frontiers in Behavioral Neuroscience 12, 79.

Gao, S., Jin, Y., Hall, K.S., Liang, C., Unverzagt, F.W., Ji, R., Murrell, J.R., Cao, J., Shen, J., Ma, F., 2007. Selenium level and cognitive function in rural elderly Chinese. American Journal of Epidemiology 165, 955-965.

Garcia, A.M.B., Martinez, R., Brandão, M.L., Morato, S., 2005. Effects of apomorphine on rat behavior in the elevated plus-maze. Physiology and Behavior 85, 440-447.

Gasbarri, A., Sulli, A., Pacitti, C., Puglisi-Allegra, S., Cabib, S., Castellano, C., Introini-Collison, I., McGaugh, J.L., 1997. Strain-dependent effects of D2 dopaminergic and muscarinic-cholinergic agonists and antagonists on memory consolidation processes in mice. Behavioural Brain Research 86, 97-104.

Gaskin, S., White, N.M., 2007. Unreinforced spatial (latent) learning is mediated by a circuit that includes dorsal entorhinal cortex and fimbria fornix. Hippocampus 17, 586-594.

Gedeon, Y., Ramesh, G.T., Wellman, P.J., Jadhav, A.L., 2001. Changes in mesocorticolimbic dopamine and D1/D2 receptor levels after low level lead exposure: a time course study. Toxicology Letters 123, 217-226.

George, M., 2010. Mineral Commodity Summaries. Selenium. US Geological Survey, Washington, DC, USA, pp. 144–145

Gerhardsson, L., Lundh, T., Minthon, L., Londos, E., 2008. Metal concentrations in plasma and cerebrospinal fluid in patients with Alzheimer's disease. Dementia and Geriatric Cognitive Disorders 25, 508-515.

Gerlai, R., 2011. Associative learning in zebrafish (*Danio rerio*), Methods in Cell Biology 101, 249-270.

Gerlai, R., 2016. Learning and memory in zebrafish (*Danio rerio*), Methods in Cell Biology 134, 551-586.

Gingrich, B., Liu, Y., Cascio, C., Wang, Z., Insel, T.R., 2000. Dopamine D2 receptors in the nucleus accumbens are important for social attachment in female prairie voles (*Microtus ochrogaster*). Behavioral Neuroscience 114, 173-183.

Gioanni, Y., Thierry, A.M., Glowinski, J., Tassin, J.P., 1998. α1-adrenergic, D1, and D2 receptors interactions in the prefrontal cortex: Implications for the modality of action of different types of neuroleptics. Synapse 30, 362-370.

Giorgi, O., Pibiri, M.G., Loi, R., Corda, M.G., 1993. Chronic treatment with SCH 23390 increases the production rate of dopamine D1 receptors in the nigro-striatal system of the rat. European Journal of Pharmacology: Molecular Pharmacology 245, 139-145.

Girault, J. A., Greengard, P., 2004. The neurobiology of dopamine signaling. Archives of Neurology 61, 641-644.

Glade, M.J., 2010. Oxidative stress and cognitive longevity. Nutrition 26, 595-603.

Gómez-Laplaza, L.M., Gerlai, R., 2010. Latent learning in zebrafish (*Danio rerio*). Behavioural Brain Research 208, 509-515.

González, B., Rivero-Echeto, C., Muñiz, J.A., Cadet, J.L., García-Rill, E., Urbano, F.J., Bisagno, V., 2016. Methamphetamine blunts Ca2+ currents and excitatory synaptic transmission through D 1/5 receptor-mediated mechanisms in the mouse medial prefrontal cortex. Addiction Biology 21, 589-602.

Goodman, A., 2008. Neurobiology of addiction: An integrative review. Biochemical Pharmacology 75, 266-322.

Grace, A., 1991. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41, 1-24.

Granado, N., Ortiz, O., Suárez, L.M., Martín, E.D., Ceña, V., Solís, J.M., Moratalla, R., 2008. D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-Induced arc and zif268 expression in the hippocampus. Cerebral Cortex 18, 1-12.

Grima, G., Benz, B., Parpura, V., Cuénod, M., Do, K.Q., 2003. Dopamine-induced oxidative stress in neurons with glutathione deficit: implication for schizophrenia. Schizophrenia Research 62, 213-224.

Grossman, L., Stewart, A., Gaikwad, S., Utterback, E., Wu, N., DiLeo, J., Frank, K., Hart, P., Howard, H., Kalueff, A.V., 2011. Effects of piracetam on behavior and memory in adult zebrafish. Brain Research Bulletin 85, 58-63.

Guarnieri, R.V., Ribeiro, R.L., de Souza, A.A.L., Galduróz, J.C.F., Covolan, L., Bueno, O.F., 2016. Effects of sulpiride on true and false memories of thematically related pictures and associated words in healthy volunteers. Frontiers in Psychiatry 7, 28.

Guilarte, T.R., 2010. Manganese and Parkinson's disease: a critical review and new findings. Environmental Health Perspectives 118, 1071-1080.

Gunzler, S.A., Shakil, S., Carlson, N.E., Nutt, J.G., Meshul, C.K., 2007. Low doses of apomorphine transiently reduce locomotor activity in MPTP-treated mice. Neuroscience Letters 428, 64-67.

Gurden, H., Takita, M., Jay, T.M., 2000. Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapses in vivo. The Journal of Neuroscience 20, 106.

Gurden, H., Tassin, J. P., Jay, T., 1999. Integrity of the mesocortical dopaminergic system is necessary for complete expression of in vivo hippocampal-prefrontal cortex long-term potentiation. Neuroscience 94, 1019-1027.

Guzowski, J.F., 2002. Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. Hippocampus 12, 86-104.

Haavik, J., Toska, K., 1998. Tyrosine hydroxylase and Parkinson's disease. Molecular Neurobiology 16, 285-309.

Hadj-Bouziane, F., Benatru, I., Brovelli, A., Klinger, H., Thobois, S., Broussolle, E., Boussaoud, D., Meunier, M., 2013. Advanced Parkinson's disease effect on goal-directed and habitual processes involved in visuomotor associative learning. Frontiers in Human Neuroscience 6, 351.

Haehnel-Taguchi, M., Fernandes, A.M., Böhler, M., Schmitt, I., Driever, W., 2018. Projections of the diencephalospinal dopaminergic system to peripheral sense organs in larval zebrafish (*Danio rerio*). Frontiers in Neuroanatomy 12, 20.

Hagmeyer, S., Mangus, K., Boeckers, T.M., Grabrucker, A.M., 2015. Effects of trace metal profiles characteristic for autism on synapses in cultured neurons. Neural Plasticity 2015, 1-17.

Hale, M.W., Crowe, S.F., 2001. The effects of apomorphine and haloperidol on memory consolidation in the day-old-chick. Behavioral Neuroscience 115, 376-383.

Halliwell, B., 2006. Oxidative stress and neurodegeneration: where are we now? Journal of Neurochemistry 97, 1634-1658.

Halliwell, B., Gutteridge, J.M., 2015. Free radicals in biology and medicine. Oxford University Press, New York, USA.

Hamilton, S.J., 2004. Review of selenium toxicity in the aquatic food chain. Science of the Total Environment 326, 1-31.

Hamilton, T.J., Wheatley, B.M., Sinclair, D.B., Bachmann, M., Larkum, M.E., Colmers, W.F., 2010. Dopamine modulates synaptic plasticity in dendrites of rat and human dentate granule cells. Proceedings of the National Academy of Sciences 107, 18185-18190.

Hare, D.J., Cardoso, B.R., Raven, E.P., Double, K.L., Finkelstein, D.I., Szymlek-Gay, E.A., Biggs, B. A., 2017. Excessive early-life dietary exposure: a potential source of elevated brain iron and a risk factor for Parkinson's disease. NPJ Parkinson's Disease, 3, 1-5.

Hasbi, A., Fan, T., Alijaniaram, M., Nguyen, T., Perreault, M.L., O'Dowd, B.F., George, S.R., 2009. Calcium signaling cascade links dopamine D1–D2 receptor heteromer to striatal BDNF production and neuronal growth. Proceedings of the National Academy of Sciences 106, 21377-21382.

Haygarth, P.M., 1994. Global importance and global cycling of selenium, in: Frankenberger, W.T. and Benson, S. (Eds.), Selenium in the Environment, Marcel Dekker, New York, USA, pp. 1-27.

Hazy, T.E., Frank, M.J., O'reilly, R.C., 2010. Neural mechanisms of acquired phasic dopamine responses in learning. Neuroscience and Biobehavioral Reviews 34, 701-720.

He, Y., Xiang, Y., Zhou, Y., Yang, Y., Zhang, J., Huang, H., Shang, C., Luo, L., Gao, J., Tang, L., 2018. Selenium contamination, consequences and remediation techniques in water and soils: A review. Environmental Research 164, 288-301.

Heath, F.C., Jurkus, R., Bast, T., Pezze, M.A., Lee, J.L., Voigt, J.P., Stevenson, C.W., 2015. Dopamine D1-like receptor signalling in the hippocampus and amygdala modulates the acquisition of contextual fear conditioning. Psychopharmacology 232, 2619-2629.

Hebb, D.O., 1949. The Organization of Behavior: A Neuropsychological Theory. John Wiley and Sons, New York, USA.

Hegde, M.L., Shanmugavelu, P., Vengamma, B., Rao, T.S., Menon, R.B., Rao, R.V., Rao, K.J., 2004. Serum trace element levels and the complexity of inter-element relations in patients with Parkinson's disease. Journal of Trace Elements in Medicine and Biology 18, 163-171.

Hemsley, D.R., 1996. Schizophrenia: a cognitive model and its implications for psychological intervention. Behavior Modification 20, 139-169.

Hensley, K., Robinson, K.A., Gabbita, S.P., Salsman, S., Floyd, R.A., 2000. Reactive oxygen species, cell signaling, and cell injury. Free Radical Biology and Medicine 28, 1456-1462.

Higa, K.K., Young, J.W., Ji, B., Nichols, D.E., Geyer, M.A., Zhou, X., 2017. Striatal dopamine D1 receptor suppression impairs reward-associative learning. Behavioural Brain Research 323, 100-110.

Holland, P.C., Schiffino, F.L., 2016. Mini-review: Prediction errors, attention and associative learning. Neurobiology of Learning and Memory 131, 207-215.

Holtz, P., 1939. Dopadecarboxylase. Naturwissenschaften 27, 724-725.

Horzmann, K.A., Freeman, J.L., 2016. Zebrafish get connected: investigating neurotransmission targets and alterations in chemical toxicity. Toxics 4, 19.

Hotte, M., Naudon, L., Jay, T.M., 2005. Modulation of recognition and temporal order memory retrieval by dopamine D1 receptor in rats. Neurobiology of Learning and Memory 84, 85-92.

Hotte, M., Thuault, S., Lachaise, F., Dineley, K.T., Hemmings, H.C., Nairn, A.C., Jay, T.M., 2006. D1 receptor modulation of memory retrieval performance is associated with changes in pCREB and pDARPP-32 in rat prefrontal cortex. Behavioural Brain Research 171, 127-133.

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

Hu, J. R., Huang, Y. H., Wang, G. X., Wu, Y. X., Xian, J. A., Wang, A. L., Cao, J. M., 2016. Deficient and excess dietary selenium levels affect growth performance, blood cells apoptosis and liver HSP70 expression in juvenile yellow catfish *Pelteobagrus fulvidraco*. Fish Physiology and Biochemistry 42, 249-261.

Huang, T. T., Leu, D., Zou, Y., 2015. Oxidative stress and redox regulation on hippocampal-dependent cognitive functions. Archives of Biochemistry and Biophysics 576, 2-7.

Huang, Y. Y., Simpson, E., Kellendonk, C., Kandel, E.R., 2004. Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. Proceedings of the National Academy of Sciences 101, 3236-3241.

Hurtado-Parrado, C., 2010. Neuronal mechanisms of learning in teleost fish. Universitas Psychologica 9, 663-678.

Hyman, S.E., Malenka, R.C., Nestler, E.J., 2006. Neural mechanisms of addiction: the role of reward-related learning and memory. Annual Review of Neuroscience 29, 565-598.

Ichihara, K., Nabeshima, T., Kameyama, T., 1988a. Effects of haloperidol, sulpiride and SCH 23390 on passive avoidance learning in mice. European Journal of Pharmacology 151, 435-442.

Ichihara, K., Nabeshima, T., Kameyama, T., 1988b. Opposite effects induced by low and high doses of apomorphine on single-trial passive avoidance learning in mice. Pharmacology Biochemistry and Behavior 30, 107-113.

Ichihara, K., Nabeshima, T., Kameyama, T., 1989. Differential effects of pimozide and SCH 23390 on acquisition of learning in mice. European Journal of Pharmacology 164, 189-195.

Ichihara, K., Nabeshima, T., Kameyama, T., 1993a. Dopaminergic agonists impair latent learning in mice: possible modulation by noradrenergic function. Journal of Pharmacology and Experimental Therapeutics 264, 122-128.

Ichihara, K., Nabeshima, T., Kameyama, T., 1993b. Mediation of dopamine D1 and D2 receptors in the effects of GBR 12909 on latent learning and locomotor activity in mice. European Journal of Pharmacology 234, 155-163.

Ihalainen, J., Riekkinen Jr, P., Feenstra, M., 1999. Comparison of dopamine and noradrenaline release in mouse prefrontal cortex, striatum and hippocampus using microdialysis. Neuroscience Letters 277, 71-74.

Irons, T., Kelly, P., Hunter, D., Macphail, R., Padilla, S., 2013. Acute administration of dopaminergic drugs has differential effects on locomotion in larval zebrafish. Pharmacology Biochemistry and Behavior 103, 792-813.

Ishige, K., Chen, Q., Sagara, Y., Schubert, D., 2001. The activation of dopamine D4 receptors inhibits oxidative stress-induced nerve cell death. Journal of Neuroscience 21, 6069-6076.

Ishrat, T., Parveen, K., Khan, M.M., Khuwaja, G., Khan, M.B., Yousuf, S., Ahmad, A., Shrivastav, P., Islam, F., 2009. Selenium prevents cognitive decline and oxidative damage in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. Brain Research 1281, 117-127.

Islam, F., Zia, S., Sayeed, I., Zafar, K.S., Ahmad, A.S., 2002. Selenium-induced alteration of lipids, lipid peroxidation, and thiol group in circadian rhythm centers of rat. Biological Trace Element Research 90, 203-214.

Iversen, S.D., Iversen, L.L., 2007. Dopamine: 50 years in perspective. Trends in Neurosciences 30, 188-193.

Iwakura, Y., Nawa, H., Sora, I., Chao, M.V., 2008. Dopamine D1 receptor-induced signaling through TrkB receptors in striatal neurons. Journal of Biological Chemistry 283, 15799-15806.

Jackson, D., Ross, S., HASHIZUME, M., 1988. Dopamine-mediated behaviours produced in naive mice by bromocriptine plus SKF 38393. Journal of Pharmacy and Pharmacology 40, 221-223.

Jamwal, A., Naderi, M., Niyogi, S., 2016. An in vitro examination of selenium–cadmium antagonism using primary cultures of rainbow trout (*Oncorhynchus mykiss*) hepatocytes. Metallomics 8, 218-227.

Janz, D.M., 2012. Selenium, in: Wood, C.M., Farrell, A.P., Brauner, C.J. (Eds.), Homeostasis and Toxicology of Essential Metals. Elsevier Academic Press Inc., San Diego, CA, USA, pp. 327-374.

Janz, D.M., DeForest, D.K., Brooks, M.L., Chapman, P.M., Gilron, G., Hoff, D., Hopkins, W.D., McIntyre, D.O., Mebane, C.A., Palace, V.P., Skorupa, J.P., Wayland, M., 2010. Selenium toxicity to aquatic organisms, in: Chapman, P.M., Adams, W.J., Brooks, M.L., Delos, C.G., Luoma, S.N., Maher, W.A., Ohlendorf, H.M., Presser, T.S., Shaw, D.P. (Eds.), Ecological Assessment of Selenium in the Aquatic Environment. CRC Press, Boca Raton, FL, USA, pp. 141-231.

Janz, D.M., Liber, K., Pickering, I.J., Wiramanaden, C.I., Weech, S.A., Gallego-Gallegos, M., Driessnack, M.K., Franz, E.D., Goertzen, M.M., Phibbs, J., 2014. Integrative assessment of selenium speciation, biogeochemistry, and distribution in a northern coldwater ecosystem. Integrated Environmental Assessment and Management 10, 543-554.

Jardine, D., Litvak, M., 2003. Direct yolk sac volume manipulation of zebrafish embryos and the relationship between offspring size and yolk sac volume. Journal of Fish Biology 63, 388-397.

Jay, T.M., 2003. Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. Progress in Neurobiology 69, 375-390.

Jensen, R., 2006. Behaviorism, latent learning, and cognitive maps: Needed revisions in introductory psychology textbooks. The Behavior Analyst 29, 187-209.

Johnson, F.O., Atchison, W.D., 2009. The role of environmental mercury, lead and pesticide exposure in development of amyotrophic lateral sclerosis. Neurotoxicology 30, 761-765.

Jones, D.C., Miller, G.W., 2008. The effects of environmental neurotoxicants on the dopaminergic system: A possible role in drug addiction. Biochemical Pharmacology 76, 569-581.

Juárez Olguín, H., Calderón Guzmán, D., Hernández García, E., Barragán Mejía, G., 2015. The role of dopamine and its dysfunction as a consequence of oxidative stress. Oxidative Medicine and Cellular Longevity 2016, 1-13.

Kabitzke, P., Simpson, E., Kandel, E., Balsam, P., 2015. Social behavior in a genetic model of dopamine dysfunction at different neurodevelopmental time points. Genes, Brain and Behavior 14, 503-515.

Kalueff, A.V., Gebhardt, M., Stewart, A.M., Cachat, J.M., Brimmer, M., Chawla, J.S., Craddock, C., Kyzar, E.J., Roth, A., Landsman, S., 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish 10, 70-86.

Kalueff, A.V., Stewart, A.M., Gerlai, R., 2014. Zebrafish as an emerging model for studying complex brain disorders. Trends in Pharmacological Sciences 35, 63-75.

Kamiński, J., Mamelak, A.N., Birch, K., Mosher, C.P., Tagliati, M., Rutishauser, U., 2018. Novelty-sensitive dopaminergic neurons in the human substantia nigra predict success of declarative memory formation. Current Biology 28, 1333-1343.

Karnik, I., Gerlai, R., 2012. Can zebrafish learn spatial tasks? An empirical analysis of place and single CS–US associative learning. Behavioural Brain Research 233, 415-421.

Karri, V., Schuhmacher, M., Kumar, V., 2016. Heavy metals (Pb, Cd, As and MeHg) as risk factors for cognitive dysfunction: A general review of metal mixture mechanism in brain. Environmental Toxicology and Pharmacology 48, 203-213.

Kebabian, J.W., Calne, D.B., 1979. Multiple receptors for dopamine. Nature 277, 93-96.

Kebabian, J.W., Petzold, G.L., Greengard, P., 1972. Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor". Proceedings of the National Academy of Sciences 69, 2145-2149.

Keefe, K., Gerfen, C., 1995. D1–D2 dopamine receptor synergy in striatum: effects of intrastriatal infusions of dopamine agonists and antagonists on immediate early gene expression. Neuroscience 66, 903-913.

Keiflin, R., Janak, P.H., 2015. Dopamine prediction errors in reward learning and addiction: from theory to neural circuitry. Neuron 88, 247-263.

Kellendonk, C., 2009. Modeling excess striatal D2 receptors in mice, In: Akira, S. (Eds.), Progress in Brain Research Vol. 179. Elsevier, New York, USA, pp. 59-65.

Kellendonk, C., Simpson, E.H., Polan, H.J., Malleret, G., Vronskaya, S., Winiger, V., Moore, H., Kandel, E.R., 2006. Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. Neuron 49, 603-615.

Kern, C.H., Stanwood, G.D., Smith, D.R., 2010. Preweaning manganese exposure causes hyperactivity, disinhibition, and spatial learning and memory deficits associated with altered dopamine receptor and transporter levels. Synapse 64, 363-378.

Kerr, J.N., Wickens, J., 2001. Dopamine D-1/D-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. Journal of Neurophysiology 85, 117-124.

Kessels, H.W., Malinow, R., 2009. Synaptic AMPA receptor plasticity and behavior. Neuron 61, 340-350.

Khan, H.A., 2010. Selenium partially reverses the depletion of striatal dopamine and its metabolites in MPTP-treated C57BL mice. Neurochemistry International 57, 489-491.

Khan, K.U., Zuberi, A., Fernandes, J.B.K., Ullah, I., Sarwar, H., 2017. An overview of the ongoing insights in selenium research and its role in fish nutrition and fish health. Fish Physiology and Biochemistry 43, 1689-1705.

Kim, H. C., Jhoo, W. K., Shin, E. J., Bing, G., 2000. Selenium deficiency potentiates methamphetamine-induced nigral neuronal loss; comparison with MPTP model. Brain Research 862, 247-252.

Kim, H. C., Jhoo, W. K., Choi, D. Y., Im, D. H., Shin, E. J., Suh, J. H., Floyd, R.A., Bing, G., 1999a. Protection of methamphetamine nigrostriatal toxicity by dietary selenium. Brain Research 851, 76-86.

Kim, Y., Kim, J. W., Ito, K., Lim, H. S., Cheong, H. K., Kim, J.Y., Shin, Y.C., Kim, K.S., Moon, Y., 1999b. Idiopathic parkinsonism with superimposed manganese exposure: utility of positron emission tomography. Neurotoxicology 20, 249-252.

Koch, M., Schmid, A., Schnitzler, H. U., 2000. Role of nucleus accumbens dopamine D 1 and D 2 receptors in instrumental and Pavlovian paradigms of conditioned reward. Psychopharmacology 152, 67-73.

Korzh, V., Sleptsova, I., Liao, J., He, J., Gong, Z., 1998. Expression of zebrafish bHLH genes ngn1and nrd defines distinct stages of neural differentiation. Developmental Dynamics 213, 92-104.

Kozorovitskiy, Y., Peixoto, R., Wang, W., Saunders, A., Sabatini, B.L., 2015. Neuromodulation of excitatory synaptogenesis in striatal development. Elife 4, e10111.

Krabbe, S., Duda, J., Schiemann, J., Poetschke, C., Schneider, G., Kandel, E.R., Liss, B., Roeper, J., Simpson, E.H., 2015. Increased dopamine D2 receptor activity in the striatum alters the firing pattern of dopamine neurons in the ventral tegmental area. Proceedings of the National Academy of Sciences 112, 1498-1506.

Krach, S., Paulus, F.M., Bodden, M., Kircher, T., 2010. The rewarding nature of social interactions. Frontiers in Behavioral Neuroscience 4, 22.

Kramar, C.P., Chefer, V.I., Wise, R.A., Medina, J.H., Barbano, M.F., 2014. Dopamine in the dorsal hippocampus impairs the late consolidation of cocaine-associated memory. Neuropsychopharmacology 39, 1645-1653.

Kreitzer, A.C., Malenka, R.C., 2005. Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. Journal of Neuroscience 25, 10537-10545.

Kumar, A., Ahmad, I., Shukla, S., Singh, B.K., Patel, D.K., Pandey, H.P., Singh, C., 2010. Effect of zinc and paraquat co-exposure on neurodegeneration: modulation of oxidative stress and expression of metallothioneins, toxicant responsive and transporter genes in rats. Free Radical Research 44, 950-965.

Kupsco, A., Schlenk, D., 2016. Molecular mechanisms of selenium-Induced spinal deformities in fish. Aquatic Toxicology 179, 143-150.

Kurylo, D.D., 2004. Effects of quinpirole on operant conditioning: perseveration of behavioral components. Behavioural Brain Research 155, 117-124.

Lambert, A.M., Bonkowsky, J.L., Masino, M.A., 2012. The conserved dopaminergic diencephalospinal tract mediates vertebrate locomotor development in zebrafish larvae. Journal of Neuroscience 32, 13488-13500.

Lavado, R., Shi, D., Schlenk, D., 2012. Effects of salinity on the toxicity and biotransformation of L-selenomethionine in Japanese medaka (*Oryzias latipes*) embryos: mechanisms of oxidative stress. Aquatic Toxicology 108, 18-22.

Le Nguyen, C., Tran, A.H., Matsumoto, J., Hori, E., Uwano, T., Ono, T., Nishijo, H., 2014. Hippocampal place cell responses to distal and proximal cue manipulations in dopamine D2 receptor-knockout mice. Brain Research 1567, 13-27.

Leal, G., Afonso, P.M., Salazar, I.L., Duarte, C.B., 2015. Regulation of hippocampal synaptic plasticity by BDNF. Brain Research 1621, 82-101.

Lee, K.H., Jeong, D., 2012. Bimodal actions of selenium essential for antioxidant and toxic pro-oxidant activities: the selenium paradox. Molecular Medicine Reports 5, 299-304.

Lemly, A.D., 1997. A teratogenic deformity index for evaluating impacts of selenium on fish populations. Ecotoxicology and Environmental Safety 37, 259-266.

Lemly, A.D., 2002. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. Aquatic Toxicology 57, 39-49.

Lemly, A.D., 2004. Aquatic selenium pollution is a global environmental safety issue. Ecotoxicology and Environmental Safety 59, 44-56.

Lemon, N., Manahan-Vaughan, D., 2006. Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. Journal of Neuroscience 26, 7723-7729.

Lenz, M., Lens, P.N., 2009. The essential toxin: the changing perception of selenium in environmental sciences. Science of the Total Environment 407, 3620-3633.

Letavayová, L., Vlčková, V., Brozmanová, J., 2006. Selenium: from cancer prevention to DNA damage. Toxicology 227, 1-14.

Levin, E.D., Buccafusco, J.J., 2006. Animal Models of Cognitive Impairment. CRC press, Boca Raton, FL, USA.

Levin, E.D., Chrysanthis, E., Yacisin, K., Linney, E., 2003. Chlorpyrifos exposure of developing zebrafish: effects on survival and long-term effects on response latency and spatial discrimination. Neurotoxicology and Teratology 25, 51-57.

Li, F., Wang, L.P., Shen, X., Tsien, J.Z., 2010. Balanced dopamine is critical for pattern completion during associative memory recall. PLoS One 5, e15401.

Li, J., Li, W., Jiang, Z. G., Ghanbari, H.A., 2013. Oxidative stress and neurodegenerative disorders. International Journal of Molecular Sciences 14, 24438-24475.

Li, P., Shah, S., Huang, L., Carr, A.L., Gao, Y., Thisse, C., Thisse, B., Li, L., 2007. Cloning and spatial and temporal expression of the zebrafish dopamine D1 receptor. Developmental Dynamics 236, 1339-1346.

Li, S., Cullen, W.K., Anwyl, R., Rowan, M.J., 2003. Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. Nature Neuroscience 6, 526.

Liang, S., Chen, J., Pierce, D.T., Zhao, J.X., 2013. A turn-on fluorescent nanoprobe for selective determination of selenium (IV). ACS Applied Materials and Interfaces 5, 5165-5173.

Lidow, M.S., Koh, P.O., Arnsten, A.F., 2003. D1 dopamine receptors in the mouse prefrontal cortex: immunocytochemical and cognitive neuropharmacological analyses. Synapse 47, 101-108.

Lin, Y., Bloodgood, B.L., Hauser, J.L., Lapan, A.D., Koon, A.C., Kim, T. K., Hu, L.S., Malik, A.N., Greenberg, M.E., 2008. Activity-dependent regulation of inhibitory synapse development by Npas4. Nature 455, 1198- 1204.

Linney, E., Upchurch, L., Donerly, S., 2004. Zebrafish as a neurotoxicological model. Neurotoxicology and Teratology 26, 709-718.

Lisman, J., Grace, A.A., Duzel, E., 2011. A neoHebbian framework for episodic memory; role of dopamine-dependent late LTP. Trends in Neurosciences 34, 536-547.

Lissek, S., Glaubitz, B., Wolf, O.T., Tegenthoff, M., 2015. The DA antagonist tiapride impairs context-related extinction learning in a novel context without affecting renewal. Frontiers in Behavioral Neuroscience 9, 238.

Liu, P.K., Arora, T., 2002. Transcripts of damaged genes in the brain during cerebral oxidative stress. Journal of Neuroscience Research 70, 713-720.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402-408.

Lodge, D.J., Grace, A.A., 2011a. Developmental pathology, dopamine, stress and schizophrenia. International Journal of Developmental Neuroscience 29, 207-213.

Lodge, D.J., Grace, A.A., 2011b. Hippocampal dysregulation of dopamine system function and the pathophysiology of schizophrenia. Trends in Pharmacological Sciences 32, 507-513.

López, J.C., Bingman, V., Rodríguez, F., Gómez, Y., Salas, C., 2000. Dissociation of place and cue learning by telencephalic ablation in goldfish. Behavioral Neuroscience 114, 687-699.

Luchiari, A.C., Salajan, D.C., Gerlai, R., 2015. Acute and chronic alcohol administration: effects on performance of zebrafish in a latent learning task. Behavioural Brain Research 282, 76-83.

Lynch, M.A., 2004. Long-term potentiation and memory. Physiological Reviews 84, 87-136.

Ma, P.M., Lopez, M., 2003. Consistency in the number of dopaminergic paraventricular organ-accompanying neurons in the posterior tuberculum of the zebrafish brain. Brain Research 967, 267-272.

Maas, D., Vallès, A., Martens, G., 2017. Oxidative stress, prefrontal cortex hypomyelination and cognitive symptoms in schizophrenia. Translational Psychiatry 7, e1171.

MacFarquhar, J.K., Broussard, D.L., Melstrom, P., Hutchinson, R., Wolkin, A., Martin, C., Burk, R.F., Dunn, J.R., Green, A.L., Hammond, R., 2010. Acute selenium toxicity associated with a dietary supplement. Archives of Internal Medicine 170, 256-261.

Mahabir, S., Chatterjee, D., Buske, C., Gerlai, R., 2013. Maturation of shoaling in two zebrafish strains: a behavioral and neurochemical analysis. Behavioural Brain Research 247, 1-8.

Maher, W., Roach, A., Doblin, M., Fan, T., Foster, S., Garrett, R., Möller, G., Oram, L., Wallschläger, D., 2010. Environmental sources, speciation, and partitioning of selenium, in: Chapman, P. M., Adams, W. J., Brooks, M. L., Delos, C. G., Luoma, S. N., Maher, W. A., Ohlendorf, H. M., Presser, T. S., Shaw, D. P. (Eds.), Ecological Assessment of Selenium in the Aquatic Environment. CRC Press, Boca Raton, FL, USA, pp. 47-92.

Maier, K.J., Knight, A.W., 1994. Ecotoxicology of selenium in freshwater systems. Reviews of Environmental Contamination and Toxicology 134, 31-48.

Mansour, A., Watson, S., 1995. Dopamine receptor expression in the central nervous system, in: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: the Fourth Generation of Progress. Lippincott Williams and Wilkins, Philadelphia, USA, pp. 207-219.

Manuel, R., Gorissen, M., Zethof, J., Ebbesson, L.O., van de Vis, H., Flik, G., van den Bos, R., 2014. Unpredictable chronic stress decreases inhibitory avoidance learning in Tuebingen long-fin zebrafish: stronger effects in the resting phase than in the active phase. Journal of Experimental Biology 217, 3919-3928.

Maraldi, T., Riccio, M., Zambonin, L., Vinceti, M., De Pol, A., Hakim, G., 2011. Low levels of selenium compounds are selectively toxic for a human neuron cell line through ROS/RNS increase and apoptotic process activation. Neurotoxicology 32, 180-187.

Marinelli, M., White, F.J., 2000. Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. The Journal of Neuroscience 20, 8876-8885.

Mariotti, M., Ridge, P.G., Zhang, Y., Lobanov, A.V., Pringle, T.H., Guigo, R., Hatfield, D.L., Gladyshev, V.N., 2012. Composition and evolution of the vertebrate and mammalian selenoproteomes. PLoS One 7, e33066.

Maroun, M., Akirav, I., 2009. Differential involvement of dopamine D1 receptor and MEK signaling pathway in the ventromedial prefrontal cortex in consolidation and reconsolidation of recognition memory. Learning and Memory 16, 243-247.

Martin, H.L., Teismann, P., 2009. Glutathione—a review on its role and significance in Parkinson's disease. The FASEB Journal 23, 3263-3272.

Martin, J. L., Finsterwald, C., 2011. Cooperation between BDNF and glutamate in the regulation of synaptic transmission and neuronal development. Communicative and Integrative Biology 4, 14-16.

Martorana, A., Koch, G., 2014. Is dopamine involved in Alzheimer's disease? Frontiers in Aging Neuroscience 6, 252.

Maximino, C., Herculano, A.M., 2010. A review of monoaminergic neuropsychopharmacology in zebrafish. Zebrafish 7, 359-378.

Maximino, C., P Costa, B., G Lima, M., 2016. A review of monoaminergic neuropsychopharmacology in zebrafish, 6 years later: Towards paradoxes and their solution. Current psychopharmacology 5, 96-138.

May, T.W., Fairchild, J.F., Petty, J.D., Walther, M.J., Lucero, J., Delvaux, M., Manring, J., Armbruster, M., 2008. An evaluation of selenium concentrations in water, sediment, invertebrates, and fish from the Solomon River Basin. Environmental Monitoring and Sssessment 137, 213-232.

McDonald, B.G., DeBruyn, A.M., Elphick, J.R., Davies, M., Bustard, D., Chapman, P.M., 2010. Developmental toxicity of selenium to dolly varden char (*Salvelinus malma*). Environmental Toxicology and Chemistry 29, 2800-2805.

Mechaly, A., Teplitsky, A., Belakhov, V., Baasov, T., Shoham, G., Shoham, Y., 2000. Overproduction and characterization of seleno-methionine xylanase T-6. Journal of Biotechnology 78, 83-86.

Mehdi, Y., Hornick, J. L., Istasse, L., Dufrasne, I., 2013. Selenium in the environment, metabolism and involvement in body functions. Molecules 18, 3292-3311.

Meiser, J., Weindl, D., Hiller, K., 2013. Complexity of dopamine metabolism. Cell Communication and Signaling 11, 34.

Méndez-Álvarez, E.a., Soto-Otero, R., Hermida-Ameijeiras, Á., López-Real, A.M.a., Labandeira-García, J.L., 2002. Effects of aluminum and zinc on the oxidative stress caused by 6-hydroxydopamine autoxidation: relevance for the pathogenesis of Parkinson's disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1586, 155-168.

Mendola, P., Selevan, S.G., Gutter, S., Rice, D., 2002. Environmental factors associated with a spectrum of neurodevelopmental deficits. Developmental Disabilities Research Reviews 8, 188-197.

Meneses, A., 2013. Identification of Neural Markers Accompanying Memory. Elsevier, San Diego, USA.

Meneses, A., Perez-Garcia, G., Ponce-Lopez, T., Tellez, R., Castillo, C., 2011. Serotonin transporter and memory. Neuropharmacology 61, 355-363.

Merritt, K.E., Bachtell, R.K., 2013. Initial d2 dopamine receptor sensitivity predicts cocaine sensitivity and reward in rats. PLoS One 8, e78258.

Meshalkina, D.A., Kizlyk, M.N., Kysil, E.V., Collier, A.D., Echevarria, D.J., Abreu, M.S., Barcellos, L.J., Song, C., Warnick, J.E., Kyzar, E.J., 2017. Zebrafish models of autism spectrum disorder. Experimental Neurology 299, 207-216.

Messias, J.P., Santos, T.P., Pinto, M., Soares, M.C., 2016. Stimulation of dopamine D1 receptor improves learning capacity in cooperating cleaner fish. Proceedings of the Royal Society B: Biological Sciences 283, 20152272.

Minatohara, K., Akiyoshi, M., Okuno, H., 2016. Role of immediate-early genes in synaptic plasticity and neuronal ensembles underlying the memory trace. Frontiers in Molecular Neuroscience 8, 78.

Misra, S., Hamilton, C., Niyogi, S., 2012a. Induction of oxidative stress by selenomethionine in isolated hepatocytes of rainbow trout (*Oncorhynchus mykiss*). Toxicology in Vitro 26, 621-629.

Misra, S., Peak, D., Chen, N., Hamilton, C., Niyogi, S., 2012b. Tissue-specific accumulation and speciation of selenium in rainbow trout (*Oncorhynchus mykiss*) exposed to elevated dietary selenomethionine. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 155, 560-565.

Misra, S., Peak, D., Niyogi, S., 2010. Application of XANES spectroscopy in understanding the metabolism of selenium in isolated rainbow trout hepatocytes: insights into selenium toxicity. Metallomics 2, 710-717.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., Caron, M.G., 1998. Dopamine receptors: from structure to function. Physiological Reviews 78, 189-225.

Miyazaki, I., Asanuma, M., 2008. Dopaminergic neuron-specific oxidative stress caused by dopamine itself. Acta Medica Okayama 62, 141-150.

Modgil, S., Lahiri, D.K., Sharma, V.L., Anand, A., 2014. Role of early life exposure and environment on neurodegeneration: implications on brain disorders. Translational Neurodegeneration 3, 9.

Mok, E. M., Munro, A., 1998. Effects of dopaminergic drugs on locomotor activity in teleost fish of the genus *Oreochromis* (Cichlidae): involvement of the telencephalon. Physiology and Behavior 64, 227-234.

Money, K.M., Stanwood, G.D., 2013. Developmental origins of brain disorders: roles for dopamine. Frontiers in Cellular Neuroscience 7, 260.

Montgomery Jr, E.B., 1995. Heavy metals and the etiology of Parkinson's disease and other movement disorders. Toxicology 97, 3-9.

Mora-Ferrer, C., Gangluff, V., 2002. D2-dopamine receptor blockade modulates temporal resolution in goldfish. Visual Neuroscience 19, 807-815.

Morice, E., Billard, J. M., Denis, C., Mathieu, F., Betancur, C., Epelbaum, J., Giros, B., Nosten-Bertrand, M., 2007. Parallel loss of hippocampal LTD and cognitive flexibility in a genetic model of hyperdopaminergia. Neuropsychopharmacology 32, 2108-2116.

Morris, J.A., 2009. Zebrafish: a model system to examine the neurodevelopmental basis of schizophrenia, in: Akira, S. (Ed.), Progress in Brain Research Vol. 179. Elsevier, New York, USA pp. 97-106.

Mouri, A., Noda, Y., Noda, A., Nakamura, T., Tokura, T., Yura, Y., Nitta, A., Furukawa, H., Nabeshima, T., 2007. Involvement of a dysfunctional dopamine-D1/N-methyl-d-aspartate-NR1 and Ca2+/calmodulin-dependent protein kinase II pathway in the impairment of latent learning in a model of schizophrenia induced by phencyclidine. Molecular Pharmacology 71, 1598-1609.

Mueller, T., 2012. What is the thalamus in zebrafish? Frontiers in Neuroscience 6, 64.

Muñoz, P., Melendez, C., Paris, I., Segura-Aguilar, J., 2015. Molecular and neurochemical mechanisms dopamine oxidation to o-quinones in Parkinson’s disease pathogenesis, in: Fuentes, J.M. (Ed.), Toxicity and Autophagy in Neurodegenerative Disorders. Springer, Zurich, CH, pp. 205-223.

Muscatello, J., Janz, D., 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Science of the Total Environment 407, 1318-1325.

Muscatello, J.R., Bennett, P.M., Himbeault, K.T., Belknap, A.M., Janz, D.M., 2006. Larval deformities associated with selenium accumulation in northern pike (*Esox lucius*) exposed to metal mining effluent. Environmental Science and Technology 40, 6506-6512.

Nabeshima, T., Nakayama, S., Ichihara, K., Yamada, K., Shiotani, T., Hasegawa, T., 1994. Effects of nefiracetam on drug-induced impairment of latent learning in mice in a water finding task. European Journal of Pharmacology 255, 57-65.

Nader, K., LeDoux, J., 1999a. The dopaminergic modulation of fear: quinpirole impairs the recall of emotional memories in rats. Behavioral Neuroscience 113, 152-156.

Nader, K., LeDoux, J., 1999b. Inhibition of the mesoamygdala dopaminergic pathway impairs the retrieval of conditioned fear associations. Behavioral Neuroscience 113, 891-901.

Naderi, M., Jamwal, A., Chivers, D.P., Niyogi, S., 2016a. Modulatory effects of dopamine receptors on associative learning performance in zebrafish (*Danio rerio*). Behavioural Brain Research 303, 109-119.

Naderi, M., Jamwal, A., Ferrari, M.C., Niyogi, S., Chivers, D.P., 2016b. Dopamine receptors participate in acquisition and consolidation of latent learning of spatial information in zebrafish (*Danio rerio*). Progress in Neuro-Psychopharmacology and Biological Psychiatry 67, 21-30.

Naderi, M., Salahinejad, A., Ferrari, M.C., Niyogi, S., Chivers, D.P., 2018. Dopaminergic dysregulation and impaired associative learning behavior in zebrafish during chronic dietary exposure to selenium. Environmental Pollution 237, 174-185.

Naderi, M., Salahinejad, A., Jamwal, A., Chivers, D.P., Niyogi, S., 2017. Chronic dietary selenomethionine exposure induces oxidative stress, dopaminergic dysfunction, and cognitive impairment in adult zebrafish (*Danio rerio*). Environmental Science and Technology 51, 12879-12888

Nasser, H.M., Calu, D.J., Schoenbaum, G., Sharpe, M.J., 2017. The dopamine prediction error: contributions to associative models of reward learning. Frontiers in Psychology 8, 244.

Nellore, J., Nandita, P., 2015. Paraquat exposure induces behavioral deficits in larval zebrafish during the window of dopamine neurogenesis. Toxicology Reports 2, 950-956.

Nelson, J.S., Grande, T.C., Wilson, M.V., 2016. Fishes of the World. John Wiley and Sons, New York, USA.

Neve, K.A., Seamans, J.K., Trantham-Davidson, H., 2004. Dopamine receptor signaling. Journal of Receptors and Signal Transduction 24, 165-205.

Neves, G., Cooke, S.F., Bliss, T.V., 2008. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nature Reviews Neuroscience 9, 65.

Newman, M., Verdile, G., Martins, R.N., Lardelli, M., 2011. Zebrafish as a tool in Alzheimer's disease research. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1812, 346-352.

Nguyen, L., Rigo, J. M., Rocher, V., Belachew, S., Malgrange, B., Rogister, B., Leprince, P., Moonen, G., 2001. Neurotransmitters as early signals for central nervous system development. Cell and Tissue Research 305, 187-202.

Nicoll, R.A., Roche, K.W., 2013. Long-term potentiation: peeling the onion. Neuropharmacology 74, 18-22.

Niimi, A., LaHam, Q., 1974. Influence of breeding time interval on egg number, mortality, and hatching of the zebrafish *Brachydanio rerio*. Canadian Journal of Zoology 52, 515-517.

Niki, E., 2008. Lipid peroxidation products as oxidative stress biomarkers. Biofactors 34, 171-180.

Nolan, E.B., Harrison, L.M., Lahoste, G.J., Ruskin, D.N., 2007. Behavioral synergism between D1 and D2 dopamine receptors in mice does not depend on gap junctions. Synapse 61, 279-287.

Norbury, A., Husain, M., 2015. Sensation-seeking: Dopaminergic modulation and risk for psychopathology. Behavioural Brain Research 288, 79-93.

Noudoost, B., Moore, T., 2011. The role of neuromodulators in selective attention. Trends in Cognitive Sciences 15, 585-591.

Noureddine, D., Miloud, S., Abdelkader, A., 2005. Effect of lead exposure on dopaminergic transmission in the rat brain. Toxicology 207, 363-368.

O'boyle, K., Gavin, K., Harrison, N., 1993. Chronic antagonist treatment does not alter the mode of interaction of dopamine with rat striatal dopamine receptors. Journal of Receptor Research 13, 329-339.

O'connell, L.A., Fontenot, M.R., Hofmann, H.A., 2011. Characterization of the dopaminergic system in the brain of an African cichlid fish, *Astatotilapia burtoni*. Journal of Comparative Neurology 519, 75-92.

Oldfield, J.E., 1987. The two faces of selenium. The Journal of Nutrition 117, 2002-2008.

Oliveira, C.E.S., Gai, B.M., Godoi, B., Zeni, G., Nogueira, C.W., 2012. The antidepressant-like action of a simple selenium-containing molecule, methyl phenyl selenide, in mice. European Journal of Pharmacology 690, 119-123.

Oliveira, R.F., Simões, J.M., Teles, M.C., Oliveira, C.R., Becker, J.D., Lopes, J.S., 2016. Assessment of fight outcome is needed to activate socially driven transcriptional changes in the zebrafish brain. Proceedings of the National Academy of Sciences 113, 654-661.

Olsen, C.M., Duvauchelle, C.L., 2001. Intra-prefrontal cortex injections of SCH 23390 influence nucleus accumbens dopamine levels 24 h post-infusion. Brain Research 922, 80-86.

Olvera-Cortés, M.E., Anguiano-Rodríguez, P., López-Vázquez, M.Á., Alfaro, J.M.C., 2008. Serotonin/dopamine interaction in learning. Progress in Brain Research 172, 567-602.

Ouchi, H., Ono, K., Murakami, Y., Matsumoto, K., 2013. Social isolation induces deficit of latent learning performance in mice: a putative animal model of attention deficit/hyperactivity disorder. Behavioural Brain Research 238, 146-153.

Owen, J.B., Butterfield, D.A., 2010. Measurement of oxidized/reduced glutathione ratio, in: Bross, P., Gregersen, N. (Eds.) Protein Misfolding and Cellular Stress in Disease and Aging: Concepts and protocols, Springer, New York, USA, pp. 269-277.

Pacitti, D., Lawan, M., Feldmann, J., Sweetman, J., Wang, T., Martin, S., Secombes, C., 2016. Impact of selenium supplementation on fish antiviral responses: a whole transcriptomic analysis in

rainbow trout (*Oncorhynchus mykiss*) fed supranutritional levels of Sel-Plex®. BMC Genomics 17, 116.

Packard, M.G., McGaugh, J.L., 1994. Quinpirole and d-amphetamine administration posttraining enhances memory on spatial and cued discriminations in a water maze. Psychobiology 22, 54-60.

Pakdel, R., Rashidy-Pour, A., 2007. Microinjections of the dopamine D2 receptor antagonist sulpiride into the medial prefrontal cortex attenuate glucocorticoid-induced impairment of long-term memory retrieval in rats. Neurobiology of Learning and Memory 87, 385-390.

Palace, V.P., Spallholz, J.E., Holm, J., Wautier, K., Evans, R.E., Baron, C.L., 2004. Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. Ecotoxicology and Environmental Safety 58, 17-21.

Pan, Y., Chatterjee, D., Gerlai, R., 2012. Strain dependent gene expression and neurochemical levels in the brain of zebrafish: focus on a few alcohol related targets. Physiology and Behavior 107, 773-780.

Panula, P., Chen, Y. C., Priyadarshini, M., Kudo, H., Semenova, S., Sundvik, M., Sallinen, V., 2010. The comparative neuroanatomy and neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases. Neurobiology of Disease 40, 46-57.

Panula, P., Sallinen, V., Sundvik, M., Kolehmainen, J., Torkko, V., Tiittula, A., Moshnyakov, M., Podlasz, P., 2006. Modulatory neurotransmitter systems and behavior: towards zebrafish models of neurodegenerative diseases. Zebrafish 3, 235-247.

Parra, K.V., Adrian, J.C., Gerlai, R., 2009. The synthetic substance hypoxanthine 3-N-oxide elicits alarm reactions in zebrafish (*Danio rerio*). Behavioural Brain Research 205, 336-341.

Pather, S., Gerlai, R., 2009. Shuttle box learning in zebrafish (*Danio rerio*). Behavioural Brain Research 196, 323-327.

Pavlov, I.P., 1927. Conditional Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex. Oxford University Press, London, UK.

Péczely, L., Ollmann, T., László, K., Kovács, A., Gálosi, R., Szabó, Á., Karádi, Z., Lénárd, L., 2014. Role of D1 dopamine receptors of the ventral pallidum in inhibitory avoidance learning. Behavioural Brain Research 270, 131-136.

Pérez-Cadahía, B., Drobic, B., Davie, J.R., 2011. Activation and function of immediate-early genes in the nervous system. Biochemistry and Cell Biology 89, 61-73.

Phibbs, J., Wiramanaden, C.I., Hauck, D., Pickering, I.J., Liber, K., Janz, D.M., 2011. Selenium uptake and speciation in wild and caged fish downstream of a metal mining and milling discharge. Ecotoxicology and Environmental Safety 74, 1139-1150.

Pillai, R., Uyehara-Lock, J.H., Bellinger, F.P., 2014. Selenium and selenoprotein function in brain disorders. IUBMB Life 66, 229-239.

Pinto, M.M.C., Marinho-Reis, A.P., Almeida, A., Ordens, C.M., Silva, M.M., Freitas, S., Simões, M.R., Moreira, P.I., Dinis, P.A., Diniz, M.L., 2017. Human predisposition to cognitive impairment and its relation with environmental exposure to potentially toxic elements. Environmental Geochemistry and Health, 1-18.

Pitsikas, N., Markou, A., 2014. The metabotropic glutamate 2/3 receptor agonist LY379268 counteracted ketamine-and apomorphine-induced performance deficits in the object recognition task, but not object location task, in rats. Neuropharmacology 85, 27-35.

Pogorelov, V.M., Rodriguiz, R.M., Insco, M.L., Caron, M.G., Wetsel, W.C., 2005. Novelty seeking and stereotypic activation of behavior in mice with disruption of the Dat1 gene. Neuropsychopharmacology 30, 1818-1831.

Pokorski, M., 2014. Neurotransmitter Interactions and Cognitive Function. Springer, Berlin, DE.

Ponnusamy, R., Nissim, H.A., Barad, M., 2005. Systemic blockade of D2-like dopamine receptors facilitates extinction of conditioned fear in mice. Learning and Memory 12, 399-406.

Popescu, A.T., Zhou, M.R., Poo, M.M., 2016. Phasic dopamine release in the medial prefrontal cortex enhances stimulus discrimination. Proceedings of the National Academy of Sciences 113, 3169-3176.

Portavella, M., Torres, B., Salas, C., 2004. Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium. Journal of Neuroscience 24, 2335-2342.

Powers, C.M., Levin, E.D., Seidler, F.J., Slotkin, T.A., 2011. Silver exposure in developing zebrafish produces persistent synaptic and behavioral changes. Neurotoxicology and Teratology 33, 329-332.

Prado, E.L., Dewey, K.G., 2014. Nutrition and brain development in early life. Nutrition Reviews 72, 267-284.

Praticò, D., Clark, C.M., Liun, F., Lee, V.Y. M., Trojanowski, J.Q., 2002. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Archives of Neurology 59, 972-976.

Pruitt, D.L., Bolanos, C.A., McDougall, S.A., 1995. Effects of dopamine D1 and D2 receptor antagonists on cocaine-induced place preference conditioning in preweanling rats. European Journal of Pharmacology 283, 125-131.

Puig, M., Rose, J., Schmidt, R., Freund, N., 2014. Dopamine modulation of learning and memory in the prefrontal cortex: insights from studies in primates, rodents, and birds. Frontiers in Neural Circuits 8, 93.

Puig, M.V., Miller, E.K., 2012. The role of prefrontal dopamine D1 receptors in the neural mechanisms of associative learning. Neuron 74, 874-886.

Puig, M.V., Miller, E.K., 2014. Neural substrates of dopamine D2 receptor modulated executive functions in the monkey prefrontal cortex. Cerebral Cortex 25, 2980-2987.

Quenon, L., de Xivry, J. J.O., Hanseeuw, B., Ivanoiu, A., 2015. Investigating associative learning effects in patients with prodromal Alzheimer's disease using the temporal context model. Journal of the International Neuropsychological Society 21, 699-708.

Qureshi, G., Qureshi, A., Memon, S., Parvez, S., 2006. Impact of selenium, iron, copper and zinc in on/off Parkinson's patients on L-dopa therapy. Journal of Neural Transmission Supplementa 71, 229-236.

Racké, K., Grosshans, A., Sirrenberg, S., Ziegler, K., 1988. Presynaptic regulation of the electrically evoked release of endogenous dopamine from the isolated neurointermediate lobe or isolated neural lobe of the rat pituitary gland in vitro. Naunyn-Schmiedeberg's Archives of Pharmacology 337, 504-511.

Ramamoorthi, K., Fropf, R., Belfort, G.M., Fitzmaurice, H.L., McKinney, R.M., Neve, R.L., Otto, T., Lin, Y., 2011. Npas4 regulates a transcriptional program in CA3 required for contextual memory formation. Science 334, 1669-1675.

Raman, A.V., Pitts, M.W., Seyedali, A., Hashimoto, A.C., Bellinger, F.P., Berry, M.J., 2013. Selenoprotein W expression and regulation in mouse brain and neurons. Brain and Behavior 3, 562-574.

Rammsayer, T.H., Rodewald, S., Groh, D., 2000. Dopamine-antagonistic, anticholinergic, and GABAergic effects on declarative and procedural memory functions. Cognitive Brain Research 9, 61-71.

Ramos, M., Goñi-Allo, B., Aguirre, N., 2005. Administration of SCH 23390 into the medial prefrontal cortex blocks the expression of MDMA-induced behavioral sensitization in rats: an effect mediated by 5-HT 2C receptor stimulation and not by D 1 receptor blockade. Neuropsychopharmacology 30, 2180-2191.

Rasekh, H., Davis, M., Cooke, L., Mazzio, E., Reams, R., Soliman, K., 1997. The effect of selenium on the central dopaminergic system: a microdialysis study. Life Sciences 61, 1029-1035.

Rayman, M.P., 2012. Selenium and human health. The Lancet 379, 1256-1268.

Reich, H.J., Hondal, R.J., 2016. Why nature chose selenium. ACS Chemical Biology 11, 821-841.

Reilly, C., 1996. Selenium in Food and Health. Springer, New York, USA.

Ren, G., Li, S., Zhong, H., Lin, S., 2013. Zebrafish tyrosine hydroxylase 2 gene encodes tryptophan hydroxylase. Journal of Biological Chemistry 31, 22451-22459.

Richardson, J.R., Taylor, M.M., Shalat, S.L., Guillot III, T.S., Caudle, W.M., Hossain, M.M., Mathews, T.A., Jones, S.R., Cory-Slechta, D.A., Miller, G.W., 2015. Developmental pesticide exposure reproduces features of attention deficit hyperactivity disorder. The FASEB Journal 29, 1960-1972.

Richie Jr, J.P., Muscat, J.E., Ellison, I., Calcagnotto, A., Kleinman, W., El-Bayoumy, K., 2011. Association of selenium status and blood glutathione concentrations in blacks and whites. Nutrition and Cancer 63, 367-375.

Rinaldi, A., Mandillo, S., Oliverio, A., Mele, A., 2007. D1 and D2 receptor antagonist injections in the prefrontal cortex selectively impair spatial learning in mice. Neuropsychopharmacology 32, 309-319.

Rink, E., Wullimann, M.F., 2001. The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). Brain Research 889, 316-330.

Rink, E., Wullimann, M.F., 2002a. Connections of the ventral telencephalon and tyrosine hydroxylase distribution in the zebrafish brain (*Danio rerio*) lead to identification of an ascending dopaminergic system in a teleost. Brain Research Bulletin 57, 385-387.

Rink, E., Wullimann, M.F., 2002b. Development of the catecholaminergic system in the early zebrafish brain: an immunohistochemical study. Developmental Brain Research 137, 89-100.

Roberts, A.C., Bill, B.R., Glanzman, D.L., 2013. Learning and memory in zebrafish larvae. Frontiers in Neural Circuits 7, 126.

Roberts, A.D., Pearce, J.M., 1999. Blocking in the Morris swimming pool. Journal of Experimental Psychology: Animal Behavior Processes 25, 225-235.

Robinson, J.E., Gradinaru, V., 2018. Dopaminergic dysfunction in neurodevelopmental disorders: recent advances and synergistic technologies to aid basic research. Current Opinion in Neurobiology 48, 17-29.

Rocchetti, J., Isingrini, E., Dal Bo, G., Sagheby, S., Menegaux, A., Tronche, F., Levesque, D., Moquin, L., Gratton, A., Wong, T.P., 2015. Presynaptic D2 dopamine receptors control long-term depression expression and memory processes in the temporal hippocampus. Biological Psychiatry 77, 513-525.

Rodríguez, F., López, J.C., Vargas, J.P., Gómez, Y., Broglio, C., Salas, C., 2002. Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. Journal of Neuroscience 22, 2894-2903.

Romero-Ramos, M., Venero, J.L., Cano, J., Machado, A., 2000. Low selenium diet induces tyrosine hydroxylase enzyme in nigrostriatal system of the rat. Molecular Brain Research 84, 7-16.

Rosenberg, T., Gal-Ben-Ari, S., Dieterich, D.C., Kreutz, M.R., Ziv, N.E., Gundelfinger, E.D., Rosenblum, K., 2014. The roles of protein expression in synaptic plasticity and memory consolidation. Frontiers in Molecular Neuroscience 7, 86.

Rossato, J.I., Bevilaqua, L.R., Izquierdo, I., Medina, J.H., Cammarota, M., 2009. Dopamine controls persistence of long-term memory storage. Science 325, 1017-1020.

Rossato, J.I., Radiske, A., Kohler, C.A., Gonzalez, C., Bevilaqua, L.R., Medina, J.H., Cammarota, M., 2013. Consolidation of object recognition memory requires simultaneous activation of dopamine D1/D5 receptors in the amygdala and medial prefrontal cortex but not in the hippocampus. Neurobiology of Learning and Memory 106, 66-70.

Rudolph, B. L., Andreller, I., Kennedy, C.J., 2008. Reproductive success, early life stage development, and survival of westslope cutthroat trout (*Oncorhynchus clarki lewisi*) exposed to elevated selenium in an area of active coal mining. Environmental Science and Technology 42, 3109-3114.

Ruhl, T., Jonas, A., Seidel, N.I., Prinz, N., Albayram, O., Bilkei-Gorzo, A., von der Emde, G., 2016. Oxidation and cognitive impairment in the aging zebrafish. Gerontology 62, 47-57.

Saili, K.S., Corvi, M.M., Weber, D.N., Patel, A.U., Das, S.R., Przybyla, J., Anderson, K.A., Tanguay, R.L., 2012. Neurodevelopmental low-dose bisphenol A exposure leads to early life-stage hyperactivity and learning deficits in adult zebrafish. Toxicology 291, 83-92.

Saito, K., Watanabe, S., 2006. Deficits in acquisition of spatial learning after dorsomedial telencephalon lesions in goldfish. Behavioural Brain Research 172, 187-194.

Salas, C., Broglio, C., Durán, E., Gómez, A., Ocaña, F.M., Jiménez-Moya, F., Rodríguez, F., 2006. Neuropsychology of learning and memory in teleost fish. Zebrafish 3, 157-171.

Salas, C., Rodríguez, F., Vargas, J.P., Durán, E., Torres, B., 1996. Spatial learning and memory deficits after telencephalic ablation in goldfish trained in place and turn maze procedures. Behavioral Neuroscience 110, 965-980.

Sánchez-Iglesias, S., Méndez-Álvarez, E., Iglesias-González, J., Muñoz-Patiño, A., Sánchez-Sellero, I., Labandeira-García, J.L., Soto-Otero, R., 2009. Brain oxidative stress and selective behaviour of aluminium in specific areas of rat brain: potential effects in a 6-OHDA-induced model of Parkinson's disease. Journal of Neurochemistry 109, 879-888.

Sarkar, S., Mukherjee, S., Chattopadhyay, A., Bhattacharya, S., 2014. Low dose of arsenic trioxide triggers oxidative stress in zebrafish brain: expression of antioxidant genes. Ecotoxicology and Environmental Safety 107, 1-8.

Sato, N., 2017. Memory processing in the nervous system, in: Schuster, A.J. (Ed), Understanding Information, Springer, Cham, CH, pp. 83-98.

Savio, L.E.B., Vuaden, F.C., Piato, A.L., Bonan, C.D., Wyse, A.T., 2012. Behavioral changes induced by long-term proline exposure are reversed by antipsychotics in zebrafish. Progress in Neuro-Psychopharmacology and Biological Psychiatry 36, 258-263.

Scerbina, T., Chatterjee, D., Gerlai, R., 2012. Dopamine receptor antagonism disrupts social preference in zebrafish: a strain comparison study. Amino Acids 43, 2059-2072.

Schantz, S.L., Widholm, J.J., 2001. Cognitive effects of endocrine-disrupting chemicals in animals. Environmental Health Perspectives 109, 1197-1206.

Schlenk, D., Zubcov, N., Zubcov, E., 2003. Effects of salinity on the uptake, biotransformation, and toxicity of dietary seleno-L-methionine to rainbow trout. Toxicological Sciences 75, 309-313.

Schmidt, R., Strähle, U., Scholpp, S., 2013. Neurogenesis in zebrafish–from embryo to adult. Neural Development 8, 3.

Schmitz, Y., Benoit-Marand, M., Gonon, F., Sulzer, D., 2003. Presynaptic regulation of dopaminergic neurotransmission. Journal of Neurochemistry 87, 273-289.

Schuler, C.A., Anthony, R.G., Ohlendorf, H.M., 1990. Selenium in wetlands and waterfowl foods at Kesterson Reservoir, California, 1984. Archives of Environmental Contamination and Toxicology 19, 845-853.

Schultz, W., 2002. Getting formal with dopamine and reward. Neuron 36, 241-263.

Schultz, W., 2016. Dopamine reward prediction error coding. Dialogues in Clinical Neuroscience 18, 23.

Schwarz, K., Foltz, C.M., 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. Journal of the American Chemical Society 79, 3292-3293.

Schwegler, H., Crusio, W.E., 1995. Correlations between radial-maze learning and structural variations of septum and hippocampus in rodents. Behavioural Brain Research 67, 29-41.

Schweitzer, J., Driever, W., 2009. Development of the dopamine systems in zebrafish, in: Pasterkamp, R.J., Smidt, M.P., Burbach, J.P.H. (Eds.), Development and Engineering of Dopamine Neurons. Springer, New York, USA, pp. 1-14.

Scimeca, J.M., Badre, D., 2012. Striatal contributions to declarative memory retrieval. Neuron 75, 380-392.

Seel, N.M., 2011. Encyclopedia of the Sciences of Learning. Springer, New York, USA.

Seeman, P., 1980. Brain dopamine receptors. Pharmacological Reviews 32, 229-313.

Semenova, S., Rozov, S., Panula, P., 2017. Distribution, properties, and inhibitor sensitivity of zebrafish catechol-O-methyl transferases (COMT). Biochemical Pharmacology 145, 147-157.

Senger, M.R., Rico, E.P., de Bem Arizi, M., Frazzon, A.P.G., Dias, R.D., Bogo, M.R., Bonan, C.D., 2006. Exposure to Hg^{2+} and Pb^{2+} changes NTPDase and ecto-5′-nucleotidase activities in central nervous system of zebrafish (*Danio rerio*). Toxicology 226, 229-237.

Shahar, A., Patel, K.V., Semba, R.D., Bandinelli, S., Shahar, D.R., Ferrucci, L., Guralnik, J.M., 2010. Plasma selenium is positively related to performance in neurological tasks assessing coordination and motor speed. Movement Disorders 25, 1909-1915.

Shams, S., Amlani, S., Buske, C., Chatterjee, D., Gerlai, R., 2018. Developmental social isolation affects adult behavior, social interaction, and dopamine metabolite levels in zebrafish. Developmental Psychobiology 60, 43-56.

Shao, F., Han, X., Li, N., Wang, W., 2010. Adolescent chronic apomorphine treatment impairs latent inhibition and reduces prefrontal cortex mGluR5 receptor expression in adult rats. European Journal of Pharmacology 649, 202-205.

Shilliam, C.S., Heidbreder, C.A., 2003. Gradient of dopamine responsiveness to dopamine receptor agonists in subregions of the rat nucleus accumbens. European Journal of Pharmacology 477, 113-122.

Sison, M., Cawker, J., Buske, C., Gerlai, R., 2006. Fishing for genes influencing vertebrate behavior: zebrafish making headway. Lab Animal 35, 33-39.

Sison, M., Gerlai, R., 2010. Associative learning in zebrafish (*Danio rerio*) in the plus maze. Behavioural Brain Research 207, 99-104.

Sison, M., Gerlai, R., 2011. Associative learning performance is impaired in zebrafish (*Danio rerio*) by the NMDA-R antagonist MK-801. Neurobiology of Learning and Memory 96, 230-237.

Skelhorn, J., Halpin, C.G., Rowe, C., 2016. Learning about aposematic prey. Behavioral Ecology 27, 955-964.

Smeets, W.J., González, A., 2000. Catecholamine systems in the brain of vertebrates: new perspectives through a comparative approach. Brain Research Reviews 33, 308-379.

Smith, L.E., Carvan, M.J., Dellinger, J.A., Ghorai, J.K., White, D.B., Williams, F.E., Weber, D.N., 2010. Developmental selenomethionine and methylmercury exposures affect zebrafish learning. Neurotoxicology and Teratology 32, 246-255.

Solovyev, N.D., 2015. Importance of selenium and selenoprotein for brain function: from antioxidant protection to neuronal signalling. Journal of Inorganic Biochemistry 153, 1-12.

Sommer, W.H., Costa, R.M., Hansson, A.C., 2014. Dopamine systems adaptation during acquisition and consolidation of a skill. Frontiers in Integrative Neuroscience 8, 87.

Souza, A.C.G., Stangherlin, E.C., Ardais, A.P., Nogueira, C.W., 2010. Diphenyl diselenide and diphenyl ditelluride: neurotoxic effect in brain of young rats, in vitro. Molecular and Cellular Biochemistry 340, 179-185.

Souza, B.R., Tropepe, V., 2011. The role of dopaminergic signalling during larval zebrafish brain development: a tool for investigating the developmental basis of neuropsychiatric disorders. Reviews in the Neurosciences 22, 107-119.

Spallholz, J.E., Palace, V.P., Reid, T.W., 2004. Methioninase and selenomethionine but not Se-methylselenocysteine generate methylselenol and superoxide in an in vitro chemiluminescent assay: implications for the nutritional carcinostatic activity of selenoamino acids. Biochemical Pharmacology 67, 547-554.

Spano, P., Govoni, S., Trabucchi, M., 1978. Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system. Advances in Biochemical Psychopharmacology 19, 155-165.

Speedie, N., Gerlai, R., 2008. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). Behavioural Brain Research 188, 168-177.

Spence, R., Gerlach, G., Lawrence, C., Smith, C., 2008. The behaviour and ecology of the zebrafish, *Danio rerio*. Biological Reviews 83, 13-34.

Spencer, G., Klumperman, J., Syed, N., 1998. Neurotransmitters and neurodevelopment. Role of dopamine in neurite outgrowth, target selection and specific synapse formation. Perspectives on Developmental Neurobiology 5, 451-467.

Squire, L.R., 1992. Declarative and nondeclarative memory: Multiple brain systems supporting learning and memory. Journal of Cognitive Neuroscience 4, 232-243.

Stadtman, T.C., 1996. Selenocysteine. Annual Review of Biochemistry 65, 83-100.

Steinbrenner, H., Sies, H., 2013. Selenium homeostasis and antioxidant selenoproteins in brain: implications for disorders in the central nervous system. Archives of Biochemistry and Biophysics 536, 152-157.

Steullet, P., Lavoie, S., Kraftsik, R., Guidi, R., Gysin, R., Cuénod, M., Do, K.Q., 2008. A glutathione deficit alters dopamine modulation of L-type calcium channels via D2 and ryanodine receptors in neurons. Free Radical Biology and Medicine 44, 1042-1054.

Stiles, J., Jernigan, T.L., 2010. The basics of brain development. Neuropsychology Review 20, 327-348.

Stouffer, E.M., Klein, J.E., 2013. Lesions of the lateral entorhinal cortex disrupt non-spatial latent learning but spare spatial latent learning. Acta Neurobiologiae Experimentalis 73, 430-437.

Stouffer, E.M., White, N.M., 2006. Neural circuits mediating latent learning and conditioning for salt in the rat. Neurobiology of Learning and Memory 86, 91-99.

Straube, B., 2012. An overview of the neuro-cognitive processes involved in the encoding, consolidation, and retrieval of true and false memories. Behavioral and Brain Functions 8, 35.

Streisinger, G., Walker, C., Dower, N., Knauber, D., Singer, F., 1981. Production of clones of homozygous diploid zebra fish (*Brachydanio rerio*). Nature 291, 293-296.

Sun, X., Lin, Y., 2016. Npas4: linking neuronal activity to memory. Trends in Neurosciences 39, 264-275.

Sweatt, J.D., 2009. Mechanisms of Memory. Academic Press, New York, USA.

Szapiro, G., Galante, J.M., Barros, D.M., De Stein, M.L., Vianna, M.R., Izquierdo, L.A., Izquierdo, I., Medina, J.H., 2002. Molecular mechanisms of memory retrieval. Neurochemical Research 27, 1491-1498.

Takahashi, M., Shirakawa, O., Toyooka, K., Kitamura, N., Hashimoto, T., Maeda, K., Koizumi, S., Wakabayashi, K., Takahashi, H., Someya, T., 2000. Abnormal expression of brain-derived neurotrophic factor and its receptor in the corticolimbic system of schizophrenic patients. Molecular Psychiatry 5, 293-300.

Talwar, P.K., 1991. Inland fishes of India and adjacent countries. Oxford and I. B. H. Publishing, Calcutta, IN.

Tang, Y. L., Wang, S. W., Lin, S. M., 2008. Both inorganic and organic selenium supplements can decrease brain monoamine oxidase B enzyme activity in adult rats. British Journal of Nutrition 100, 660-665.

Tang, Y.P., Noda, Y., Nabeshima, T., 1997. Involvement of activation of dopamineraic neuronal system in learning and memory deficits associated with experimental mild traumatic brain injury. European Journal of Neuroscience 9, 1720-1727.

Tashjian, D.H., Teh, S.J., Sogomonyan, A., Hung, S.S., 2006. Bioaccumulation and chronic toxicity of dietary l-selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*). Aquatic Toxicology 79, 401-409.

Tay, T.L., Ronneberger, O., Ryu, S., Nitschke, R., Driever, W., 2011. Comprehensive catecholaminergic projectome analysis reveals single-neuron integration of zebrafish ascending and descending dopaminergic systems. Nature Communications 2, 171.

Tekin, I., Roskoski, R., Carkaci-Salli, N., Vrana, K.E., 2014. Complex molecular regulation of tyrosine hydroxylase. Journal of Neural Transmission 121, 1451-1481.

Teles, M.C., Cardoso, S.D., Oliveira, R.F., 2016. Social plasticity relies on different neuroplasticity mechanisms across the brain social decision-making network in zebrafish. Frontiers in Behavioral Neuroscience 10, 16.

Teles, M.C., Dahlbom, S.J., Winberg, S., Oliveira, R.F., 2013. Social modulation of brain monoamine levels in zebrafish. Behavioural Brain Research 253, 17-24.

Terriente, J., Pujades, C., 2013. Use of zebrafish embryos for small molecule screening related to cancer. Developmental Dynamics 242, 97-107.

Thomas, J., Janz, D., 2011. Dietary selenomethionine exposure in adult zebrafish alters swimming performance, energetics and the physiological stress response. Aquatic Toxicology 102, 79-86.

Thomas, J., Janz, D., 2016. Embryo microinjection of selenomethionine reduces hatchability and modifies oxidant responsive gene expression in zebrafish. Scientific Reports 6, 26520.

Thomas, J.K., Janz, D.M., 2014. In ovo exposure to selenomethionine via maternal transfer increases developmental toxicities and impairs swim performance in F1 generation zebrafish (*Danio rerio*). Aquatic Toxicology 152, 20-29.

Thomas, J.K., Janz, D.M., 2015. Developmental and persistent toxicities of maternally deposited selenomethionine in zebrafish (*Danio rerio*). Environmental Science and Technology 49, 10182-10189.

Ting-A-Kee, R., Mercuriano, L.E., Vargas-Perez, H., George, S.R., van der Kooy, D., 2013. Dopamine D1 receptors are not critical for opiate reward but can mediate opiate memory retrieval in a state-dependent manner. Behavioural Brain Research 247, 174-177.

Tolman, E.C., Honzik, C.H., 1930. Introduction and removal of reward, and maze performance in rats. University of California Publications in Psychology 4, 257-275.

Tournier, B.B., Steimer, T., Millet, P., Moulin-Sallanon, M., Vallet, P., Ibañez, V., Ginovart, N., 2013. Innately low D2 receptor availability is associated with high novelty-seeking and enhanced behavioural sensitization to amphetamine. International Journal of Neuropsychopharmacology 16, 1819-1834.

Tran, A.H., Tamura, R., Uwano, T., Kobayashi, T., Katsuki, M., Matsumoto, G., Ono, T., 2002. Altered accumbens neural response to prediction of reward associated with place in dopamine D2 receptor knockout mice. Proceedings of the National Academy of Sciences 99, 8986-8991.

Tran, S., Nowicki, M., Muraleetharan, A., Gerlai, R., 2015. Differential effects of dopamine D 1 and D 2/3 receptor antagonism on motor responses. Psychopharmacology 232, 795-806.

Truong, L., Mandrell, D., Mandrell, R., Simonich, M., Tanguay, R.L., 2014. A rapid throughput approach identifies cognitive deficits in adult zebrafish from developmental exposure to polybrominated flame retardants. Neurotoxicology 43, 134-142.

Tsuang, M.T., Bar, J.L., Stone, W.S., Faraone, S.V., 2004. Gene-environment interactions in mental disorders. World Psychiatry 3, 73-83.

Tsunoda, M., Johnson, V., Sharma, R., 2000. Increase in dopamine metabolites in murine striatum after oral exposure to inorganic but not organic form of selenium. Archives of Environmental Contamination and Toxicology 39, 32-37.

USEPA, 2016. Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater 2016. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC, USA.

Valente, A., Huang, K. H., Portugues, R., Engert, F., 2012. Ontogeny of classical and operant learning behaviors in zebrafish. Learning and Memory 19, 170-177.

Van Cutsem, J., Van Gerven, F., Fransen, J., Schrooten, P., Janssen, P., 1990. The in vitro antifungal activity of ketoconazole, zinc pyrithione, and selenium sulfide against Pityrosporum and their efficacy as a shampoo in the treatment of experimental pityrosporosis in guinea pigs. Journal of the American Academy of Dermatology 22, 993-998.

Verstraeten, S.V., Aimo, L., Oteiza, P.I., 2008. Aluminium and lead: molecular mechanisms of brain toxicity. Archives of Toxicology 82, 789-802.

Vidal-Gadea, A., Topper, S., Young, L., Crisp, A., Kressin, L., Elbel, E., Maples, T., Brauner, M., Erbguth, K., Axelrod, A., 2011. *Caenorhabditis elegans* selects distinct crawling and swimming gaits via dopamine and serotonin. Proceedings of the National Academy of Sciences, 108, 17504-17509.

Vijayraghavan, S., Wang, M., Birnbaum, S.G., Williams, G.V., Arnsten, A.F., 2007. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nature Neuroscience 10, 376-384.

Vinceti, M., Bonvicini, F., Bergomi, M., Malagoli, C., 2010. Possible involvement of overexposure to environmental selenium in the etiology of amyotrophic lateral sclerosis: a short review. Annali dell'Istituto Superiore di Sanita 46, 279-283.

Vinceti, M., Chiari, A., Eichmüller, M., Rothman, K.J., Filippini, T., Malagoli, C., Weuve, J., Tondelli, M., Zamboni, G., Nichelli, P.F., 2017. A selenium species in cerebrospinal fluid predicts conversion to Alzheimer's dementia in persons with mild cognitive impairment. Alzheimer's Research and Therapy 9, 100.

Vinceti, M., Mandrioli, J., Borella, P., Michalke, B., Tsatsakis, A., Finkelstein, Y., 2014. Selenium neurotoxicity in humans: bridging laboratory and epidemiologic studies. Toxicology Letters 230, 295-303.

Vinceti, M., Solovyev, N., Mandrioli, J., Crespi, C.M., Bonvicini, F., Arcolin, E., Georgoulopoulou, E., Michalke, B., 2013. Cerebrospinal fluid of newly diagnosed amyotrophic lateral sclerosis patients exhibits abnormal levels of selenium species including elevated selenite. Neurotoxicology 38, 25-32.

Wang, H., Zhong, X., Shi, W. Y., Guo, B., 2011. Study of malondialdehyde (MDA) content, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in chickens infected with avian infectious bronchitis virus. African Journal of Biotechnology 10, 9213-9217.

Wang, K., Peng, C., Huang, J., Huang, Y., Jin, M., Geng, Y., 2013. The pathology of selenium deficiency in *Cyprinus carpio* L. Journal of Fish Diseases 36, 609-615.

Wang, Q., Yu, Y., Li, J., Wan, Y., Huang, Q., Guo, Y., Li, H., 2017. Effects of different forms of selenium fertilizers on Se accumulation, distribution, and residual effect in winter wheat–summer maize rotation system. Journal of Agricultural and Food Chemistry 65, 1116-1123.

Wang, X.H., Souders II, C.L., Zhao, Y.H., Martyniuk, C.J., 2018. Paraquat affects mitochondrial bioenergetics, dopamine system expression, and locomotor activity in zebrafish (*Danio rerio*). Chemosphere 191, 106-117.

Wang, Y., Ge, W., 2004a. Cloning of zebrafish ovarian P450c17 (CYP17, 17α-hydroxylase/17, 20-lyase) and characterization of its expression in gonadal and extra-gonadal tissues. General and Comparative Endocrinology 135, 241-249.

Wang, Y., Ge, W., 2004b. Developmental profiles of activin βA, βB, and follistatin expression in the zebrafish ovary: evidence for their differential roles during sexual maturation and ovulatory cycle. Biology of Reproduction 71, 2056-2064.

Wang, Z., Becker, H., 2013. Ratios of S, Se and Te in the silicate Earth require a volatile-rich late veneer. Nature 499, 328-331.

Ward, R.D., Simpson, E.H., Richards, V.L., Deo, G., Taylor, K., Glendinning, J.I., Kandel, E.R., Balsam, P.D., 2012. Dissociation of hedonic reaction to reward and incentive motivation in an animal model of the negative symptoms of schizophrenia. Neuropsychopharmacology 37, 1699-1 707.

Watson, D.J., Loiseau, F., Ingallinesi, M., Millan, M.J., Marsden, C.A., Fone, K.C., 2012. Selective blockade of dopamine D3 receptors enhances while D2 receptor antagonism impairs social novelty discrimination and novel object recognition in rats: a key role for the prefrontal cortex. Neuropsychopharmacology 37, 770-786.

Wells, P.G., McCallum, G.P., Chen, C.S., Henderson, J.T., Lee, C.J., Perstin, J., Preston, T.J., Wiley, M.J., Wong, A.W., 2009. Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. Toxicological Sciences 108, 4-18.

Westerfield, M., 2007. The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (*Danio rerio*). University of Oregon press, Eugene, OR, USA.

White, F.J., Wang, R.Y., 1984. Pharmacological characterization of dopamine autoreceptors in the rat ventral tegmental area: microiontophoretic studies. Journal of Pharmacology and Experimental Therapeutics 231, 275-280.

White, P.J., Bowen, H.C., Parmaguru, P., Fritz, M., Spracklen, W., Spiby, R., Meacham, M., Mead, A., Harriman, M., Trueman, L., 2004. Interactions between selenium and sulphur nutrition in Arabidopsis thaliana. Journal of Experimental Botany 55, 1927-1937.

Williams, G.V., Goldman-Rakic, P.S., 1995. Modulation of memory fields by dopamine Dl receptors in prefrontal cortex. Nature 376, 572-575.

Wirth, E.K., Conrad, M., Winterer, J., Wozny, C., Carlson, B.A., Roth, S., Schmitz, D., Bornkamm, G.W., Coppola, V., Tessarollo, L., 2010. Neuronal selenoprotein expression is required for interneuron development and prevents seizures and neurodegeneration. The FASEB Journal 24, 844-852.

Wise, R.A., 2004. Dopamine, learning and motivation. Nature Reviews Neuroscience 5, 483-494.

Wiseman, S., Thomas, J.K., Higley, E., Hursky, O., Pietrock, M., Raine, J.C., Giesy, J.P., Janz, D.M., Hecker, M., 2011a. Chronic exposure to dietary selenomethionine increases gonadal steroidogenesis in female rainbow trout. Aquatic Toxicology 105, 218-226.

Wiseman, S., Thomas, J.K., McPhee, L., Hursky, O., Raine, J.C., Pietrock, M., Giesy, J.P., Hecker, M., Janz, D.M., 2011b. Attenuation of the cortisol response to stress in female rainbow trout chronically exposed to dietary selenomethionine. Aquatic Toxicology 105, 643-651.

Woock, S.E., Garrett, W.R., Partin, W.E., Bryson, W.T., 1987. Decreased survival and teratogenesis during laboratory selenium exposures to bluegill, *Lepomis macrochirus*. Bulletin of Environmental Contamination and Toxicology 39, 998-1005.

Wullimann, M.F., Mueller, T., 2004. Teleostean and mammalian forebrains contrasted: evidence from genes to behavior. Journal of Comparative Neurology 475, 143-162.

Xu, T. X., Sotnikova, T.D., Liang, C., Zhang, J., Jung, J.U., Spealman, R.D., Gainetdinov, R.R., Yao, W. D., 2009. Hyperdopaminergic tone erodes prefrontal long-term potential via a D2 receptor-operated protein phosphatase gate. Journal of Neuroscience 29, 14086-14099.

Xu, X., Scott-Scheiern, T., Kempker, L., Simons, K., 2007. Active avoidance conditioning in zebrafish (*Danio rerio*). Neurobiology of Learning and Memory 87, 72-77.

Xu, X., Weber, D., Burge, R., VanAmberg, K., 2016. Neurobehavioral impairments produced by developmental lead exposure persisted for generations in zebrafish (*Danio rerio*). Neurotoxicology 52, 176-185.

Xu, X., Weber, D., Carvan, M.J., Coppens, R., Lamb, C., Goetz, S., Schaefer, L.A., 2012. Comparison of neurobehavioral effects of methylmercury exposure in older and younger adult zebrafish (*Danio rerio*). Neurotoxicology 33, 1212-1218.

y Cajal, S.R., 1894. The Croonian lecture.—La fine structure des centres nerveux. Proceedings of the Royal Society of London 55, 444-468.

Yamamoto, K., Ruuskanen, J.O., Wullimann, M.F., Vernier, P., 2011. Differential expression of dopaminergic cell markers in the adult zebrafish forebrain. Journal of Comparative Neurology 519, 576-598.

Yamamoto, K., Vernier, P., 2011. The evolution of dopamine systems in chordates. Frontiers in Neuroanatomy 5, 21.

Yang, X., Bao, Y., Fu, H., Li, L., Ren, T., Yu, X., 2014. Selenium protects neonates against neurotoxicity from prenatal exposure to manganese. PloS One 9, e86611.

Yang, X., Yu, X., Fu, H., Li, L., Ren, T., 2013. Different levels of prenatal zinc and selenium had different effects on neonatal neurobehavioral development. Neurotoxicology 37, 35-39.

Yanik, M., Kocyigit, A., Tutkun, H., Vural, H., Herken, H., 2004. Plasma manganese, selenium, zinc, copper, and iron concentrations in patients with schizophrenia. Biological Trace Element Research 98, 109-117.

Yawata, S., Yamaguchi, T., Danjo, T., Hikida, T., Nakanishi, S., 2012. Pathway-specific control of reward learning and its flexibility via selective dopamine receptors in the nucleus accumbens. Proceedings of the National Academy of Sciences 109, 12764-12769.

Young, E.A., Dreumont, S.E., Cunningham, C.L., 2014. Role of nucleus accumbens dopamine receptor subtypes in the learning and expression of alcohol-seeking behavior. Neurobiology of Learning and Memory 108, 28-37.

Zala, S.M., Määttänen, I., 2013. Social learning of an associative foraging task in zebrafish. Naturwissenschaften 100, 469-472.

Zald, D.H., Cowan, R.L., Riccardi, P., Baldwin, R.M., Ansari, M.S., Li, R., Shelby, E.S., Smith, C.E., McHugo, M., Kessler, R.M., 2008. Midbrain dopamine receptor availability is inversely associated with novelty-seeking traits in humans. Journal of Neuroscience 28, 14372-14378.

Zee, J., Patterson, S., Wiseman, S., Hecker, M., 2016. Is hepatic oxidative stress a main driver of dietary selenium toxicity in white sturgeon (*Acipenser transmontanus*)? Ecotoxicology and Environmental Safety 133, 334-340.

Zhang, Y., Zhou, Y., Schweizer, U., Savaskan, N.E., Hua, D., Kipnis, J., Hatfield, D.L., Gladyshev, V.N., 2007. Comparative analysis of selenocysteine machinery and selenoproteome gene expression in mouse brain identifies neurons as key functional sites of selenium in mammals. Journal of Biological Chemistry 283, 2427-2438.

Zubieta, J. K., Huguelet, P., Ohl, L.E., Koeppe, R.A., Kilbourn, M.R., Carr, J.M., Giordani, B.J., Frey, K.A., 2000. High vesicular monoamine transporter binding in asymptomatic bipolar I disorder: sex differences and cognitive correlates. American Journal of Psychiatry 157, 1619-1628.

Zündorf, G., Reiser, G., 2011. Calcium dysregulation and homeostasis of neural calcium in the molecular mechanisms of neurodegenerative diseases provide multiple targets for neuroprotection. Antioxidants and Redox Signaling 14, 1275-1288.