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Anatomy and Function of Synaptic Zinc in the Striatum

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by

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Abstract

Synaptic zinc is located in many regions of the brain. One area that contains a high amount is the input center of the basal ganglia: the striatum. Environmental enrichment was used to examine potential changes in morphology of striatal cells of mice with (ZnT3 wildtype) and without (ZnT3 knockout) the zinc transporter (ZnT3) necessary to load zinc into vesicles. No changes were found in dendritic length for any regions of the striatum. However, all regions of the striatum showed an increase in spine density in both genotypes, with enriched ZnT3 KO mice having a greater increase in the nucleus accumbens region. ZnT3 mice were also tested on a battery of motor behavioural tasks. ZnT3 KO mice may be hyperactive, as evidenced by their performance on the pole task, and impulsive, as evidenced by their inaccurate performance on a skilled reach task, when compared to ZnT3 WT mice.

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List of Symbols, Abbreviations, and Nomenclature

5CSRTT: 5 choice serial reaction time task;

6-OHDA: 6-hydroxydopamine;

ADHD: attention deficit hyperactivity disorder;

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid;

ASD: autism spectrum disorder;

BDNF: brain-derived neurotrophic factor;

DA: dopamine;

DLS: dorsolateral striatum;

DMS: dorsomedial striatum;

GABA: gamma-aminobutyric acid;

GPR: g-protein coupled receptor;

KO: knockout;

LTD: long-term depression;

LTP: long-term potentiation;

MPP+: 1-methyl-4-phenylpyridinium;

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine;

MSN: medium spiny neuron;

NAc: nucleus accumbens;

NMDA: N-methyl-D-aspartate;

OCD: obsessive-compulsive disorder;

SN: substantia nigra;

TrkB: tropomyosin-related kinase B;

WT: wildtype;

ZnT3: zinc transporter 3;

Chapter One: **General Introduction**

1.1 Introduction

Zinc is found in all cells of the body and is essential for life (Chasapis et al., 2012). In the brain, a proportion of zinc is located in vesicles inside axonal boutons of neurons (called zincergic neurons) and can be released alongside glutamate (Frederickson et al., 2000; Frederickson et al., 2005). This synaptic zinc is found mostly in the forebrain, including the input center for the basal ganglia: the striatum (Brown and Dyck, 2004a). The basal ganglia are a group of nuclei located within the forebrain that are involved in fine motor control, motor learning and memory, and certain aspects of cognition (reviewed by Liljeholm and O'Doherty, 2012). The purpose of this thesis is to expand current knowledge of both the anatomy and function of synaptic zinc in the striatum. This chapter will begin with an overview of research done on synaptic zinc, then discuss research on the striatum, and finish with an overview of what is currently known about synaptic zinc in the striatum.

1.2 Zincergic signaling in the brain

1.2.1 Zinc location in brain

As mentioned above, synaptic zinc is found in almost all areas of the forebrain (Frederickson et al., 1992). Zinc is packaged into synaptic vesicles by zinc transporter 3 (ZnT3; Cole et al., 1999). The zinc is released from the vesicles into the synaptic cleft (synaptic zinc) and can act on post-synaptic neurons (reviewed by Nakashima & Dyck, 2009). There are two ways to examine synaptic zinc distribution: stain for zinc that is present in the synapse or do tract tracing studies that show zincergic cell bodies. Staining for synaptic zinc has historically been

done using an autometallographic technique (Danscher, 1982) or more recently by using fluorescent probes (Frederickson et al., 1992; Fahrni and O'Halloran, 1999; Hirano et al., 2002; Lim et al., 2005). The autometallographic technique involves injecting sodium selenite into the animal, usually intraperitoneallly, and waiting approximately an hour for the selenite to bind to the zinc present in the synapse and form a zinc-selenide crystal. The crystals can then be visualized by developing brain slices in a silver-lactate solution (Danscher, 1981). A study comparing the autometallographic technique with a fluorescent probe for zinc found that both methods stained approximately the same areas, with the exception being that areas stained lightly with the autometallographic technique did not show reliably with the fluorescent technique. Synaptic zinc was found in the hippocampus, amygdala, septal nuclei, bed nucleus of the stria terminalis, regions of the hypothalamus, neocortex (high amounts of staining in layers I-III and V, with lower amounts of staining in layer IV), perirhinal cortex, caudate-putamen, nucleus accumbens, fundus, and olfactory tubercle (Frederickson et al., 1992).

To image zincergic cell bodies, sodium selenite is injected either intraperitoneally to image all cell bodies or intracranially to image cell bodies for a specific region. Slices are stained the same way as above (using the autometallographic technique) or using a silver enhancement kit. However, the staining will label both the zincergic cell bodies and the synaptic zinc. Pre-treating brain slices with hydrogen peroxide (H_2O_2) 24 hours after sodium selenite treatment allows visualization of only the cell bodies without having the synaptic zinc stained as well (Brown and Dyck, 2003). Using this method, Brown and Dyck (2004) showed that zincergic neurons were found mainly in the cerebral cortex, hippocampus, and amygdala, but also in anterior olfactory nuclei, claustrum, tenia tecta, endopiriform region, lateral ventricle, lateral septum, zona incerta, superior colliculus, and periaqueductal grey. In the cerebral cortex, cell

bodies were located in layers II/III, V, and VI with no zincergic neurons in layer IV. However, no cell bodies were found in striatum (caudate-putamen, nucleus accumbens; Slomianka et al., 1990; Brown and Dyck, 2004a) indicating that the zinc found there must be coming in from neurons outside the striatum.

1.2.2 Receptor modulation

Zinc has been found to modulate several types of receptors including glutamatergic, GABAergic, dopaminergic, serotonergic, purinergic, glycinergic, and nicotinic receptors (reviewed by Nakashima and Dyck, 2009). Recently, two orphan G-protein coupled receptors have been found to be zinc-specific: GPR39 (Holst et al., 2007; Yasuda et al., 2007; Cohen et al., 2012) and GPR83 (Muller et al., 2013). In addition, zinc has been implicated in the tropomyosin-related kinase signaling pathway which is activated by brain derived neurotrophic factor (BDNF; Hwang et al., 2005). Zinc can also enter postsynaptic cells and affect second messenger systems including cAMP, CAMKII, and PKA (reviewed by Nakashima and Dyck, 2009). Figure 1.1 provides an overview of zincergic signalling at a synapse. Zinc's effects on the glutamatergic, dopaminergic and BDNF systems will be discussed in more detail below as they are most relevant to this thesis.

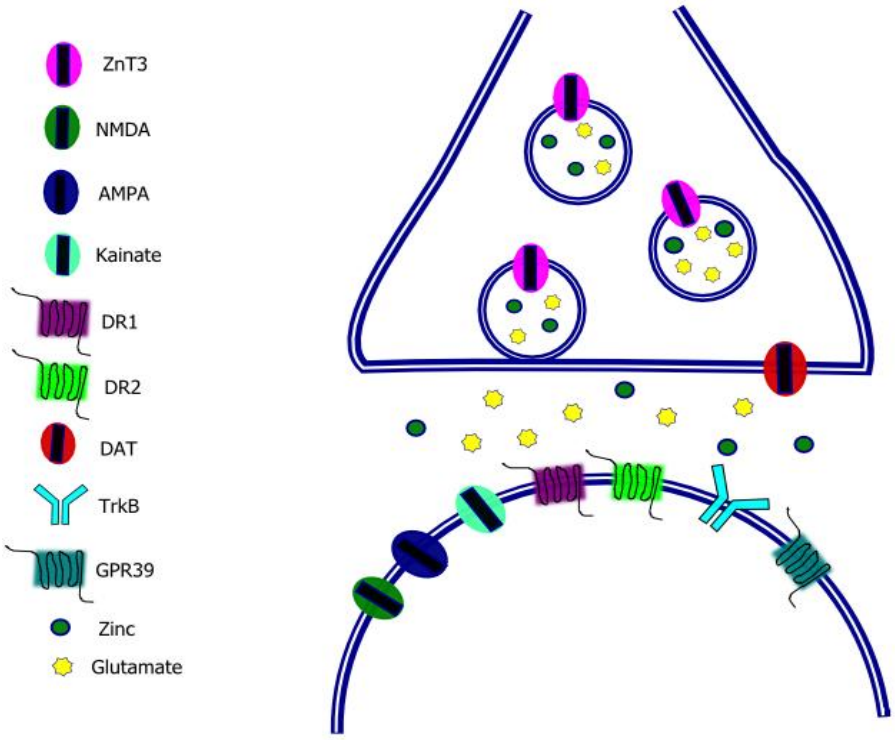

Figure 1.1. Zincergic signaling in the brain. Acronyms: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, DAT, dopamine transporter, DR1, dopamine receptor 1, DR2, dopamine receptor 2, GPR39, g protein-coupled receptor 39, NMDA, n-methyl-d-aspartate, TrkB, tropomyosin-related kinase B, ZnT3, zinc transporter 3

1.2.2.1 Zinc and glutamate

Glutamate acts on 3 receptors: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate. Zinc can modulate all 3 types; however, most studies focus on zinc's effect on the NMDA receptor. NMDA receptors are usually composed of four subunits, two NR1 and two NR2 subunits. Eight variants of the NR1 subunit exist as well as four variants of the NR2 subunit (A-D; Paoletti and Neyton, 2007). Zinc inhibits NMDA receptors, but how much it does so depends on the composition of subunits (Williams, 1996; Paoletti and Neyton, 2007); the NR2 subunit is where the main difference in amount of inhibition lies (Rachline et al., 2005).

NMDA receptors play an important role in long-term potentiation (LTP) and long-term depression (LTD); mechanisms involved in synaptic plasticity. LTP and LTD can be modulated differently depending on the composition of NMDA subunits (Massey et al., 2004; Liu et al., 2007). Since zinc also differentially modulates NMDA receptors based on subunit composition, this is a potential way for zinc to modulate synaptic plasticity.

Zinc has many opposite functions that depend on which receptors/transporters, and the composition of their subunits, are present at the synapse, making it difficult to know the exact effect zinc will have on any given cell (for a comprehensive review see Nakashima and Dyck, 2009). The role of zinc in synaptic plasticity has mainly been examined at the mossy fibre pathway of the hippocampus and the results are diverse and often contradictory. Zinc's role in synaptic plasticity has also been examined in the amygdala and in the CA3-CA1 pathway in the hippocampus with more consistent results (reviewed by Nakashima and Dyck, 2009). While other areas of the brain also exhibit synaptic plasticity (one of which will be discussed below in Section 1.3.4), the effect(s) of zinc has not been examined.

Research on the effect of zinc on the AMPA receptor has found a differential effect based on subunit composition (Blakemore and Trombley, 2004), similar to that found for the NMDA receptor. In some cases, zinc potentiates a response (Bresink et al., 1996; Lin et al., 2001). However, it can also cause no effect or inhibit AMPA receptors (Dreixler and Leonard, 1994; Bresink et al., 1996). Very little research has been done on the effect of zinc on the kainate receptor.

1.2.2.2 Zinc and dopamine

Dopamine is an important neuromodulator in the striatum (discussed below in Section 1.3.4). Although not much research has been done examining zinc and the dopaminergic system, zinc binding sites have been found on dopamine receptors (Schetz and Sibley, 2001; Liu et al., 2006), as well as on the dopamine uptake transporter (Norregaard et al., 1998). Zinc binding to these sites has been found to modulate binding of dopamine receptor antagonists in a manner that is dose-dependent, allosteric, and reversible (Schetz and Sibley, 1997; Schetz et al., 1999). Zinc potentiates dopamine release at what is arguably considered physiological concentration (3-300μM); however, it inhibits release at higher (>1mM) concentrations (Koizumi et al., 1995). In addition, Bjorkland et al. (2007) found that zinc inhibits dopamine transport through the dopamine transporter. Also, when examining cocaine or cocaine analogues, drugs that affect the dopaminergic system, zinc was found to potentiate the drugs' action (Richfield, 1993; Bjorklund et al., 2007). Therefore, it seems that zinc acts in such a way as to increase efflux and block uptake of dopamine, presumably leading to more dopamine in the synaptic cleft (Koizumi et al., 1995; Norregaard et al., 1998). It follows then that if zinc were depleted in the brain, the dopaminergic system would likely be affected. However, most of these studies use *in vitro*

methods rather than *in vivo*, so the actual effect of zinc on the dopaminergic system may be different in live animals than what has been found.

One set of studies has examined the role of zinc on the survival of dopaminergic neurons with contradictory results. Lo et al. (2004) found that zinc and dopamine applied together could induce cell death in PC12 cells from a rat. However, Eibl et al. (2010) and Gauthier et al. (2008) found that zinc enhanced survival of dopaminergic neurons, most likely through metallothionein binding, in oxidative environments where dopamine normally forms toxic products.

The nigrostriatal dopaminergic system is important in the etiology of Parkinson's disease. As such, several studies have examined the interaction between zinc and dopamine within the substantia nigra (SN) or through the use of drugs that mimic Parkinson's disease in animals. One study found that infusing zinc into the SN led to degeneration of the nigrostriatal dopamine system (Lin, 2001). Another study found that zinc accumulation in the SN indicated degeneration of dopaminergic neurons (Lee et al., 2009). This leads to a chicken or egg conundrum. Does death of the dopaminergic neurons cause the increase in zinc or is the increased zinc leading to the death of dopaminergic neurons?

Drugs such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridinium (MPP+), and 6-hydroxydopamine **(**6-OHDA) are considered dopaminergic neurotoxins because they cause depletion of dopamine in the brain. As such, they are frequently used in research on Parkinson's disease. One study found that zinc caused no changes in dopamine levels when administered alone, but could potentiate dopamine depletion in MPTP treated animals (Hussain and Ali, 2002). Other studies looked at the effects of drugs on zinc levels in the brain. Sheline et al. (2013) found increases in zinc in the presence of MPP+, while Rojas et al. (2005) found that MPP+ caused decreases of zinc in the striatum. Administration of

6-OHDA was found to increase zinc levels in the substantia nigra, globus pallidus, putamen, and amygdala (Tarohda et al., 2005).

The results from all of these studies point to a complex relationship between zinc and dopamine, the understanding of which is further complicated by the fact that some studies were done *in vitro* while others were done *in vivo*. This complicated relationship between zinc and dopamine may also depend on the presence or absence of other molecules (such as metallothioneins).

1.2.2.3 Zinc and BDNF

Zinc has been found to modulate brain derived neurotrophic factor (BDNF; Hwang et al., 2005; Travaglia et al., 2013). Specifically, zinc was found to increase phosphorylation of the BDNF receptor, tropomyosin-related kinase B (TrkB), which led to an increase in the BDNF precursor, pro-BDNF (Hwang et al., 2005). Later, it was found that zinc could activate the TrkB pathway in the absence of BDNF (Huang et al., 2008). The BDNF pathway has close ties to NMDA-related plasticity.

Mice that are lacking the protein required to package zinc into vesicles (ZnT3) have been shown to have lower levels of pro-BDNF and the BDNF receptor, TrkB (Adlard et al., 2010). Since BDNF has been shown to influence dendritic growth and synaptic plasticity (McAllister et al., 1997), it is possible that changes in the amount of synaptic zinc in the brain may affect neuronal morphology, as well as synaptic plasticity. A recent study in our lab, also using ZnT3 knockout mice (discussed further in Section 1.2.3.2), found that zinc is necessary for the increases in BDNF seen in animals raised in enriched environments (Chrusch, 2015).

1.2.3 Function

As discussed above (section 1.2.2.1), synaptic zinc has been shown to be a modulator of synaptic plasticity in various regions of the brain. However, *ex vivo* synaptic plasticity studies are not the only or best way to examine zincergic functions. Behavioural studies provide a clearer understanding of function in the living animal. These studies can be done by altering experiences of wildtype animals or by studying knockout/transgenic animals.

1.2.3.1 Zinc and experience-dependent plasticity

There are different ways of changing how an animal experiences the world; most commonly used are deprivation studies and enrichment studies. Both ways have been used to look at zinc in the brain, specifically in the somatosensory (barrel) cortex and in the hippocampus.

One model commonly used to study experience-dependent plasticity is the vibrissae/barrel cortex system. Rodents have a specific number of vibrissae (whiskers), each of which maps to a specific region (barrel) in the somatosensory cortex. Plucking or trimming vibrissae results in changes in the barrel cortex. Previous studies in our lab have shown changes in zinc staining in the barrel cortex following vibrissae plucking (Nakashima and Dyck, 2010). This suggests that zinc is involved in the plastic changes taking place in the barrel cortex in response to the loss of sensory input from the vibrissae.

Many benefits, both anatomical and behavioural, have been found from environmental enrichment. These benefits include increases in capillaries (Sirevaag et al., 1988) and hippocampal neurons (Kempermann et al., 1997), improvements in LTP (Foster and Dumas, 2001; Abraham et al., 2002; Artola et al., 2006; Irvine et al., 2006), and increased abilities in

spatial behavioral tasks (Venable et al., 1988; Leggio et al., 2005). For rodents, an enriched environment consists of a larger cage with more animals and more toys including tunnels and running wheels. This is compared to standard housing which consists of a shoebox cage. Using enriched environments, Nakashima and Dyck (2008) examined changes in synaptic zinc levels in the mouse barrel cortex of deprived and non-deprived vibrissae. They found greater changes in synaptic zinc levels in mice that were housed in the enriched environment, indicating that enrichment is able to affect zincergic function.

1.2.3.2 ZnT3 knockout mice

To examine the role of synaptic zinc in the brain, a knockout mouse was created that lacked the zinc transporter (ZnT3) required to package zinc into vesicles to be released. Therefore, these mice lacked synaptic zinc. When the ZnT3 knockout (KO) mice were first tested for a behavioural phenotype, none was found apart from their being slightly more susceptible to kainic acid-induced seizures (Cole et al., 2001). However, more recent studies from our lab and others are beginning to find deficits in certain behavioural tasks. In 2010, Adlard and colleagues found that older (6 months) ZnT3 KO mice performed worse on the Morris water task than younger (3 months) ZnT3 KO (Adlard et al., 2010). Other tasks that the ZnT3 KO have been found deficient on include conditioned (but not innate) fear memory (Martel et al., 2010; Sindreu et al., 2011), t-alternation maze (Sindreu et al., 2011), and the Morris water task with alternate platform locations (Martel et al., 2011).

A recent study in our lab (mentioned briefly in section 1.2.2.3) examined the effects of environmental enrichment on hippocampal neurogenesis in ZnT3 KO mice. They found that enrichment significantly increased neurogenesis and cell survival, and reduced cell death in

ZnT3 wildtype (WT) but not ZnT3 KO mice. Environmental enrichment also improved performance on two hippocampal dependent tasks (spatial object recognition and Morris water task) in ZnT3 WT but not ZnT3 KO mice. Another finding from this study (mentioned above) was that the increase in BDNF seen in enriched animals is dependent on the presence of zinc (Chrusch, 2015).

1.2.4 Zinc deficiency in humans

As mentioned earlier, zinc is essential for life. Alterations in the amount of zinc in the body can affect many systems and has been linked to many diseases/disorders. While some of these are whole-body issues, such as cancer, diabetes, and cardiovascular disease, many are neurological issues, such as depression, Alzheimer's disease, amyotrophic lateral sclerosis, epilepsy, and ischemia (reviewed by Frederickson et al., 2005; Bitanihirwe and Cunningham, 2009; Chasapis et al., 2012). Zinc deficiency has also recently been linked to autism spectrum disorder (Grabrucker et al., 2014) and obsessive-compulsive disorder (Sayyah et al., 2012; Shohag et al., 2012). While the focus of this thesis does not involve studying any one of these specifically, it is important to recognize possible implications that dysfunction of the zincergic system in the brain may have on health.

1.3 Striatum

1.3.1 Anatomical and functional divisions

The striatum consists of the caudate nucleus, the putamen, the nucleus accumbens, and the olfactory tubercle (McGeorge and Faull, 1989). However, it can be viewed in many different ways. The striatum can be divided into 2 compartments, striosomes and matrix, using various

histochemical markers, such as acetylcholinesterase, enkephalin, and tyrosine hydroxylase (Graybiel and Ragsdale, 1978; Graybiel, 1984; Graybiel and Chesselet, 1984; Graybiel et al., 1986). The striatum can also be divided into 2 afferent pathways, direct and indirect, that have opposite effects with the direct pathway distinguished by the presence of dopamine D1 receptors and acetylcholine M4 receptors, while the indirect pathway is distinguished by dopamine D2 receptors and adenosine A2A receptors. In motor function, the direct pathway serves to facilitate movement while the indirect pathway serves to prevent movement (Freeze et al., 2013). Another division of the striatum is into 2 streams: dorsal and ventral. The dorsal stream is involved in learning what to do, while the ventral stream has more to do with when and where an action is performed (Hart et al., 2013). It can also be divided into 3 functionally-distinct regions: dorsolateral, dorsomedial, and ventral. For most of the remainder of this thesis, I will be referring to the 3 functionally-distinct regions.

The dorsolateral region (DLS) mainly consists of the putamen; the dorsomedial region (DMS) is mainly the caudate nucleus; and the ventral striatum is usually considered to be the nucleus accumbens (NAc) and olfactory tubercle. Each region has particular functions attributed to it and receives slightly different input (McGeorge and Faull, 1989; Alexander et al., 1990).

1.3.2 Connectivity

As the input center for the basal ganglia, the striatum receives excitatory afferent connections from many areas of the cortex, as well as from the thalamus, hippocampus, and amygdala. It also receives dopaminergic input from the substantia nigra and ventral tegmental area (VTA). The DLS receives input from sensory and motor cortices; the DMS receives input from prefrontal and parietal cortices; and the NAc receives input from medial orbitofrontal and

anterior cingulate cortices (Alexander et al., 1990). Efferent connections are to other members of the basal ganglia, namely, the globus pallidus (internus and externus), substantia nigra (pars reticulate), and the subthalamic nucleus (Mengual et al., 1995; Sorensen et al., 1995). These output centers of the basal ganglia project to various regions of the thalamus which project back to cortex (Figure 1.2).

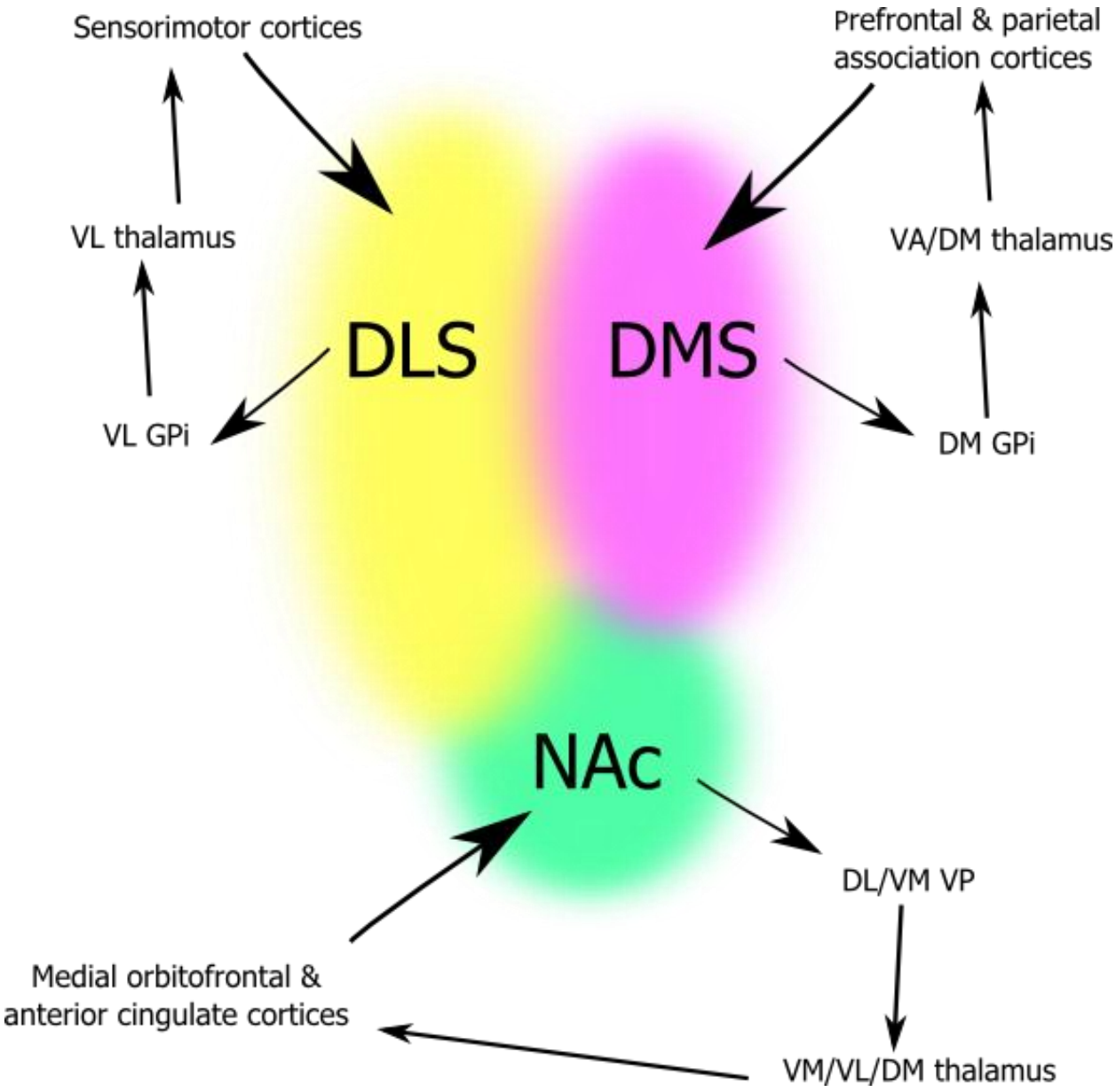

Figure 1.2. Striatal Connectivity. A schematic of the functional loops of the striatum. Acronyms: DL, dorsolateral, DLS, dorsolateral striatum, DM, dorsomedial, DMS, dorsomedial striatum, NAc, nucleus accumbens, VA, ventral anterior, VL, ventrolateral, VM, ventromedial

1.3.3 Function

As part of the extrapyramidal motor system, the basal ganglia in general are involved in refinement of movements (Alexander, 1994; Nelson and Kreitzer, 2014). However, they have also been found to have cognitive functions (reviewed by Packard and Knowlton, 2002). The different functions seem to be segregated and correspond to the 3 different regions of striatum discussed above: DLS, DMS, and NAc. The DLS region is thought to be part of the motor circuit and involved with fine motor control as well as motor learning (Alexander et al., 1990). It is also involved in the learning of habits (reviewed by Liljeholm and O'Doherty, 2012). The DMS region is thought to be involved in goal-directed learning (Yin et al., 2005). The NAc is involved in the reward pathway and in motivation (Ikemoto and Panksepp, 1999; Carelli, 2002). More recently, it has been proposed that the NAc also plays a role in aversive situations in addition to its role in rewarding situations (Carlezon Jr and Thomas, 2009).

1.3.4 Dopamine and Striatal Plasticity

The majority of the cells within the striatum are GABAergic projection neurons called medium spiny neurons (MSN). They make up ~95% of all the neurons located in the striatum. The other ~5% are GABAergic or cholinergic interneurons (Matamales et al., 2009). Since the striatum is involved in motor, habit, and goal-directed learning (as mentioned above), plastic changes must be taking place either within the neurons of the striatum itself or in its afferent or efferent connections. Because zinc has been found to act as modulator of plasticity in both the barrel cortex and the hippocampus, it seems likely that it will play a similar role in the striatum.

Dopamine is a very important neuromodulator/neurotransmitter in the striatum. It is involved in the modulation of striatal plasticity. Mechanisms of plasticity, including LTP and LTD, have been shown to occur at corticostriatal synapses of both the direct and indirect pathway neurons (reviewed by Kreitzer and Malenka, 2008). Dopamine, particularly the D1-receptor, has been shown to modulate these processes in different ways depending on the stage of learning; it is more critical in early stages than in later stages (reviewed by Costa, 2007). Also, relative levels of dopamine versus adenosine and acetylcholine determine whether LTD occurs in the direct or indirect pathway (reviewed by Lerner and Kreitzer, 2011).

1.3.5 Striatum and enriched environments

As mentioned earlier in reference to zinc (Section 1.2.3.1), environmental enrichment has been shown to have many benefits. Few studies have been done examining the effects of enrichment on the striatum. One study looked at repetitive behaviour in deer mice and found that it is attenuated by being raised in an enriched environment and is associated with changes in the cortico-basal ganglia circuits; similar changes were not seen in the hippocampus (Lewis, 2004). Other studies have used the Golgi-cox stain to examine morphological changes in the striatum after enrichment and found an increase in spine density of MSNs in the striatum (Comery et al., 1995; Comery et al., 1996; Kolb et al., 2003). Another study, also using Golgi-cox stain, examined dendrite length after enrichment, as well as after exposure to a running wheel alone. While differences in dendritic length were found in the hippocampus, no differences were found in layer V cortex pyramidal neurons or in striatal medium spiny neurons (Faherty et al., 2003). Therefore, environmental enrichment seems to have different effects depending on the brain region and variable measured.

1.3.6 Disorders of the striatum

Dysfunction of the striatum can lead to many problems. Most people generally think of the hyper- and hypokinetic disorders, such as Huntington's disease and Parkinson's disease (reviewed by Afifi, 1994). However, many other disorders have now been linked to striatal dysfunction. These include other motor disorders (such as dystonia; Pappas et al., 2014), drug addiction (Yager et al., 2015), Tourette's syndrome (Pappas et al., 2014), attention deficit hyperactivity disorder (ADHD; Dalley et al., 2008), obsessive-compulsive disorder (OCD; Pappas et al., 2014), and autism spectrum disorder (ASD; Maloney et al., 2013). Again, while this thesis does not directly deal with any of these disorders, it is important to keep possible implications of this research in mind.

1.4 Zinc in the striatum

1.4.1 Anatomy

Other than the studies on the relationship of zinc and dopamine discussed above (section 1.2.2.2), only 2 studies have looked specifically at zinc in the striatum. Early zinc staining studies in rats showed that there were zinc-containing terminals in the caudate-putamen complex and visual inspection of images from those early papers reveals a heterogeneous staining pattern as shown in Figure 1.3 (Danscher et al., 1973; Danscher, 1981; Perez-Clausell and Danscher, 1985). But, as mentioned, while many researchers have proceeded to study regions of intense zinc staining, such as the hippocampus and somatosensory cortex, very few studies have examined the caudate-putamen.

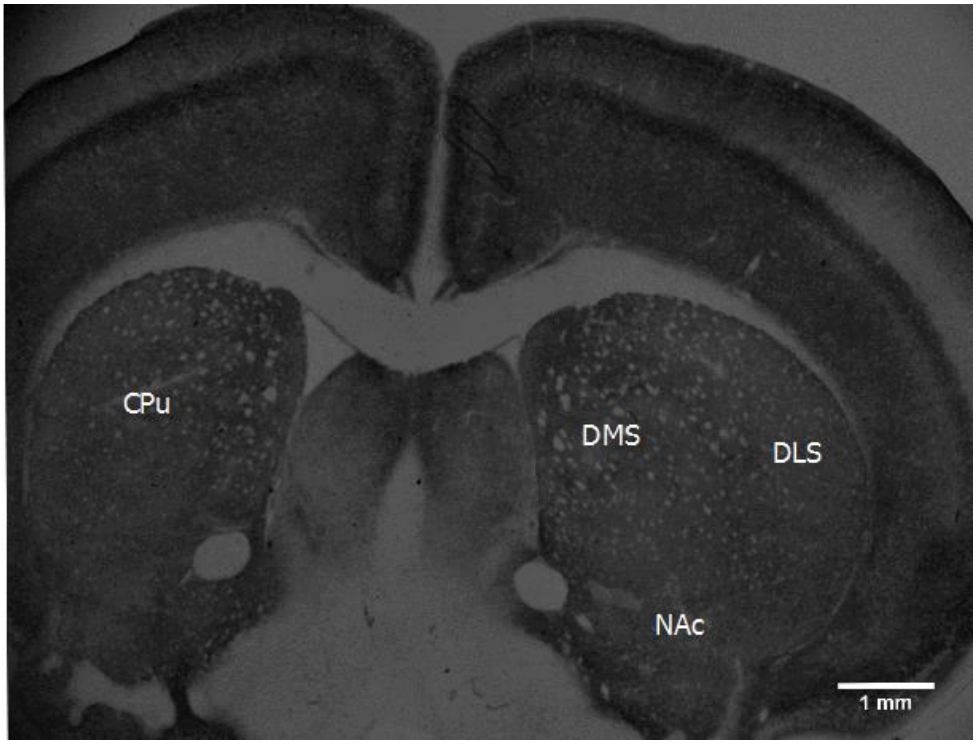

Figure 1.3. Autometallographic zinc staining of a coronal section of mouse brain showing staining in the caudate/putamen (CPu) with functional regions labelled on the right hemisphere. Acronyms: CPu, caudate/putamen, DLS, dorsolateral striatum, DMS, dorsomedial striatum, NAc, nucleus accumbens

Zinc staining of the adult striatum shows a heterogeneous distribution. The patchy distribution that is found somewhat coincides with the patch-matrix distribution found when staining for other histochemical markers of the striatum, such as acetlycholinesterase or Calbindin-D_{28K} (Mengual et al., 1995). However, there was no consistent pattern in overlap between the zinc stained patch-matrix and the acetylcholinesterase or Calbindin-D_{28K} patch-matrices. It was also found that the rim of the caudate-putamen was darkly stained while the inner region was less intensely stained, but showed areas of high and low intensity staining. The intensity of the staining varied along the rostral-caudal axis (Mengual et al., 1995). Retrograde tracing studies of zincergic innervation of the striatum in rats revealed that the cell bodies of the neurons are located in motor cortex, somatosensory cortex, cingulate gyrus, and amygdala, depending on the site of injection (dorsomedial, dorsolateral, or ventrolateral striatum). In the neocortex, zincergic cells were mainly found at the border between deep layer V and superficial layer VI; however, some zincergic cells were also found in layer II/III and deep layer VI. Zincergic cells were found in layers II and III in the cingulate cortex with few in layer VI (Figure 1.4). The basolateral and basomedial nuclei of the amygdala also contained several zincergic cells that projected to the striatum (Sorensen et al., 1995).

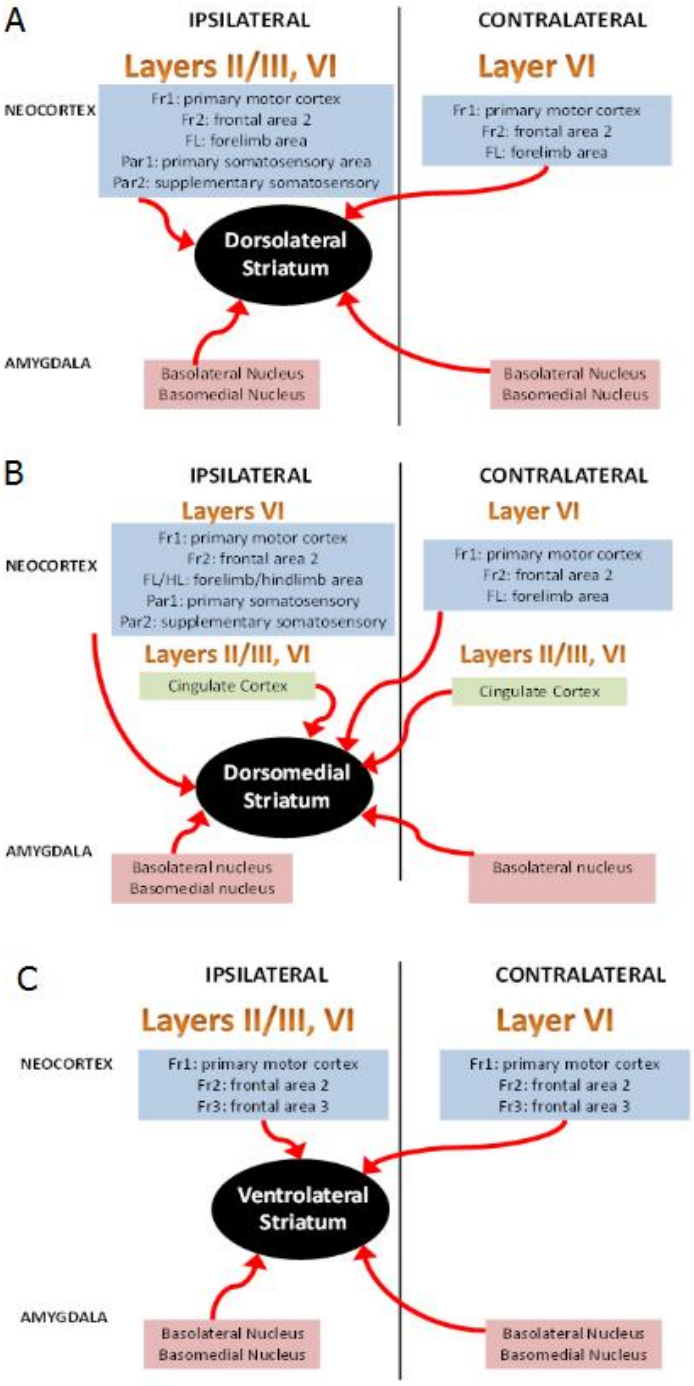

Figure 1.4. Zincergic afferent connections to striatum. Zincergic input to A) dorsolateral, B) dorsomedial, and C) ventrolateral striatum (adapted from Sorensen et al., 1995).

As mentioned earlier, in 2003, Brown and Dyck discovered that by incubating brain tissue in H_2O_2 prior to zinc staining, the synaptic zinc was eliminated leaving only the zinc in cell bodies stained (Brown and Dyck, 2003). Using this method to stain the whole brain, they found that there was no staining in the striatum, indicating that while zinc was released into the synapses of the striatum, the cell bodies that packaged the zinc were located outside of the striatum. Therefore, the zinc containing axons must be coming from somewhere else in the brain, likely from the connections listed above.

1.4.2 Behaviour

No studies to date have been done with the specific intention of examining behavioural outcomes of depleting synaptic zinc in the striatum. However, some behavioural tests used to examine other behaviours have also been shown to be affected during striatal dysfunction. Section 1.2.3.2 highlighted behavioural studies done using ZnT3 KO mice. Deficits were found on conditioned (but not innate) fear memory (Martel et al., 2010; Sindreu et al., 2011), the t-alternation maze (Sindreu et al., 2011), and the Morris water task with alternate platform locations (Martel et al., 2011). Most of the deficits in these tasks were attributed to the loss of synaptic zinc in either the hippocampus or the amygdala. However, many of the deficits seen in the behaviour of the ZnT3 KO mice could also potentially be attributed to striatal dysfunction. Many of the tasks involve forming an association. The first time the mouse learned the task, they had no problems; the difficulty arose when the association was changed. Studies looking at striatal damage have had similar results. Rats with lesions to the striatum (but not to the hippocampus) had weak deficits in tone-shock pairing (Ferreira et al., 2003). A later study showed that it is the connection between the striatum and amygdala that is important in tone fear

conditioning. Rats with lesions to the striatum in one hemisphere and to the amygdala in the other had significant deficits in learning the tone-shock association (Ferreira et al., 2008). More recently, it has been shown that NMDA receptors and protein synthesis in the striatum are necessary for consolidation of a weak (using a lower intensity foot shock) fear memory (Kishioka et al., 2013). Another study used the Morris water task to test rats with a dorsal striatal lesion; rats successfully found the platform when visible and hidden but failed to find it when the location was changed (McDonald and White, 1994; Furtado and Mazurek, 1996). This is not to suggest that the loss of zinc in the hippocampus and amygdala has no or little effect on the ZnT3 KO mice, but only to suggest that the picture presented in previous studies of these mice may not be as simple as the authors have painted it to be. To date, no published studies of ZnT3 KO mice have examined skilled motor behaviour, which is striatal-dependent.

1.5 Overview and Objectives

Synaptic zinc is found in many areas of the brain, including the striatum. The striatum is involved in motor learning, fine motor skills, and cognition. Zinc has been found to affect synaptic plasticity in other parts of the brain and is able to modulate dopamine – a key player in striatal plasticity-, suggesting a possible role for synaptic zinc in striatal plasticity. Therefore, my hypothesis is that synaptic zinc plays a role in modulating plasticity in the striatum. Removal of synaptic zinc, using the ZnT3 knockout mouse, will affect plasticity within the striatum and cause anatomical and behavioural differences. The purpose of this thesis is to expand on current knowledge of synaptic zinc in the striatum, both anatomically and functionally. Chapter 2 addresses the anatomical aspect with an experiment using ZnT3 WT and KO mice raised in standard or enriched conditions and examines changes in dendritic morphology after Golgi-cox

staining. Chapter 3 addresses the behavioural aspect by testing ZnT3 WT and KO mice on various motor behavioral tasks, both striatal-dependent and -independent. The final chapter, Chapter 4, contains a general discussion of what was found in both of the studies, as well as future directions for this area of research. A third study is included in the appendix which examined circadian expression of cFos in the striata of ZnT3 WT and KO mice and found that there are no differences between genotypes.

Chapter Two: **Neuronal Morphology**

2.1 Acknowledgements

This project was done in collaboration with Brendan McAllister. We contributed equally during the course of the experiment. In terms of quantification, his focus was on tracing cells in the cortex, while my focus was on cells in the striatum.

2.2 Introduction

The bivalent metal zinc is an essential component of all cells (Chasapis et al., 2012). A proportion of the zinc found in the brain is loaded into synaptic vesicles by zinc transporter 3 (ZnT3; Cole et al., 1999). These vesicles can be released from presynaptic cells into the synaptic cleft (Frederickson et al., 2000; Frederickson et al., 2005). This synaptic zinc can modulate many receptors on the postsynaptic membrane or can enter the postsynaptic cell and affect second messenger systems inside the cell (reviewed by Nakashima and Dyck, 2009). Acting in this way, synaptic zinc has been found to modulate plasticity in the hippocampus and amygdala (reviewed by Nakashima and Dyck, 2009). Plasticity refers to changes taking place within the brain and can occur at molecular, cellular, circuit, or whole-brain levels.

Staining the brain for synaptic zinc reveals that it is located in high concentration in neocortex, hippocampus, amygdala, and striatum, as well as in other regions (Brown and Dyck, 2004a). Location of zincergic cell bodies using retrograde tracing shows that, while there is a high concentration of synaptic zinc within the striatum, there are no zincergic cell bodies there (Brown and Dyck, 2003, 2004b). This indicates that the synaptic zinc located there must be coming from other regions of the brain. This fact makes it an interesting area to examine; however, research on the role of synaptic zinc in the striatum is sorely lacking.

The striatum, which includes caudate-putamen and nucleus accumbens, is part of the basal ganglia, a group of nuclei involved in the modulation of fine motor skills, goal-directed learning, habit formation, motivation, and reward (reviewed by Liljeholm and O'Doherty, 2012). The nucleus accumbens region is also involved in aspects of novelty (reviewed by Ikemoto and Panksepp, 1999). Functionally, the striatum can be divided into 3 regions, dorsolateral, dorsomedial and ventral/nucleus accumbens. Each region receives input from different areas of neocortex and is therefore involved in different aspects of behaviour (Alexander et al., 1986; McGeorge and Faull, 1989). The afferent input converges on the medium spiny neurons (MSNs), which are the main projection neurons of the striatum and make up approximately 95% of all neurons there (Matamales et al., 2009). Most input to the striatum is glutamatergic. However, the striatum also receives dopaminergic input from the substantia nigra and ventral tegmental area (reviewed by Silberberg and Bolam, 2015). Dopamine is a very important neurotransmitter in the striatum (reviewed by Costa, 2007; Do et al., 2012; Haber, 2014) and plays a role in striatal plasticity (reviewed by Kreitzer and Malenka, 2008; Lerner and Kreitzer, 2011). Alterations in striatal dopamine levels lead to diseases/disorders that can have both motor and cognitive dysfunction. Parkinson's disease (Afifi, 1994) and Huntington's disease are most often discussed as outcomes of striatal dysfunction (reviewed by Dayalu and Albin, 2015); however, other disorders such as obsessive-compulsive disorder (OCD; Pappas et al., 2014), autism spectrum disorders (ASD; Maloney et al., 2013), addiction (Yager et al., 2015), and attention deficit hyperactivity disorder (ADHD; Dalley et al., 2008) have also been linked to striatal dysfunction.

As mentioned above, synaptic zinc has been found to affect many different receptors as well as many second messenger systems (reviewed by Nakashima and Dyck, 2009). Of particular relevance to the striatum, zinc binding sites have been found on dopamine receptors

(Schetz and Sibley, 1997; Liu et al., 2006) and the dopamine transporter (Norregaard et al., 1998), indicating that zinc may have a modulatory role on the dopaminergic system. Because research in this area is varied and often contradictory, the specific effect that zinc is having on the dopaminergic system *in vivo* is unknown. Another important system within the striatum is the glutamatergic system, as input from cortex uses glutamate as its main neurotransmitter (Silberberg and Bolam, 2015). Synaptic zinc has strong ties to the glutamatergic system and can modulate all 3 glutamate receptors (reviewed by Nakashima and Dyck, 2009). These two neurotransmitter systems – dopaminergic and glutamatergic – interact within the striatum to influence output (reviewed by Gardoni and Bellone, 2015). Since synaptic zinc is capable of modulating both groups of receptors, it is likely that it is doing so in the striatum and that alterations of synaptic zinc levels may affect functioning of both systems.

One system frequently used to examine zincergic plasticity is the vibrissae-barrel cortex system. Each vibrissae (whisker) projects to a specific barrel in the part of the somatosensory cortex that receives input from the vibrissae (named for the barrel-shaped sections of cortex). Staining for synaptic zinc reveals higher amounts of zinc in the areas between the barrels (Brown and Dyck, 2002). Removing or stimulating vibrissae can thus lead to changes in zinc levels in the barrel cortex (Brown and Dyck, 2002, 2005). Mice raised in enriched environments were found to have greater increases in the amount of synaptic zinc present in the barrel cortex after vibrissae plucking (Nakashima and Dyck, 2008), indicating that environment type can modulate zincergic function.

In addition to the effects on zinc in the barrel cortex, environmental enrichment has been found to have other beneficial effects, both behaviourally and anatomically. For mice, an enriched environment consists of a large, 2-leveled chamber with toys, running wheels, and more

mice. There is also a novel aspect to enriched environments as the toys, as well as the location of food and water, are changed every week. Rodents raised in enriched environments have been found to have improved abilities on spatial tasks (Venable et al., 1988; Leggio et al., 2005). They have also been found to have anatomical changes including increases in hippocampal neurons (Kempermann et al., 1997), in angiogenesis (Sirevaag et al., 1988), and in dendritic length and spine density (Leggio et al., 2005). In the striatum specifically, enrichment has controversial effects on dendritic length of MSNs, but increases spine density on terminal dendrites of MSNs. Kolb et al. (2003) found that raising rats in enriched environments for 3-3.5 months increased dendritic length in the nucleus accumbens. However, Faherty et al. (2003) found that mice raised in enriched environments or with just a running wheel for 4-5 months had no difference in dendritic length in the ventrolateral striatum (which may or may not include the nucleus accumbens). Studies on spine density of MSNs found increases in the dorsolateral striatum (Comery et al., 1995; Comery et al., 1996) and in the nucleus accumbens (Kolb et al., 2003) following enrichment.

To examine the effects that environmental enrichment has on striatum, as well as how this interaction is affected by synaptic zinc, we utilised ZnT3 knockout (KO) mice. These mice lack synaptic zinc (Cole et al., 1999) and are therefore a good tool to determine how synaptic zinc, or lack of synaptic zinc, affects the brain. A recent study in our lab using adult ZnT3 mice found that they lacked plasticity in the hippocampus (Chrusch, 2015). The mice were raised in either standard or enriched environments for 3-6 weeks. Then measures of neurogenesis, including cell survival, proliferation, and death, were determined. There were no differences between genotypes raised in standard environments. Enriched ZnT3 wildtype (WT) mice had increases in cell survival and proliferation, and decreases in cell death. Enriched ZnT3 KO mice,

on the other hand, were no different than the standard housed mice (Chrusch, 2015). This suggests that, at least in terms of hippocampal neurogenesis, the ZnT3 KO mice do not benefit from enrichment and therefore seem to lack plasticity.

We hypothesized that synaptic zinc in the striatum modulates plasticity and that removal of synaptic zinc via the ZnT3 knockout mouse would result in a lack of plasticity. The current study used ZnT3 WT and KO mice raised in standard or enriched environments to determine whether ZnT3 KO mice show benefits from enrichment as measured by changes in dendritic length or spine density. We predicted that enriched ZnT3 WT mice would have increases in dendritic length and in spine density in the striatum compared to standard housed animals and that enriched ZnT3 KO mice would be no different than standard housed animals.

2.3 Methods

2.3.1 Animals

C57BL/129sv mice heterogeneous for the ZnT3 allele were bred to produce wildtype, heterozygous, and knockout offspring. Three month old female ZnT3 WT and KO mice were used for this experiment. Mice were raised in either standard shoebox cages their entire life (ZnT3 WT: n = 6 and ZnT3 KO: n = 6) or raised in an enriched environment from one month of age onward (ZnT3 WT: n = 8 and ZnT3 KO: n = 7). All mice were in a 12 h light/dark cycle and had access to food and water ad libitum. All procedures were approved by the Animal Care Committee of the University of Calgary and conformed to the guidelines set out by the Canadian Council for Animal Care.

2.3.2 Procedure

The enrichment cages are large Plexiglas boxes with two levels that are filled with toys including running wheels and tunnels. Each cage of enriched animals held 8-10 mice. At 3 months of age, mice were perfused using 100 ml of saline, brains were removed and weighed, then placed in Golgi-cox solution. The brains remained in the solution for 10 days before being moved to sucrose for a minimum of 3 days. Brains were sectioned on a vibratome at 250 μm 3-7 days after being placed in sucrose. Slices were mounted on 1.5% gelatin-coated slides. Once mounted, slides were left in a humidity chamber for no more than 5 but no less than 2 days. The staining protocol involved rinsing the slides in distilled water (dH2O) for 1 minute before placing in ammonium hydroxide for 30 minutes in the dark. Slides were then rinsed in dH2O for 1 min before being placed in Kodak fixative for film for 30 minutes in the dark. After rinsing again in dH2O, slides were dehydrated in an ascending series of ethanol (50%, 70%, 95%) for 1 minute each, then were placed in 100% ethanol (3 x 5 minutes). Slides were then placed in a solution consisting of equal parts Xylene, Chloroform, and 100% ethanol. Last, slides were cleared in Xylene (2 x 15 minutes). Slides were then coverslipped with permount and allowed to dry.

2.3.3 Dendritic length

Once the slides were dry, a Zeiss microscope was used to find cells in specific regions of the striatum that were good candidates for tracing. A good candidate for tracing was determined by whether the whole cell was visible within the section and whether there was sufficient distance between it and other cells in the area so as to be able to determine which dendrites belonged to which cell. Figure 2.1 shows a representation of how a cell from the striatum is

chosen. Cells (n = 4 per hemisphere) were chosen from the dorsolateral (DLS) and dorsomedial (DMS) striatum, as well as from the nucleus accumbens (NAc). Medium spiny neurons (MSNs) were analyzed because they make up ~95% of the cells in the striatum and are the main projection neurons (Matamales et al., 2009). Once a cell was chosen, it was then traced onto white computer paper by camera lucida using the 40X objective lens. Sholl analysis was performed to determine the amount of dendritic branching. This analysis consists of placing a series of concentric circles equidistance apart around the soma of the cell and counting how many dendrites cross each circle. Summing the number of crosses from each circle gives an idea of how much branching has occurred and can be converted into micrometers of branching. Lengths for each region in each hemisphere were averaged. A 2x2 (genotype x environment) factorial analysis of variance (ANOVA) was used for each region of the striatum.

2.3.4 Spine Density

Spine density was determined in a similar way as dendritic length. A MSN was located in the desired region of striatum. Two randomly chosen terminal dendrites from each cell (n = 4/hemisphere/region) were traced at 1000X magnification. Number of spines located on a given length of dendrite was determined. Dendritic spines can have different morphologies but generally consist of a spine shaft and a head (Hering and Sheng, 2001). For the purpose of this study, a spine was counted if a shaft and head were visible regardless of the shape of the head. Length of section was determined using the measurement tool in ImageJ software (ImageJ 1.48; National Institutes of Health, Bethesda, MD, USA). Spine densities were averaged for each region for each hemisphere. A 2x2 (genotype x environment) factorial analysis of variance (ANOVA) was used for each region of the striatum.

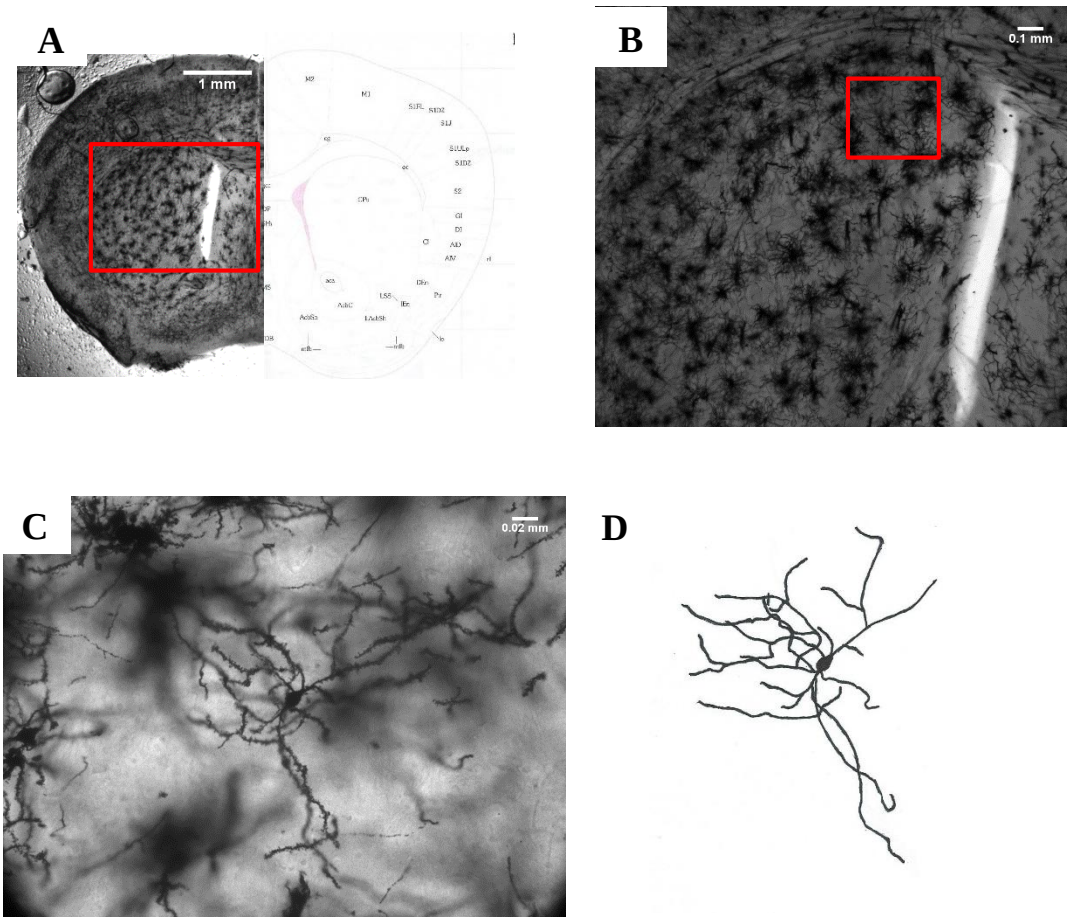

Figure 2.1. Process of finding and tracing cells in the striatum. A) Section at 2.5X magnification. B) Same section at 10X magnification. C) Labeled medium spiny neuron at 40X magnification. D) Same neuron as (C) traced onto white computer paper.

2.4 Results

Female ZnT3 wildtype (WT) and knockout (KO) mice were raised either in standard housing or in an enriched environment until 3 months of age. Golgi-cox stain was used to examine neuronal morphology, specifically dendritic length and spine density, in 3 regions of the striatum: dorsolateral (DLS), dorsomedial (DMS), and nucleus accumbens (NAc).

2.4.1 Dendritic Length

Sholl analysis was used to determine dendrite length (n = 4 cells/area/hemisphere). No differences were found in the DLS between housing conditions, $F(1,50)=.03$, $p=.854$, or between genotypes, $F(1,50)=.39$, $p=.533$ (Figure 2.2). There was also no significant interaction between housing condition and genotype, $F(1,50)=.17$, $p=.684$. No differences were found in the DMS between housing conditions, $F(1,50)=1.00$, $p=.323$, or between genotypes, $F(1,50)=.29$, $p=.594$ (Figure 2.3). There was also no significant interaction between housing condition and genotype, $F(1,50)=.70$, $p=.407$. No differences were found in the NAc between housing conditions, $F(1,50)=.45$, $p=.506$, or between genotypes, $F(1,50)=.50$, $p=.483$. There was also no significant interaction between housing condition and genotype, $F(1,50)=.46$, $p=.501$ (Figure 2.4).

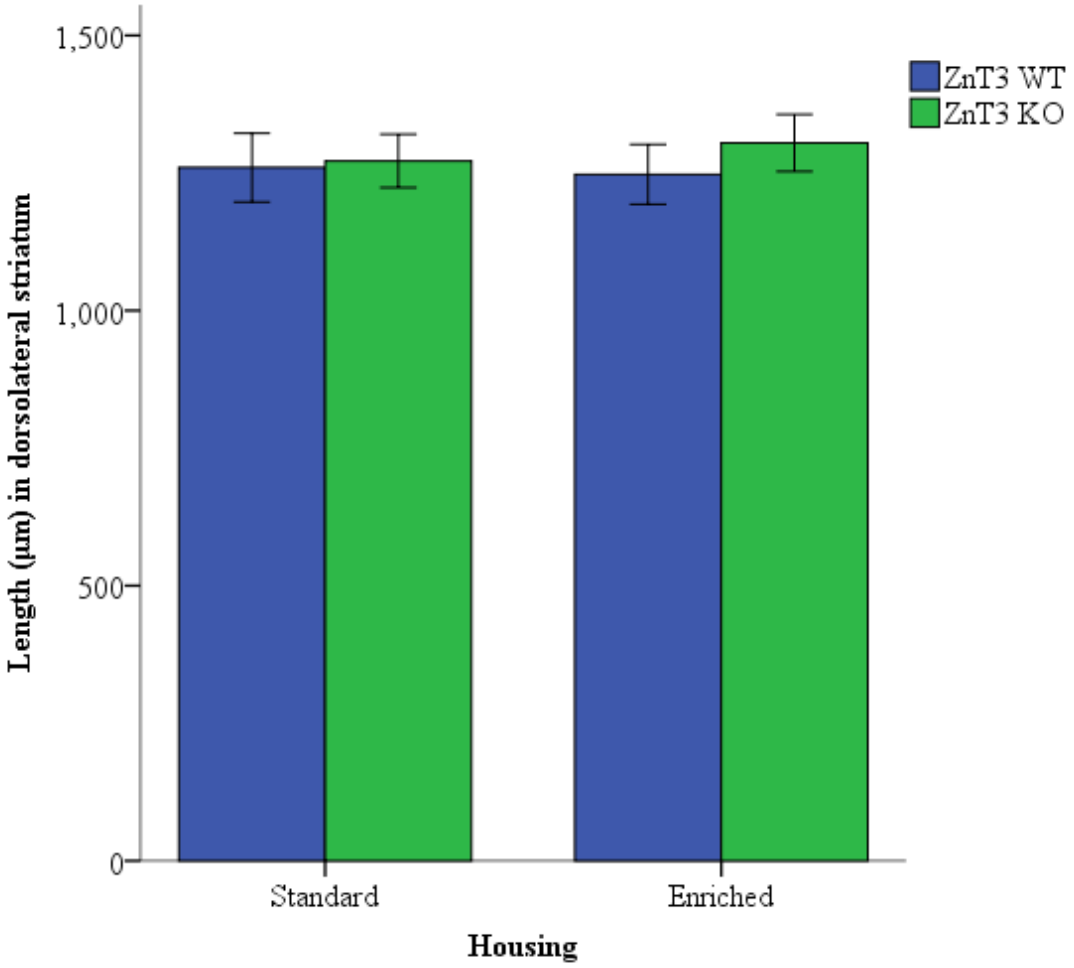

Figure 2.2. Mean dendritic length of medium spiny neurons in the dorsolateral striatum for ZnT3 wildtype (WT) and knockout (KO) mice raised in standard (n=6 per genotype) or enriched environments (WT: n=8; KO: n=7). Error bars represent ± SEM.

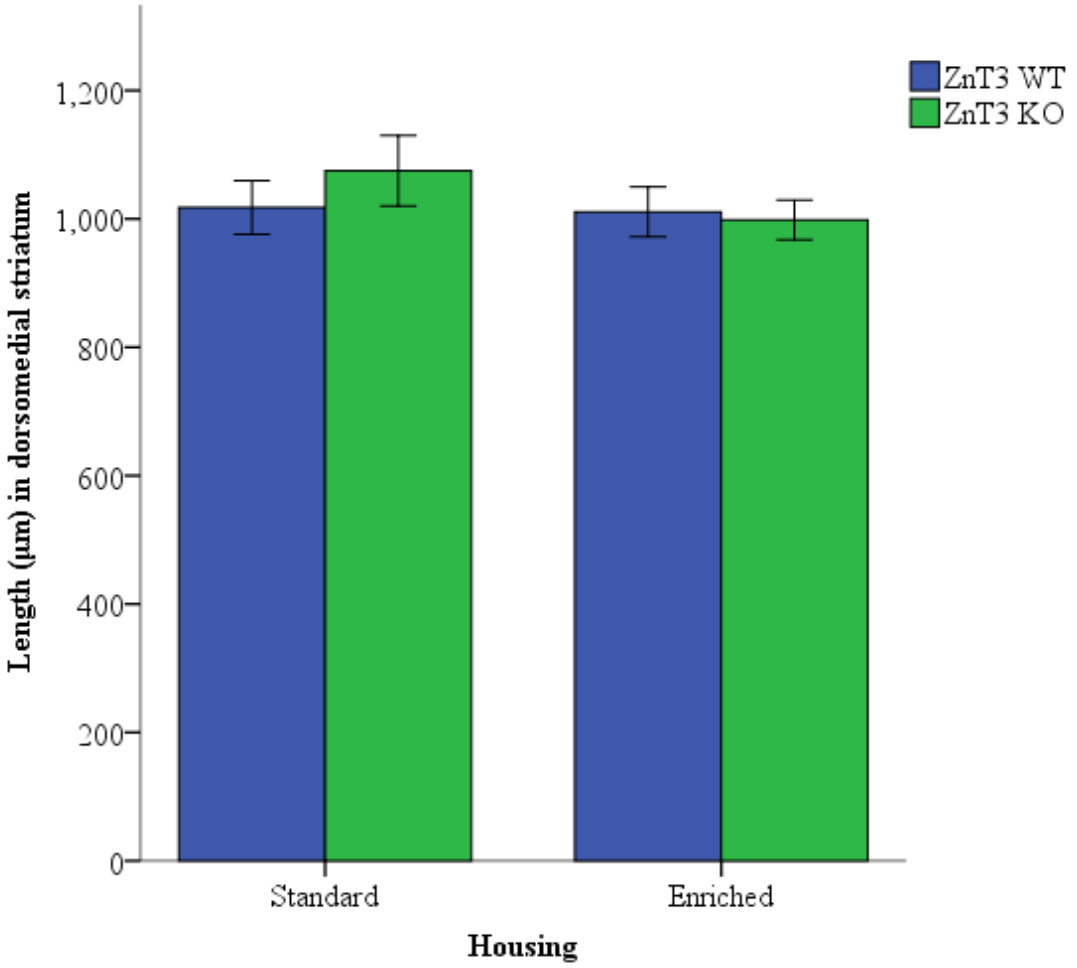

Figure 2.3. Mean dendritic length of medium spiny neurons in the dorsomedial striatum for ZnT3 wildtype (WT) and knockout (KO) mice raised in standard (n=6 per genotype) or enriched environments (WT: n=8; KO: n=7). Error bars represent ± SEM.

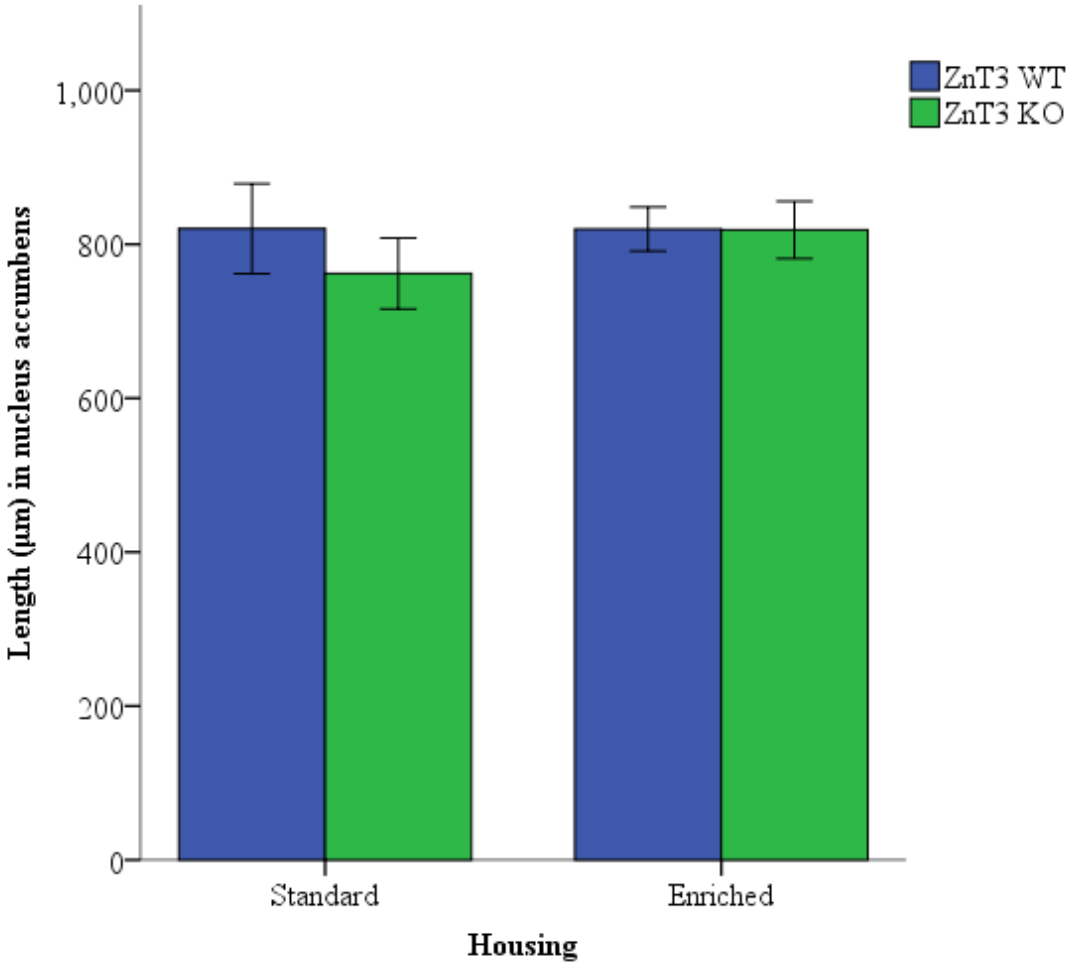

Figure 2.4. Mean dendritic length of medium spiny neurons in the ventral striatum for ZnT3 wildtype (WT) and knockout (KO) mice raised in standard (n=6 per genotype) or enriched environments (WT: n=8; KO: n=7). Error bars represent ± SEM.

2.4.2 Spine Density

Spine density was determined by tracing sections of the terminal ends of dendrites (n=4/area/hemisphere). Figure 2.5 shows a representative medium spiny neuron under the 100X objective with a terminal dendrite visible. In the DLS, a 2x2 (genotype x environment) factorial ANOVA found a main effect of environment, $F(1,49)=9.11$, $p=.004$, with all animal showing increased spine density when raised in an enriched environment (Figure 2.6). There was no significant interaction, $F(1,49)=.43$, $p=.515$, and no main effect of genotype, $F(1,49)=1.53$, $p=.222$. In the DMS, a 2x2 (genotype x environment) factorial ANOVA found a main effect of environment, $F(1,50)=9.92$, $p=.003$ (Figure 2.7). There was no significant interaction, $F(1,50)=1.08$, $p=.304$, and no main effect of genotype, $F(1,50)=2.04$, $p=.160$. In the NAc, a 2x2 (genotype x environment) factorial ANOVA found a significant interaction between genotype and environment, $F(1,50)=5.29$, $p=.026$ (Figure 2.8). Follow up tests were performed using the Least Significant Difference test. In the standard environment, there was no difference between genotypes, $t(11)=1.32$, $p=.195$. In the enriched environment, there was also no significant difference, $t(14)=1.98$, $p=.053$. However, visual examination of the data finds a cross-over interaction which suggests that the ZnT3 KO mice show greater changes in spine density between standard and enriched environments than do ZnT3 WT mice. A main effect of environment was also found, $F(1,50)=37.12$, $p<.001$. There was no main effect of genotype, $F(1,50)=.12$, $p=.734$.

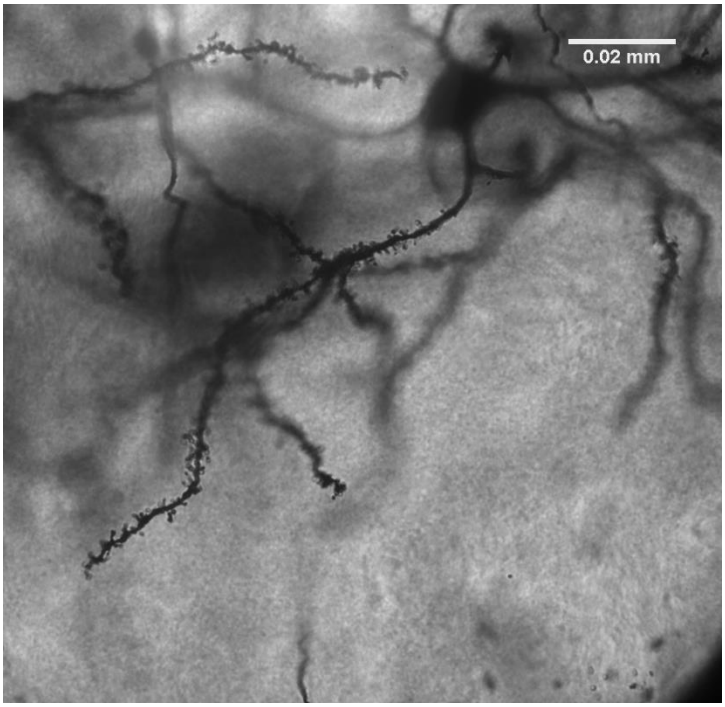

Figure 2.5. Representative terminal dendrite of a medium spiny neuron under the 100X objective.

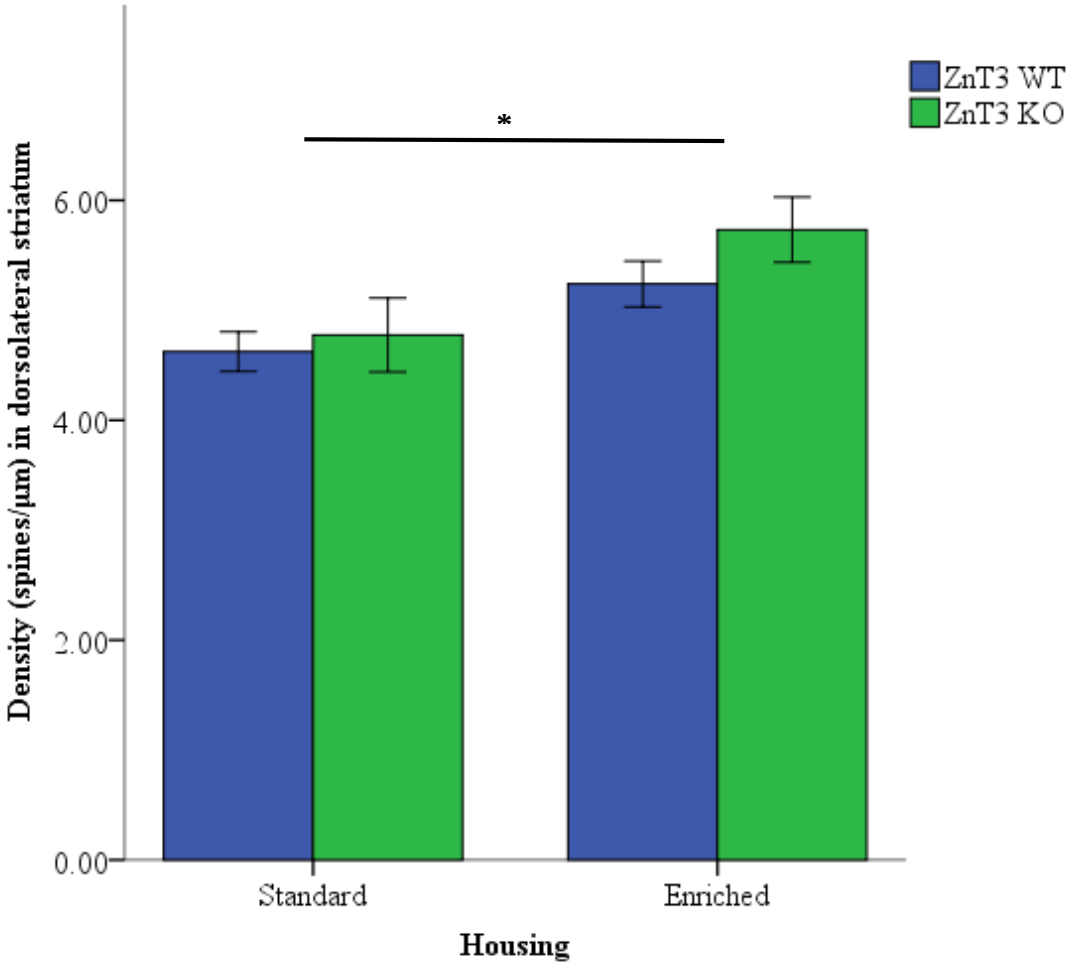

Figure 2.6. Spine density (spines/µm) in the dorsolateral striatum of ZnT3 wildtype (WT) and knockout (KO) mice raised in standard (n=6 per genotype) or enriched environments (WT: n=8; KO: n=7). Error bars represent ± SEM.

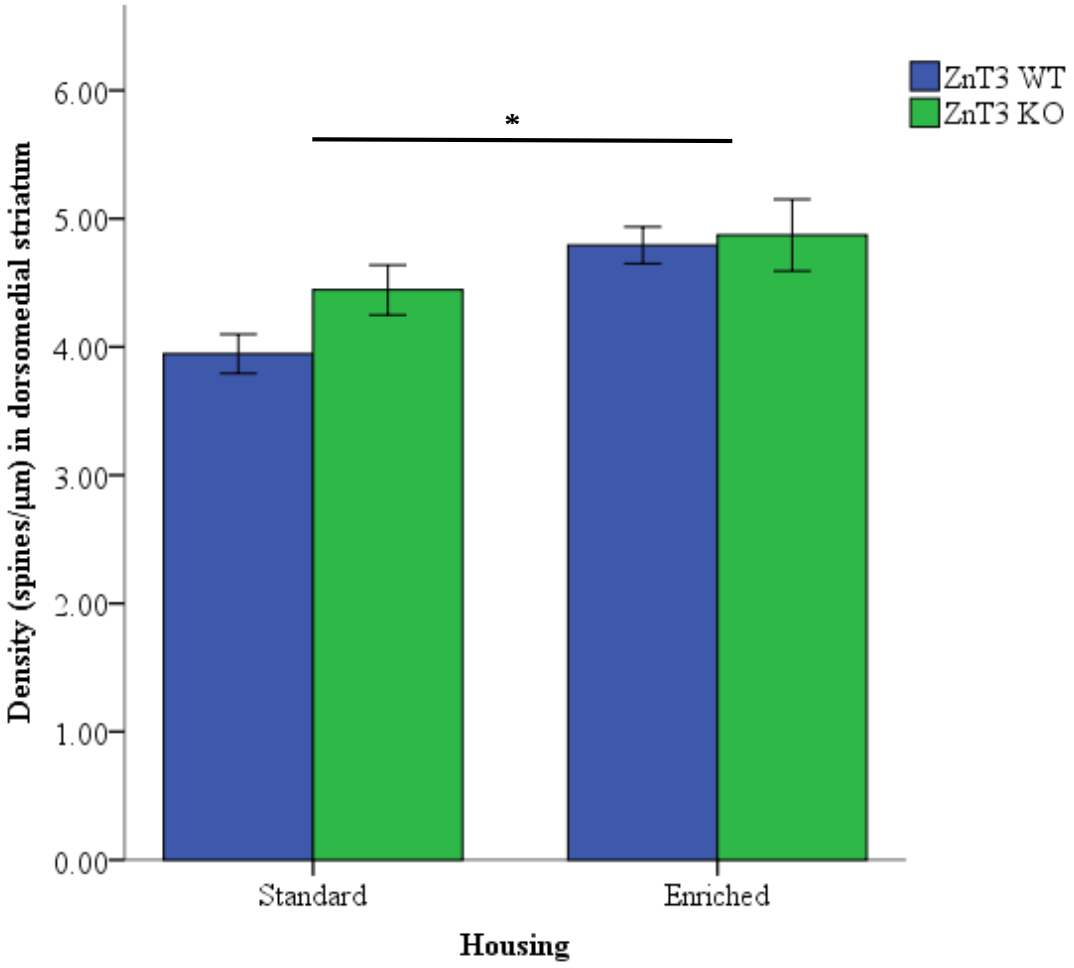

Figure 2.7. Spine density (spines/µm) in the dorsomedial striatum of ZnT3 wildtype (WT) and knockout (KO) mice raised in standard (n=6 per genotype) or enriched environments (WT: n=8; KO: n=7). Error bars represent ± SEM.

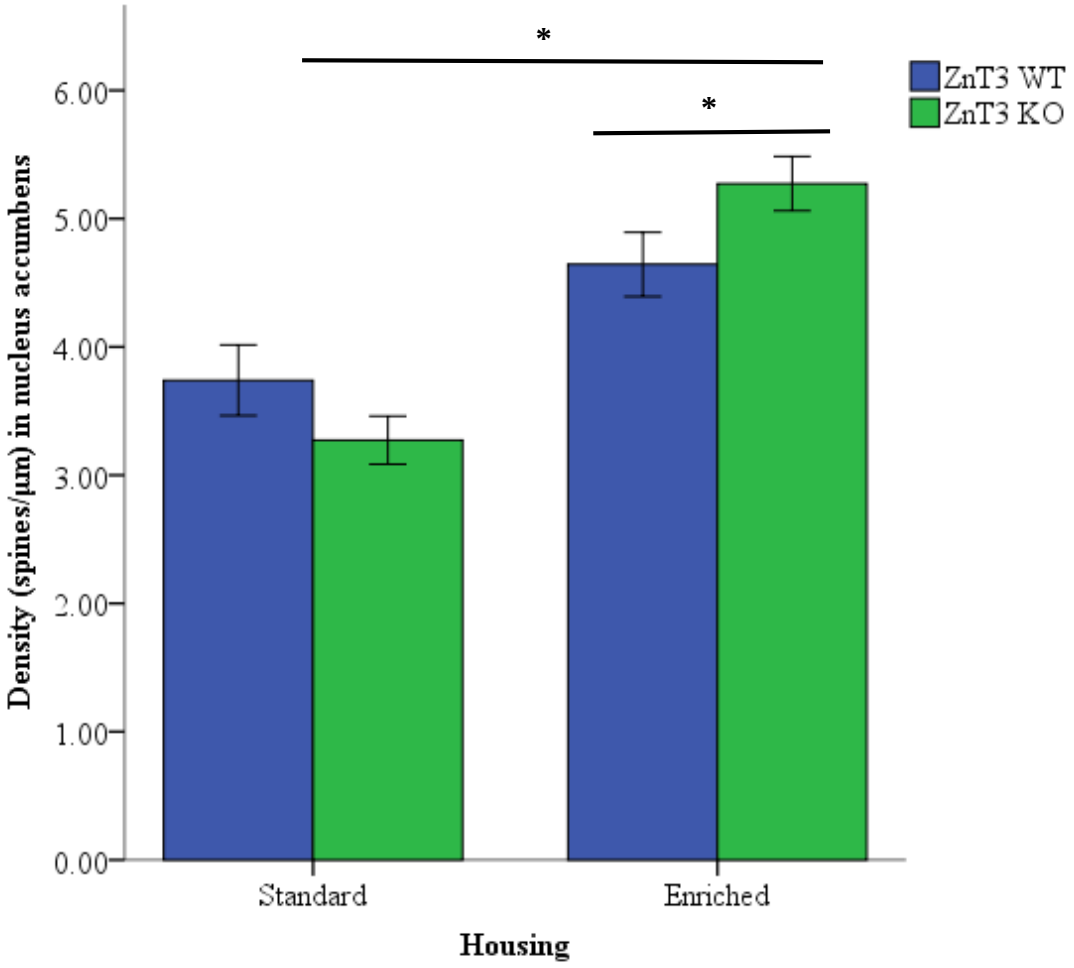

Figure 2.8. Spine density (spines/µm) in the nucleus accumbens of ZnT3 wildtype (WT) and knockout (KO) mice raised in standard (n=6 per genotype) or enriched environments (WT: n=8; KO: n=7). Error bars represent ± SEM.

2.5 Discussion

ZnT3 wildtype (WT) and knockout (KO) mice were raised in either standard or enriched environments. At 3 months of age, their brains were removed and placed in Golgi-cox solution. Cell tracings were used to examine dendritic length (through Sholl analysis) and spine density in three areas of striatum: dorsolateral (DLS), dorsomedial (DMS), and nucleus accumbens (NAc). No changes were found in dendritic length for genotype or environment. However, differences were found in spine density. Environmental enrichment increased spine density in DLS and DMS, irrespective of genotype. In the NAc, while enrichment increased spine density, it did so more for the ZnT3 KO mice than the ZnT3 WT mice. In the standard environment, there was no significant difference between genotypes, while in the enriched environment there was a significant difference between genotypes with ZnT3 KO mice showing a greater increase in spine density. This suggests that the ZnT3 KO mice may benefit more from environmental enriched than their WT littermates, at least in the NAc.

A limitation of using Golgi-cox stain to examine striatal morphology is that cells stain in clumps making it very difficult to find isolated cells ideal for tracing. Also, because no one is entirely sure how the Golgi stain works, there is no way of knowing why specific cells stained in clumps and why others are isolated. Therefore, sampling of cells in the Golgi-stained striatum may not be random. Few studies have used Golgi-cox stain to examine changes in morphology of animals in enriched environments. However, several studies have used the stain to examine other factors that affect morphology of medium spiny neurons (MSNs), such as disease or drugs.

With respect to dendritic length, what we found adds to what has been previously found. Faherty et al. (2003) found no difference in dendritic length of MSNs in the ventrolateral

striatum of mice raised in standard cages, cages with a running wheel only, and enriched environments for 4-5 months. However, Kolb et al. (2003) found a significant increase in dendritic length of MSNs in the NAc of rats raised in enriched environments for 3-3.5 months compared to rats raised in standard environments. This may be indicative of a species difference or brain region difference, as ventrolateral striatum may or may not include the NAc, in effects of environmental enrichment. Other studies of MSNs have found that patients with Parkinson's disease (McNeill et al., 1988; Zaja-Milatovic et al., 2005) and Huntington's disease (Spires et al., 2004) have decreased dendritic length. Several drugs of addiction, including nicotine (McDonald et al., 2005), amphetamine (Robinson and Kolb, 1997, 1999b), and cocaine (Robinson and Kolb, 1999b; Robinson et al., 2001) cause an increase in dendritic length or complexity in the NAc; while others, such as morphine (Robinson and Kolb, 1999a), decrease complexity. No differences were found in either genotype in our study which is consistent with the finding that enriched mice have no changes in dendritic length in the striatum (Faherty et al., 2003), suggesting that enrichment has differential effects based on species. It also suggests that environmental enrichment has similar effects on spine density as some drugs of addiction.

Several studies have found similar increases in spine density on MSNs in the DLS (Comery et al., 1995; Comery et al., 1996) and NAc (Kolb et al., 2003) in rats raised in enriched environments. These studies differ in the amount of time animals were enriched, ranging from 30 days (Comery et al., 1995; Comery et al., 1996) up to 3-3.5 months (Kolb et al., 2003). Our time in enrichment fits right in the middle of this range. It is possible that a shorter amount of time in enrichment may be sufficient to induce the changes in spine density found in this study. However, the studies using only 30 days of enrichment had a glaring confounding variable present. Rats housed in enriched environments were in groups while rats housed in standard

cages were alone (Comery et al., 1995; Comery et al., 1996). Since rats are social creatures, isolation is very stressful for them (Fone and Porkess, 2008). It is, therefore, possible that the changes seen in spine density in the Comery et al. (1995; 1996) studies were due to housing individually versus in groups and not due to housing in standard versus enriched conditions. Either way, our study supports the increase in spine density in enriched animals in the DLS and NAc, and shows that this increase extends to the DMS as well.

As the most interesting finding from this study was the interaction between genotype and environment in the NAc, the rest of this discussion will focus on this finding. The NAc has strong ties to the limbic system and has been shown to be an important component of the brain's reward circuit (reviewed by Ikemoto and Panksepp, 1999). The NAc itself is divided into core and shell regions which have different connections and functions. Afferent connections to the NAc include hippocampus, amygdala, and prefrontal cortex, as well as dopaminergic input from the ventral tegmental area (VTA). Its efferent connections include sites in the midbrain, hypothalamus, and ventral pallidum, as well as motor effector sites. As such, it is thought to be the connection between the limbic system and the motor system. Studies have found that, more than just reward, the NAc is involved in choosing correct actions and suppressing incorrect actions in an effort to make goal-directed learning more efficient (reviewed by Floresco, 2015).

In addition to the enrichment studies examining spine density in the NAc, other enrichment studies have been done examining aspects other than neuronal morphology. One study found increases in extracellular dopamine levels in the NAc in enriched animals compared to standard housed animals (Segovia et al., 2010). Many studies have examined the effects of amphetamine on animals in enriched environments. While these do not specifically examine the NAc, as part of the reward circuit, the NAc is involved in several aspects of drug taking and drug

addiction (reviewed by Yager et al., 2015). Rats raised in enriched environments are more sensitive to the locomotor effects of amphetamine (Bowling et al., 1993; Bowling and Bardo, 1994). However, enriched rats are less likely to self-administer amphetamine (Bardo et al., 2001).

A slightly different approach to this finding is to examine other ways of altering spine density on MSNs besides enrichment. Since the NAc is strongly linked to addiction, a number of studies have examined how various drugs affect spine density on MSNs. Amphetamine increased spine density on distal dendrites of MSNs (the site where dopamine and glutamate converge) in the NAc and dorsal striatum under standard conditions (Robinson and Kolb, 1999b; Li et al., 2003). Similar results were found in the NAc with cocaine administration (Robinson and Kolb, 1999b; Lee et al., 2006). Both amphetamine and cocaine act to increase dopamine in the synapse. Conversely, using a drug that decreases dopamine in the synapse, 6-hydroxydopamine (6-OHDA), causes a decrease in spine density in the NAc (Ingham et al., 1989). These studies suggest that the amount of dopamine present in the NAc is proportional to and may cause, changes in spine density. Since zinc interacts with both dopamine and glutamate, it is possible that in the NAc, zinc modulates this interaction. It seems that ZnT3 KO mice may have increased dopamine in the NAc. Environmental enrichment has a novelty aspect to it as the location of food, water, and toys are rearranged every week. The increased spine density in ZnT3 KO mice may be reflective of this and may indicate that ZnT3 KO mice are more sensitive to novelty than ZnT3 WT mice. Given their seeming hypersensitivity to novelty, it would be interesting to see how ZnT3 KO mice would react to various drugs of addiction.

Our findings also show that ZnT3 KO mice retain some degree of plasticity within the striatum. This is contrary to what was found in the hippocampus (mentioned above; Chrusch,

2015). Although the two studies measure different aspects, morphology versus neurogenesis, the enriched ZnT3 KO mice lacked hippocampal plasticity. Put together, these findings suggest that synaptic zinc may have differential effects in the brain.

One limitation of this study is that only female mice were used. Sex differences have been found in brain anatomy (Cahill, 2006), therefore it is possible that male ZnT3 mice would have a different response to enrichment. A future study should test the effects of enrichment on male ZnT3 mice to determine if they also show changes in spine density in the striatum.

Another area to examine would be other mouse models that have synaptic zinc dysfunction. Two g-protein coupled receptors, GPR39 (Holst et al., 2007; Yasuda et al., 2007; Cohen et al., 2012) and GPR83 (Muller et al., 2013), have been found to be activated by zinc. Knockout mouse models exist for both. It would be interesting to see if these mice also show similar increases in spine density in the striatum after enrichment.

In summary, environmental enrichment has no effect on dendritic length within the striatum; however, it increases spine density in all 3 regions of striatum (DLS, DMS, NAc). In particular, the enriched ZnT3 KO mice show a greater increase in spine density in the NAc than their enriched WT counterparts. This implies that ZnT3 KO mice retain some plasticity within the striatum and may indicate a hypersensitivity to novelty. Future studies should examine how ZnT3 KO mice react to other aspects of novelty and/or rewards, such as various drugs of addiction.

Chapter Three: **Behaviour**

3.1 Introduction

Zinc is an essential metal in the body (Chasapis et al., 2012), including the brain where it can be loaded into synaptic vesicles by zinc transporter 3 (ZnT3; Cole et al., 1999) and released into the synapse (Frederickson et al., 2000; Frederickson et al., 2005). Synaptic zinc acts on post-synaptic neurons by modulating receptors or by entering the cell and modulating second messenger systems (reviewed by Nakashima and Dyck, 2009). High amounts of synaptic zinc are found in the neocortex (layers II/III, V, and IV), hippocampus, amygdala, and striatum; lower amounts are found in several other brain regions as well (Brown and Dyck, 2004a). Research on the role of synaptic zinc in the hippocampus, amygdala, and somatosensory cortex suggest that it modulates plasticity (reviewed by Nakashima and Dyck, 2009).

In 1999, a mouse model was created by knocking out the ZnT3 gene. As a result, the ZnT3 knockout (ZnT3 KO) mice lack synaptic zinc in their brains (Cole et al., 1999). The mice were tested on a battery of behavioural tasks, including tests of olfaction, audition, motor coordination, nociception, anxiety, and tasks involving different types of learning and memory (i.e. fear conditioning, Morris water task, radial arm maze). Surprisingly, ZnT3 KO mice were found to perform at the same level as ZnT3 wildtype (WT) mice on all of these tasks (Cole et al., 2001). The ZnT3 mice were later tested again on fear conditioning paradigms and found to have deficits in learning the tone-shock pairing (Martel et al., 2010; Sindreu et al., 2011). They also seem to have difficulty on tasks that require behavioural flexibility, such as the t-alternation maze (Sindreu et al., 2011) and a version of the Morris water task that involves learning an initial platform location and subsequently learning a new platform location (Martel et al., 2011). Also,

older ZnT3 KO mice (6 months of age) were found to perform poorer than ZnT3 WT mice of the same age on the Morris water task (Adlard et al., 2010).

A potential problem with the initial testing of the ZnT3 KO mice is that data included both male and female mice (Cole et al., 2001). Studies have shown that males and females perform differently on some tasks, particularly on motor tasks (reviewed by Field and Pellis, 2008). This calls into question the conclusion that ZnT3 KO mice are no different than ZnT3 WT mice on tests of motor function, as differences may be present for one sex but not the other; averaging results from both sexes might mask these differences. Because synaptic zinc is present in several brain regions that are considered part of the motor system (Brown and Dyck, 2004a), it is possible that the ZnT3 KO mice show deficits on one or more aspect of motor function.

The motor system is divided into the pyramidal motor system (consisting of motor cortex projecting to spinal cord) and the extra-pyramidal motor system (consisting of the basal ganglia and cerebellum). Each system and brain region makes a different contribution to overall motor output. For example, while the pyramidal system is mainly concerned with controlling the muscles that cause movement, the cerebellum is involved with coordination of those movements and the basal ganglia are involved in fine motor skills and motor learning (reviewed by Doya, 2000; Middleton and Strick, 2000). One area of the brain that contains high levels of synaptic zinc is the input center of the basal ganglia, the striatum. As part of the basal ganglia, the striatum is involved in many behavioural tasks including fine motor skills, motor task learning and memory, as well as other aspects of cognition such as motivation and reward (reviewed by Liljeholm and O'Doherty, 2012). The striatum is divided into 3 functional regions: dorsolateral (DLS; mainly putamen), dorsomedial (DMS; mainly caudate nucleus), and ventral/nucleus accumbens (NAc). Different regions of the striatum have different afferent connections and,

therefore, different functions. With respect to synaptic zinc, the striatum is an interesting area to examine because while synaptic zinc is present within the striatum, retrograde tracing studies have found that there are no zincergic cell bodies in the striatum. This means that the synaptic zinc must be coming in from other brain regions, most likely from neocortex.

There are behavioural tests available to examine the different aspects of the motor system. The current study used seven of these tasks to examine the behaviour of female ZnT3 WT and KO mice. Muscle strength and neuromuscular coordination were tested using the inverted wire hang test (reviewed by Balkaya et al., 2013). Gait was analysed by footprint analysis (reviewed by Brooks and Dunnett, 2009; Balkaya et al., 2013). The limb asymmetry test examined forelimb usage during rearing (reviewed by Brooks and Dunnett, 2009; Balkaya et al., 2013). The pole test examined motor coordination, as well as bradykinesia (slowness of movement; reviewed by Balkaya et al., 2013). Motor coordination, as well as balance and motor learning, can also be tested using balance beams (reviewed by Brooks and Dunnett, 2009; Luong et al., 2011). The horizontal ladder tested forelimb-hindlimb coordination and is a test of skilled walking (Metz and Whishaw, 2009). The skilled reach test examined skilled forelimb use and goal-directed learning and had some aspects of motivation and reward (Whishaw, 1996; Gholamrezaei and Whishaw, 2009).

The skilled reach task is one of only a few behavioural tests for rodents that examine striatal-dependent motor tasks. The skilled reach task involves training an animal to reach through a slit at the front of a Plexiglas box to retrieve a food pellet sitting on a ledge outside of the box. It is largely done with rats, however, it is possible to train mice on this task as well. With mice, the task can take as little as 7 days (Marques and Olsson, 2010) or can continue on for up to or over a month (Whishaw, 1996; Chen et al., 2014). Endpoint measures include

latency to first reach attempt, latency to successfully retrieve the first pellet, number of reach attempts, and reach accuracy. These are determined each day and rodents generally improve on these outcomes over the course of training. Training is complete when rodents are reaching with an accuracy of 50% or greater. A more detailed analysis of reaching behaviour can also be done that examines kinematic aspects of reaching such as posture, as well as forearm and forepaw movements.

The purpose of this study was to examine general and skilled motor behaviour in ZnT3 WT and KO mice. Because of the presence of synaptic zinc in the motor system, including striatum, and because of its role in modulating plasticity in other brain regions, we hypothesized that the lack of synaptic zinc in the striatum of ZnT3 KO mice would cause them to perform poorly on striatal-dependent behavioural tasks, specifically on the skilled reach task. Due to the fact that the ZnT3 KO mice have no obvious motor impairments and that there is little synaptic zinc present in the cerebellum (Wall, 2005), it is unlikely that deficits will be found on general motor tasks and tasks involving mainly cerebellum – i.e. striatal-independent tasks (balance beams, pole test, etc).

3.2 Methods

3.2.1 Animals

Mice on a C57BL/129sv background heterozygous for the ZnT3 allele were bred for this study. Two-three month-old ZnT3 WT and KO female mice were used. ZnT3 heterozygous mice were tested as well, but were removed from analysis because our interest lies in what a complete lack of synaptic zinc will do. Mice were group-housed under a 12 h light/dark cycle and have ad libitum access to food (LabDiet Mouse Diet 9F, #5020) and water for most of the testing period

(see below). All procedures were approved by the Animal Care Committee of the University of Calgary and conformed to the guidelines set out by the Canadian Council for Animal Care.

3.2.2 Food restriction

Mice were food restricted for the skilled reach task. During food restriction, the mice were given enough food to maintain 85-90% of their body weight (2g of food/animal/day). The food was provided after the testing session for the day was completed. Sugar pellets (0.2 g/animal/day; Noyes precision Formula F sucrose pellets, Lancaster, NH) were given alongside the food.

3.2.3 Procedure

Mice were subjected to a battery of behavioural tasks to test both general motor function as well as the function of the striatum. All testing occurred during the day. All tasks, except for footprint analysis, were videotaped for use in scoring. Mice were acclimatized to the testing room for 3 days prior to testing.

3.2.3.1 Limb use asymmetry test

The limb use asymmetry test is used to examine possible impairments in forepaw usage during rearing. Mice (WT: n=14; KO: n=12) were placed in a clear glass cylinder with a mirror behind it for optimal viewing and recorded for 5 minutes. Limb use during rearing was scored based on independent or simultaneous use of forelimbs (Schallert et al., 2000). A repeated-measures analysis of variance (ANOVA) was used to compare groups for left, right, and both paw usage. Paired sample t-tests were used post hoc.

3.2.3.2 Footprint analysis.

Footprint analysis is done to examine gait. Mice (WT: n=14; KO: n=12) were trained to walk along a paper-covered runway (40 cm long by 8 cm wide). Their paws were dipped in food colouring so that footprints were visible. Mice had 3 trials back-to-back. From each trial 3 consecutive strides were used to measure stride length (distance from back of hind paw on one stride to back of same hind paw on next stride) and stride width (distance from middle of left hind paw to middle of right hind paw). Average stride length and width was compared between groups using independent samples t-tests.

3.2.3.3 Pole test.

The pole test is a measure of motor coordination and can also be used as a test of bradykinesia (slowness of movement). Mice (WT: n=18; KO: n=16) were placed head facing upwards at the top of a 50 cm vertical pole with a tennis ball attached to the top to prevent mice from climbing up. The pole was wrapped in gauze to prevent slipping. Latency to turn around so their head is facing down and latency to climb down the pole to the ground was recorded. Mice had 3 back-to-back trials (Sedelis et al., 2000; Cole et al., 2001; Doeppner et al., 2014). Average latencies were compared between groups using independent samples t-tests.

3.2.3.4 Horizontal ladder.

The horizontal ladder is a test of skilled walking. Mice (WT: n=14; KO: n=12) were trained to walk across a horizontal ladder (1 m long with 99 metal rungs evenly spaced) into their home cage. Each mouse had 3 practice trials back-to-back before being returned to their home

cage. Once all mice completed the practice trials, each mouse was then required to cross once with all rungs in place and once with 13 rungs removed from the middle portion of the ladder (no two consecutive rungs were removed). Time to cross and number of footfalls were recorded for both trials. A repeated measures ANOVA was used to compare time to cross between groups and again for number of footfalls with the repeated factor being all rungs present or 13 rungs removed.

3.2.3.5 Inverted wire-hang test.

The inverted wire hang test examines coordination and muscle strength. Mice (WT: n=14; KO: n=12) were placed on a wire mesh grid (13.5 cm by 13.5 cm) with 7.5 cm high walls, 35 cm off the ground. The grid was rotated so that the mouse was hanging upside down. Mice had 3 trials in succession. Average latency to fall was recorded (Tillerson and Miller, 2003). Independent t-tests were used to compare fall time between groups. As weight of the mouse might affect fall time, all mice were weighed prior to the test. Unstandardized fall time as well as fall time standardized to weight (reported as impulse = weight x fall time) are reported.

3.2.3.6 Beam walking.

Beam walking tests motor coordination and motor learning, and has been found to be more sensitive to deficits than the rotarod which tests similar functions (Stanley et al., 2005). Mice (WT: n=14; KO: n=12) were trained to walk across two 50 cm wooden beams (1.2 and 0.6 cm in diameter, 80 cm off the ground) to a wooden box that the mice were habituated to at the start of training. Training occurred over 3 days (Luong et al., 2011). Mice were first trained to cross the 1.2 cm diameter beam. They had 3 consecutive trials. After a 10 minute break, mice

were then trained to cross the 0.6 cm diameter beam. After 3 consecutive trials they were returned to their home cage. Time to cross beam and number of foot-slips were recorded (Linden et al., 2007). A repeated measure ANOVA was used to compare across the 3 days for each size of beam on both time to cross and number of foot-slips. Paired sample t-tests were used post hoc.

3.2.3.7 Skilled reach task.

The skilled reach task is a test of motor learning and fine motor skills. There are also motivation and reward aspects to the test. Mice (WT: n=14; KO: n=12) were food restricted as described above for the duration of the test period. Mice were placed in a modified Plexiglas reaching box (13 cm wide x 15 cm long x 40 cm high) and trained to reach through a slit (1 cm) in the box to retrieve a sugar pellet on a ledge (1.5 cm above ground) in front of the slit. Mice were habituated to the sugar pellets by placing them in their home cages for 5 days prior to training. Mice were habituated to the reaching box over 3 days. The first day mice were placed in littermate pairs in the reaching box for 10 minutes with access to sugar pellets on the floor of the box as well as on the ledge outside the box within tongue distance. The second day the mice were placed individually in the box for 10 minutes with access to sugar pellets on the floor and on the ledge within tongue distance. On the third day, pellets were only available on the ledge and were gradually moved so that the mouse was forced to reach for the pellet. Preferred paw for each mouse was determined and the pellets were moved to the contralateral side on the ledge so that the mouse was forced to only reach with its preferred paw. Days 4-8 consisted of daily sessions that lasted 10 minutes. Mice were placed into groups each day determined by their willingness and ability to perform the task. Four groups were determined: non-learners were mice that would not eat the sugar pellets that were readily available to them; mediocre learners

would eat sugar pellets within tongue distance but made no attempt to retrieve pellets at reaching distance; eager learners would eat sugar pellets within tongue distance and try either by tongue or by reaching to retrieve pellets at reaching distance but remained unsuccessful; and successful learners, for the purpose of this classification, were able to successfully retrieve at least one pellet during the course of the 10 minute training session. The Kruskal-Wallis test was used to determine whether or not mice improved (in terms of group membership) over the 5 days of testing. Because not all mice learned the task within the 8 days of training, only mice that were successful over at least 3 days of training (n=6 per genotype) were included in the statistical analyses. Latency to first reach attempt (the first time the mouse makes a reaching attempt), number of reaching attempts (only counted when a sugar pellet was present and in the correct location on the shelf), reaching accuracy (number of pellets retrieved/number of reach attempts x 100), and latency to retrieve one pellet (latency to the first successful retrieval of the day) were analyzed using repeated measures ANOVAs (Marques and Olsson, 2010). Paired sample t-tests were used post hoc for significant main effects. Independent sample t-tests were used post hoc for significant interactions.

3.3 Results

3.3.1 Limb asymmetry

When examining paw usage during rearing in a glass cylinder, a repeated-measures analysis of variance (ANOVA) was used to compare groups for left, right, and both paw usage. Tukey's LSD test was used post hoc. A significant main effect of paw was found, $F(1.50,35.88)=141.20$, $p<.001$. Mice (WT: n=14; KO: n=12) used both paws during rearing

significantly more often than just left paw, $t(25)=15.43$, $p<.001$, or right paw, $t(25)=11.53$, $p<.001$ alone. There was no difference between left and right paw usage, $t(25)=.66$, $p=.519$. There was also no significant difference between genotypes (Figure 3.1).

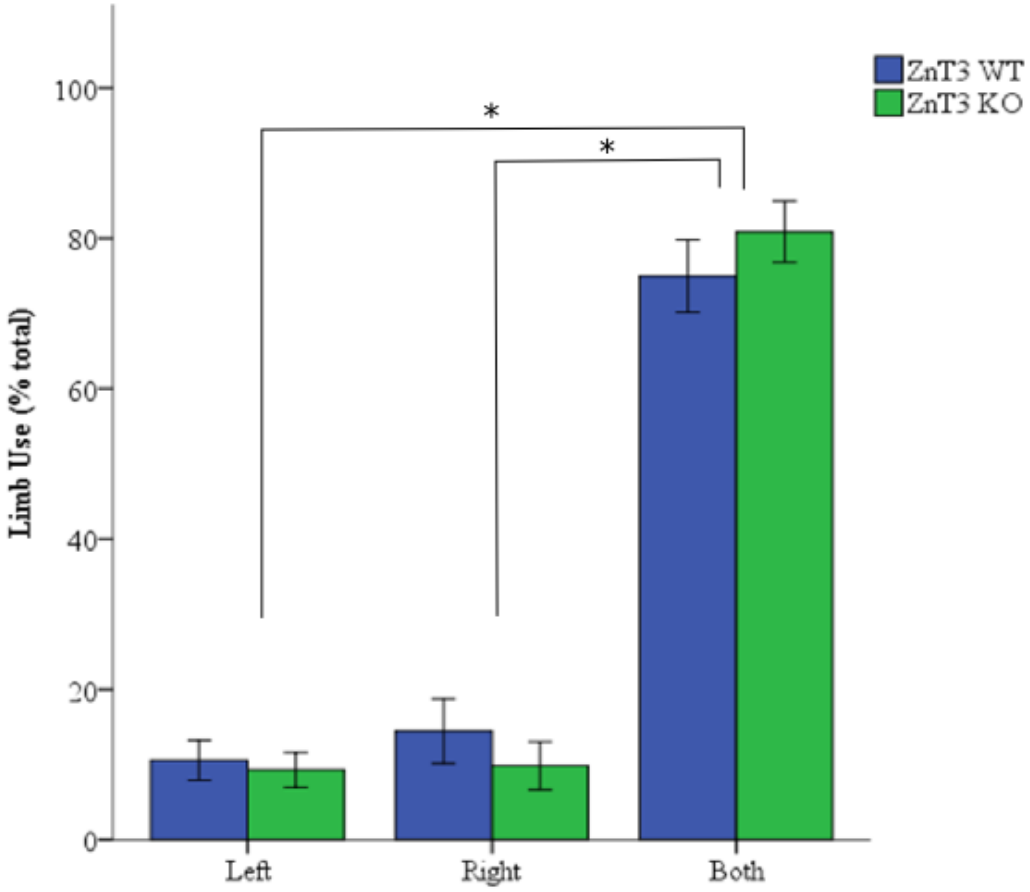

Figure 3.1. Limb use in ZnT3 wildtype (WT; n=14) and knockout (KO; n=12) mice during rearing in a glass cylinder over the course of 5 minutes. Error bars represents ±SEM.

3.3.2 Footprint analysis

Stride length and width were examined using footprint analysis. An independent samples t-test found no significant differences found between genotypes (WT: n=14; KO: n=12) for length, $t(24)=.80$, $p=.430$, or width, $t(24)=.51$, $p=.511$ (Figure 3.2).

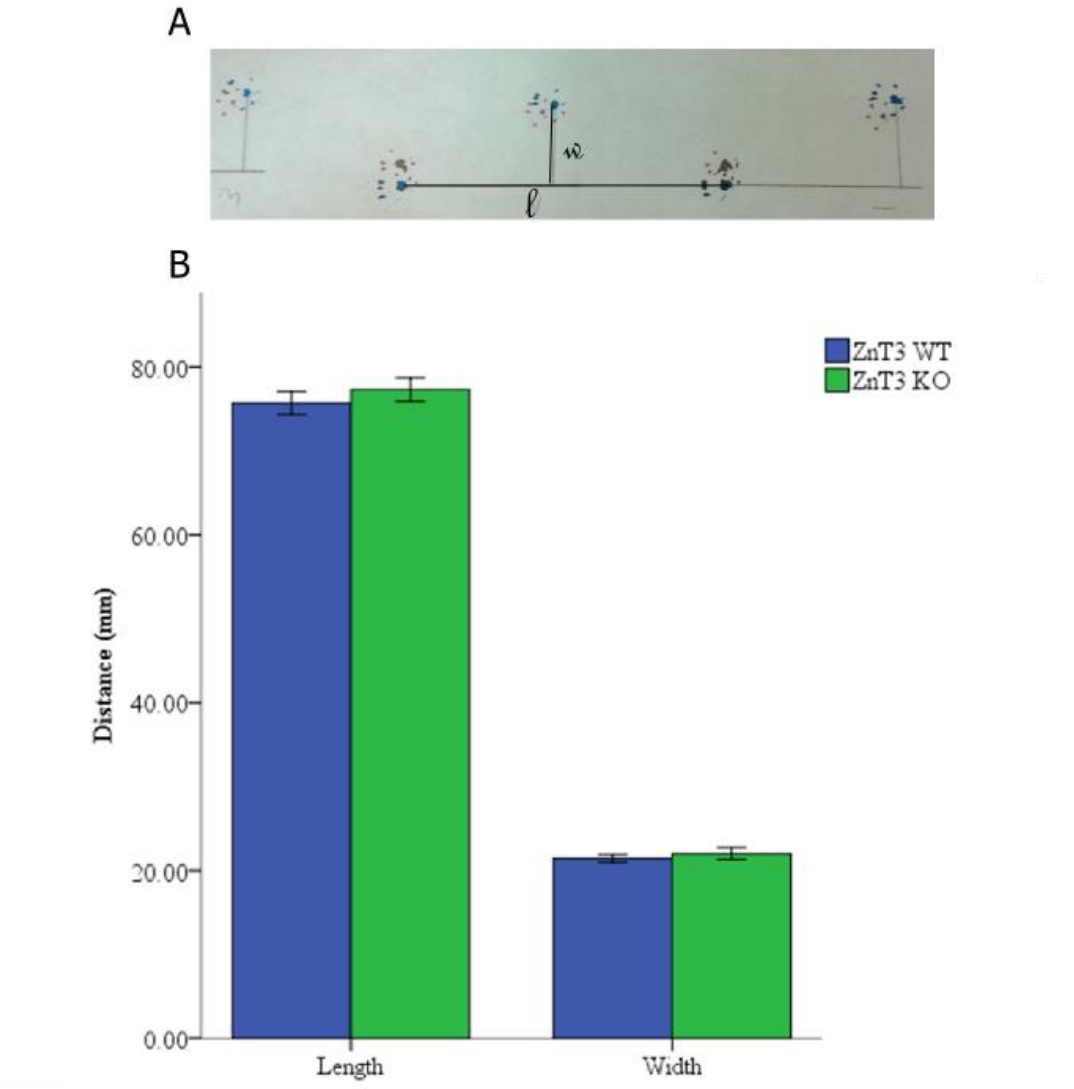

Figure 3.2. A) Representation of footprint analysis raw data showing how length (ℓ) and width (𝓌) were measured. B) Stride length and width for ZnT3 wildtype (WT; n=14) and knockout (KO; n=12) mice as measured using footprint analysis. Error bars represents ±SEM.

3.3.3 Pole test

Mice (WT: n=18; KO: n=16) were placed at the top of a 50 cm tall gauze-covered pole face-up. Time to turn around and face downward and total time to reach the bottom of the pole were recorded. Independent samples t-tests were used to compare between genotypes. Levene's test for equality of variances was significant for turn time, F=7.30, p=.011, and for total time, F=15.95, p<.001, therefore degrees of freedom were adjusted accordingly. Turning time approached significance with the ZnT3 KO mice turning slightly faster, t(22.83)=2.02, p=.055. Total time (including turn time) to descend the pole was significantly faster for ZnT3 KO mice, t(22.09)=2.70, p = .013 (Figure 3.3).

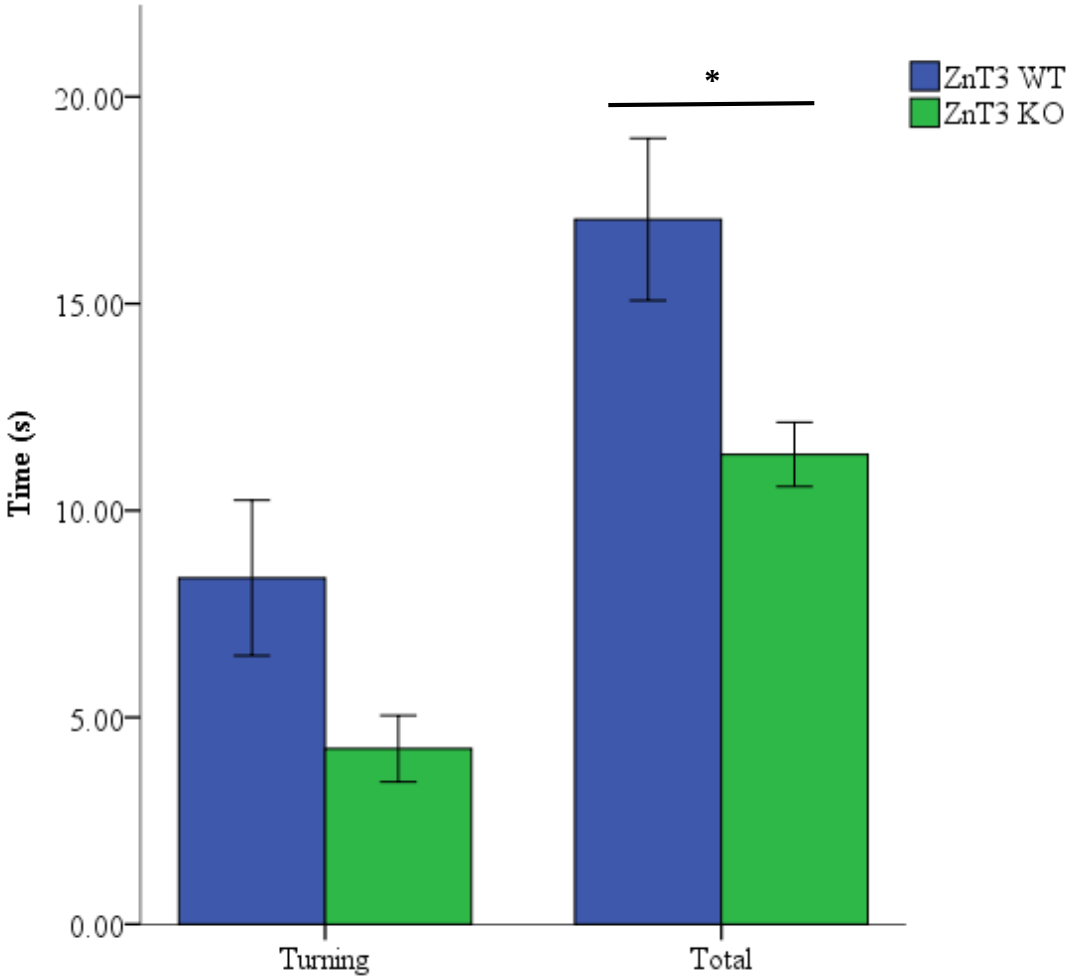

Figure 3.3. Time it took for ZnT3 wildtype (WT; n=18) and knockout (KO; n=16) mice to turn facing downward and descend to the bottom of a 50 cm tall gauze-covered pole. Total time includes turning time. Error bars represents ±SEM.

3.3.4 Horizontal ladder

Mice (WT: n=14; KO: n=12) were trained to run across a horizontal ladder first with all rungs in place, then with 13 rungs removed from the middle portion of the ladder. Time to cross and number of foot slips were recorded for each trial. A repeated measures ANOVA was used to compare time to cross between groups and again for number of footfalls with the repeated factor being all rungs present or 13 rungs removed. There was a significant main effect of trial in that mice were significantly slower when the 13 rungs were removed, $F(1,24)=31.26$, $p<.001$ (Figure 3.4 panel A). However, there were no differences between genotypes. Mice also had significantly more foot slips when the 13 rungs were removed, $F(1,24)=8.46$, $p=.008$ (Figure 3.4 panel B). This did not differ between genotypes.

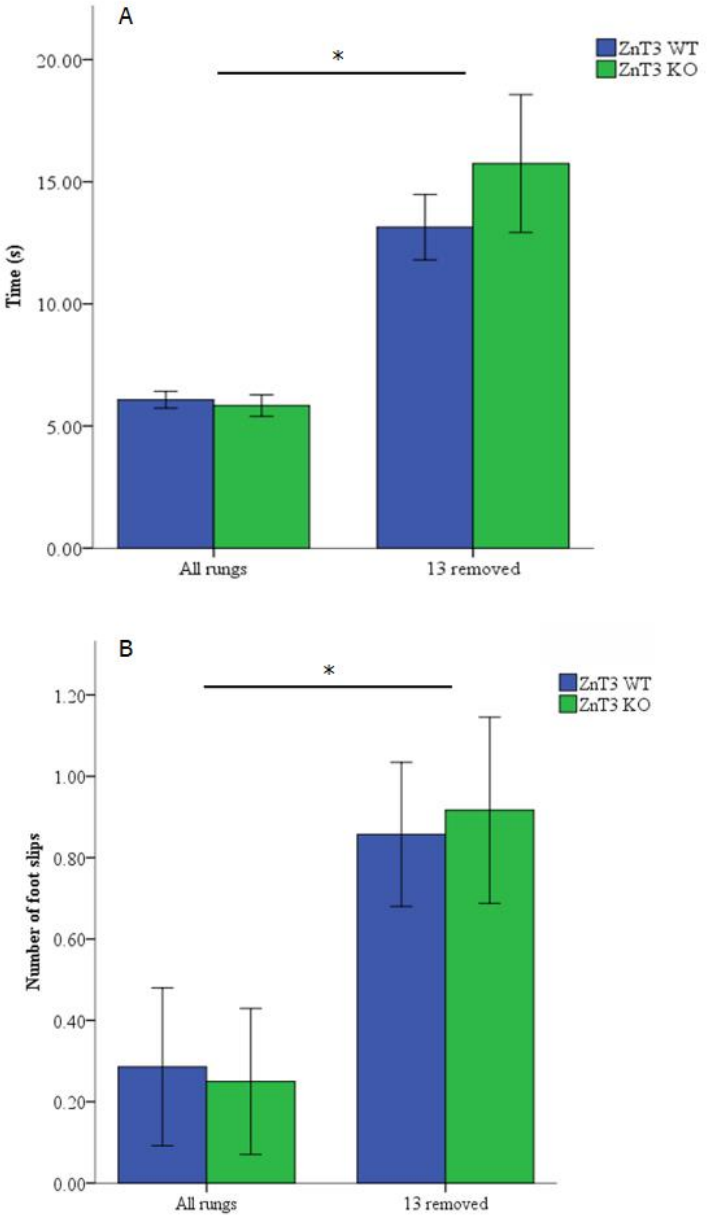

Figure 3.4. Horizontal ladder. A) Time taken for ZnT3 wildtype (WT; n=14) and knockout (KO; n=12) mice to cross a horizontal ladder with all rungs in and with 13 rungs removed. B) Number of foot slips that occurred when ZnT3 WT and KO mice crossed a horizontal ladder with all rungs present and with 13 rungs removed. Error bars represents ±SEM.

3.3.5 Inverted wire hang test

Mice (WT: n=14; KO: n=12) were placed on a wire mesh grid and turned upside down. Average time to fall during 3 consecutive trials was determined. Independent t-tests were used to compare fall time between groups. There were no significant differences between genotypes in weight, $t(24)=.934$, $p=.360$ (Figure 3.5), unstandardized fall time, $t(24)=.12$, $p=.903$ (Figure 3.6 panel A), or fall time standardized to weight (impulse = weight x fall time), $t(22.40)=.202$, $p=.842$ (Figure 3.6 panel B).

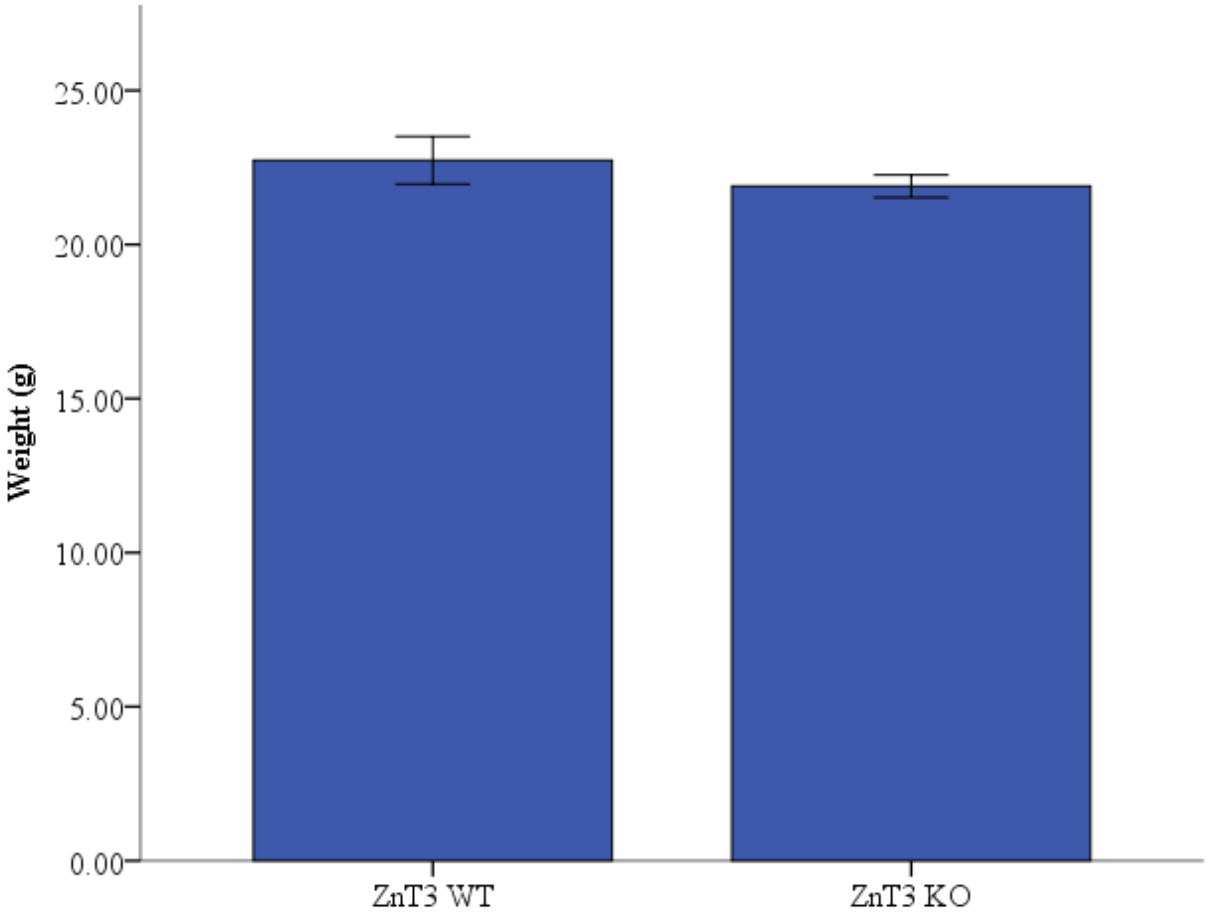

Figure 3.5. Weight in grams of ZnT3 wildtype (WT; n=14) and knockout (KO; n=12) mice prior to wire hang test. Error bars represents ±SEM.

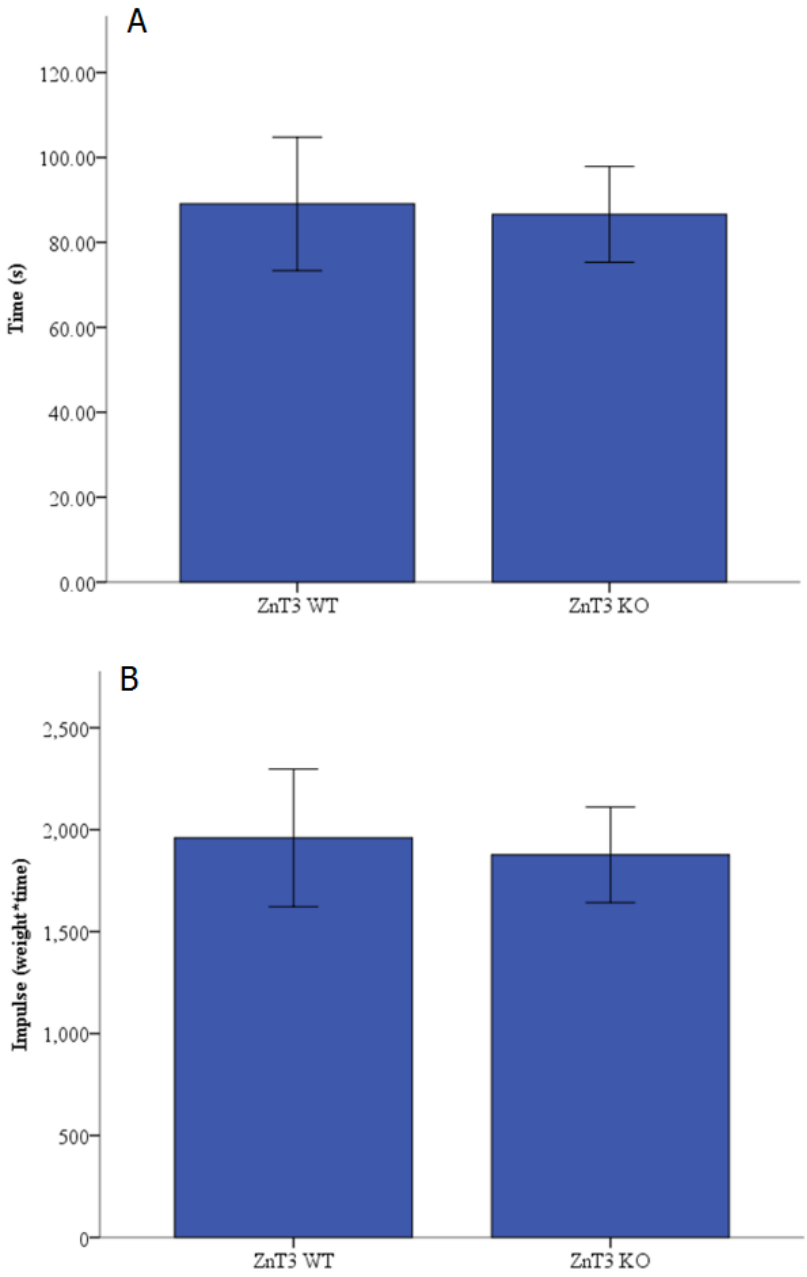

Figure 3.6. Inverted wire hang test. Average time to fall from wire grid for ZnT3 wildtype (WT; n=14) and knockout (KO; n=12) mice, A) unstandardized and B) standardized to weight. Error bars represents ±SEM.

3.3.6 Balance beam test

Mice (WT: n=14; KO: n=12) were trained to walk across beams of two different sizes (1.2 cm and 0.6 cm diameter). Training took place over 3 days. Time to cross the beam and number of foot slips were recorded. A repeated measure ANOVA was used to compare across the 3 days for each size of beam on both time to cross and number of foot-slips. All mice significantly improved their time to cross both the large beam, $F(2,48)=6.12$, $p=.004$ (Figure 3.7 panel A), and the small beam, $F(2,48)=3.56$, $p=.036$ (Figure 3.7 panel B), over the 3 days of training. Follow up paired samples t-tests (corrected $\alpha=.017$) found that for the large beam there were significant differences between day 1 and day 3, $t(25)=3.16$, $p=.004$, and day 2 and day 3, $t(25)=3.67$, $p=.001$. For the small beam, there was a significant difference between day 1 and day 3, $t(25)=2.65$, $p=.014$. However, there was no difference between genotypes. In terms of foot slips, mice did not improve on the large beam over the 3 days, $F(1.33,31.98)=2.13$, $p=.149$ (Figure 3.7 panel C). However, they did improve on the small beam over the 3 days, $F(1.55,37.28)=4.77$, $p=.021$ (Figure 3.7 panel D). Follow up paired t-tests (corrected $\alpha=.017$) found a significant difference between day 1 and day 3, $t(25)=2.78$, $p=.010$. There were no differences between genotypes.

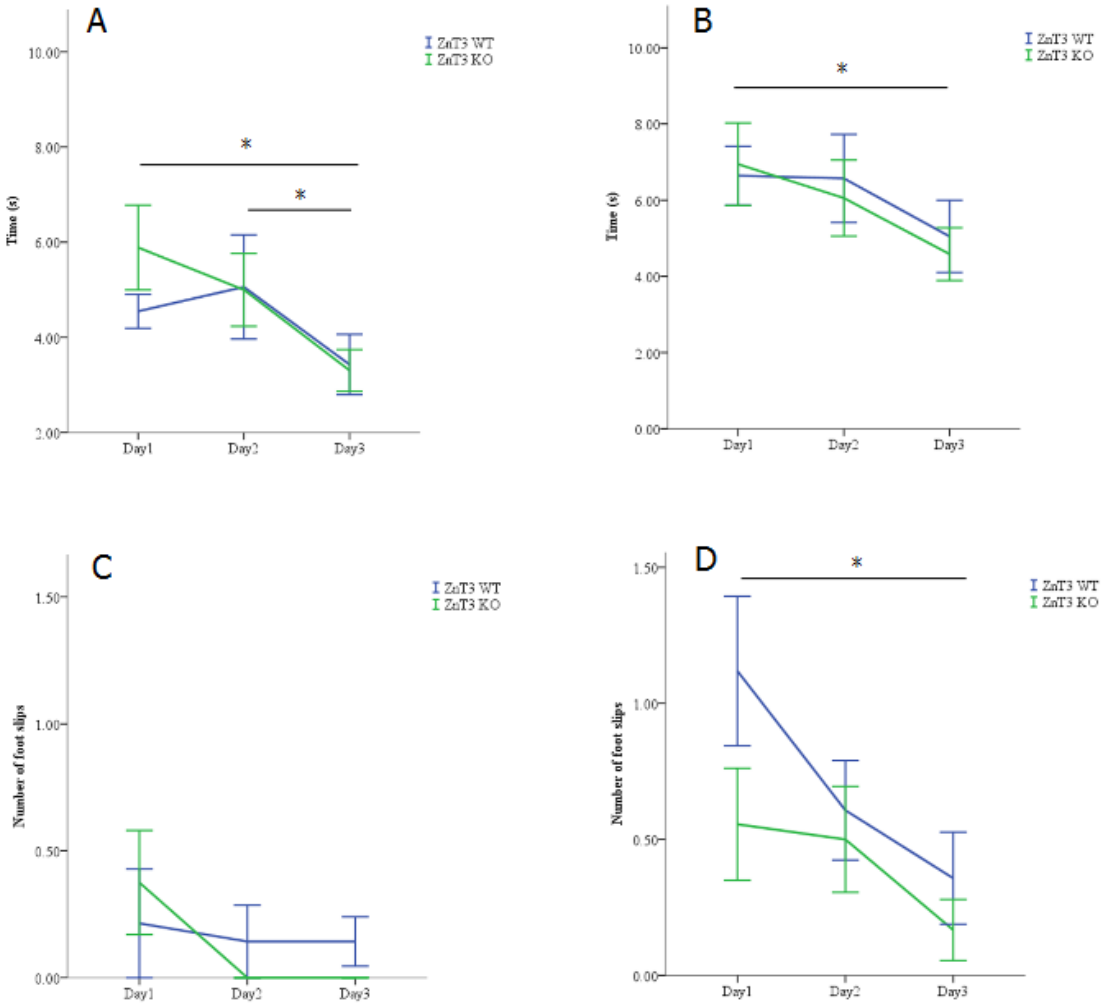

Figure 3.7. Balance beams. Time taken for ZnT3 wildtype (WT; n=14) and knockout (KO; n=12) mice to cross A) large (1.2 cm diameter) and B) small (0.6 cm diameter) balance beams. Number of foot slips ZnT3 WT and KO mice had while crossing a C) large (1.2 cm diameter) and small (0.6 cm diameter) balance beam. Error bars represents ±SEM.

3.3.7 Skilled reach task

Over 8 days, mice (WT: n=14; KO: n=12) were trained to reach through a slit in the front of a reaching box to retrieve a sugar pellet. During the course of training, mice were food restricted (2 g of food per animal per day) to 85-90% of their starting weights. The weight of each mouse was determined prior to food restriction and every other day throughout training. A repeated measures ANOVA was used to ensure mice of both genotypes were losing weight equally. All mice lost weight (as expected) with a main effect of day, $F(4,96)=195.47$, $p<.001$; however, there was no difference between genotypes, $F(4,96)=2.24$, $p=.108$. Figure 3.8 show weight of mice in grams (panel A) as well as in percent of initial weight (panel B).

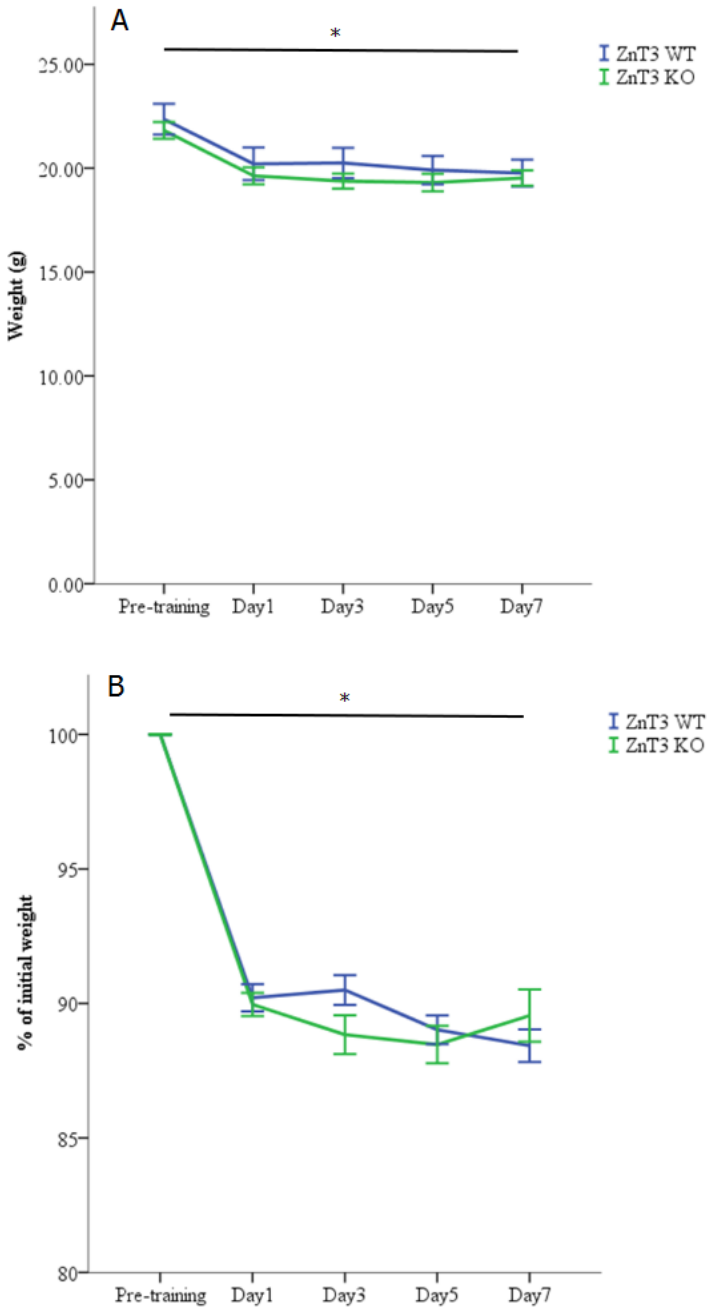

Figure 3.8. Skilled reach. Weight A) in grams and B) as a percentage of initial weight of ZnT3 wildtype (WT; n=14) and knockout (KO; n=12) mice prior to and over the course of skilled reach training. Error bars represents ±SEM.

To determine whether mice could learn the skilled reach task, each mouse was assigned to one of four groups on each day of testing: non-learners were mice that would not eat the sugar pellets that were readily available to them; mediocre learners would eat sugar pellets within tongue distance but made no attempt to retrieve pellets at reaching distance; eager learners would eat sugar pellets within tongue distance and try either by tongue or by reaching to retrieve pellets at reaching distance but remained unsuccessful; and successful learners were able to successfully retrieve at least one pellet during the course of the 10 minute training session. The Kruskal-Wallis test showed that the mice in general learned the task over the 5 days, $\chi^2=19.46$, $p=.001$. Figure 3.9 shows number of mice in each group (by genotype) on each day of testing.

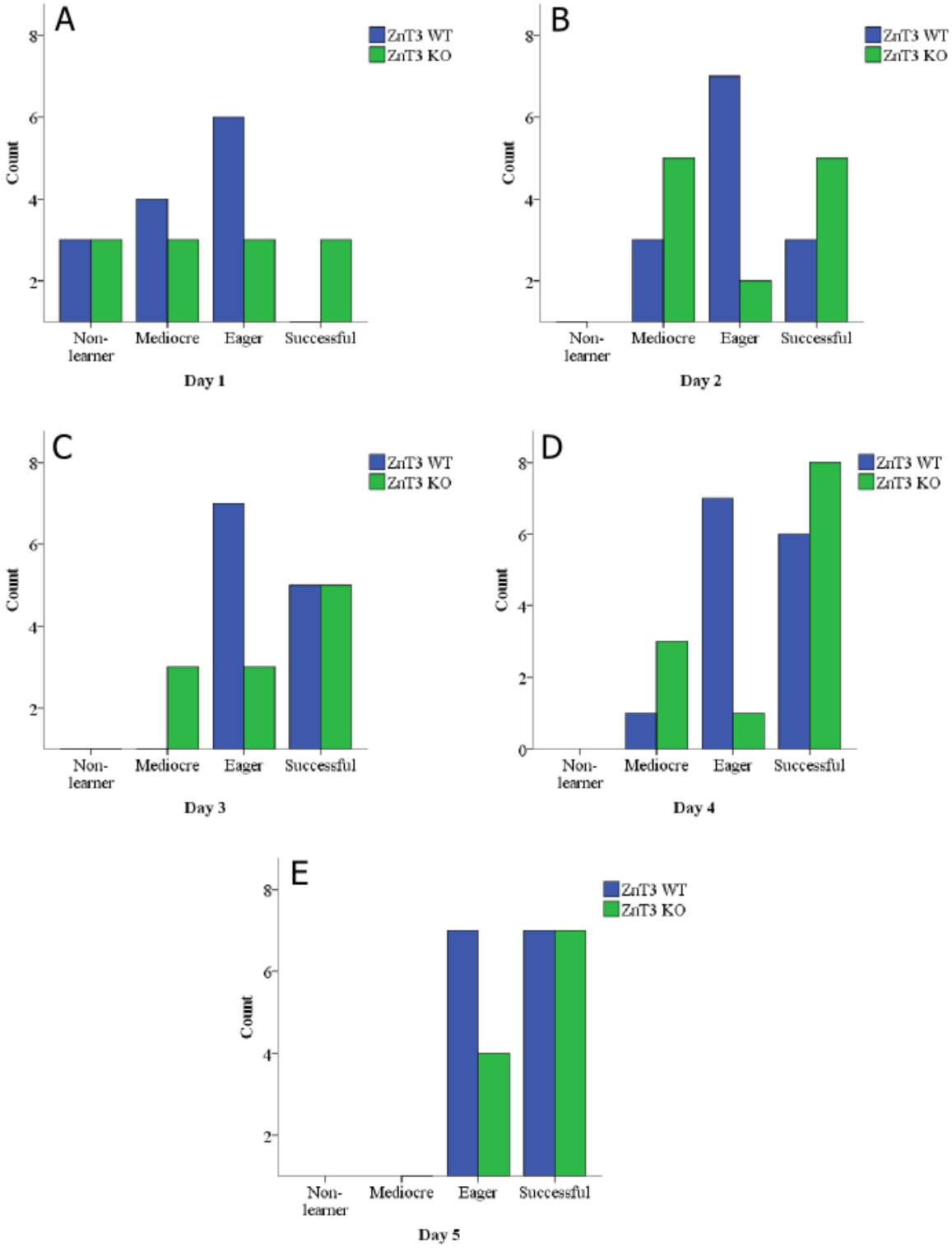
A
ZnT3 WT
ZnT3 KO
Count
8
6
4
2
Non-learner
Mediocre
Eager
Successful
Day 1
B
ZnT3 WT
ZnT3 KO
Count
8
6
4
2
Non-learner
Mediocre
Eager
Successful
Day 2
C
ZnT3 WT
ZnT3 KO
Count
8
6
4
2
Non-learner
Mediocre
Eager
Successful
Day 3
D
ZnT3 WT
ZnT3 KO
Count
8
6
4
2
0
Non-learner
Mediocre
Eager
Successful
Day 4
E
ZnT3 WT
ZnT3 KO
Count
8
6
4
2
Non-learner
Mediocre
Eager
Successful
Day 5

Figure 3.9. ZnT3 wildtype (WT; n=14) and knockout (KO; n=12) mice were trained on the skilled reach task over 5 days (A-E). They were placed in groups depending on how well they performed. Non-learners were mice that would not eat the sugar pellets that were readily available to them; mediocre learners would eat sugar pellets within tongue distance but made no attempt to retrieve pellets at reaching distance; eager learners would eat sugar pellets within tongue distance and try either by tongue or by reaching to retrieve pellets at reaching distance but remained unsuccessful; and successful learners, for the purpose of this classification, were able to successfully retrieve at least one pellet during the course of the 10 minute training session.

Of the mice trained in the task, only mice that were successfully retrieving pellets on at least 3 days (WT: n=6; KO: n=6) were included in statistical analyses. Repeated measure ANOVAs were used to compare between genotypes over 3 days. Latency to first reach attempt significantly decreased over the 3 days, $F(2,20)=15.47$, $p<.001$ (Figure 3.10 panel A). Paired sample t-tests showed significant differences between day 1 and day 2, $t(11)=4.19$, $p=.002$, and day 1 and day 3, $t(11)=4.79$, $p=.001$; no difference was found between day 2 and day 3. No difference was found between genotypes. Latency to successfully retrieve first pellet also significantly decreased over the 3 days, $F(2,20)=11.83$, $p<.001$ (Figure 3.10 panel B). Paired samples t-tests showed significant differences between day 1 and day 2, $t(11)=3.16$, $p=.009$, and day 1 and day 3, $t(11)=5.18$, $p<.001$; no differences were found between day 2 and day 3. No differences were found between genotype. Number of reach attempts increased significantly over the 3 days, $F(2,20)=6.45$, $p=.007$ (Figure 3.10 panel C). Paired sample t-tests showed significant differences between day 1 and day 2, $t(11)=3.12$, $p=.010$, and day 1 and day 3, $t(11)=4.15$, $p=.002$; no differences were found between day 2 and day 3. No differences were found between genotype. A significant interaction was found for accuracy (number of pellets retrieved / number of reach attempts x 100), $F(2,20)=4.36$, $p=.027$ (Figure 3.10 panel D). Independent samples t-tests found no significant difference between genotypes for day 1, $t(10)=.99$, $p=.347$, or day 2, $t(10)=1.41$, $p=.189$. However, there was a significant difference between genotypes on day 3, $t(10)=3.81$, $p=.003$, with ZnT3 WT mice steadily increasing in accuracy over the 3 days while ZnT3 KO mice steadily decreased in accuracy.

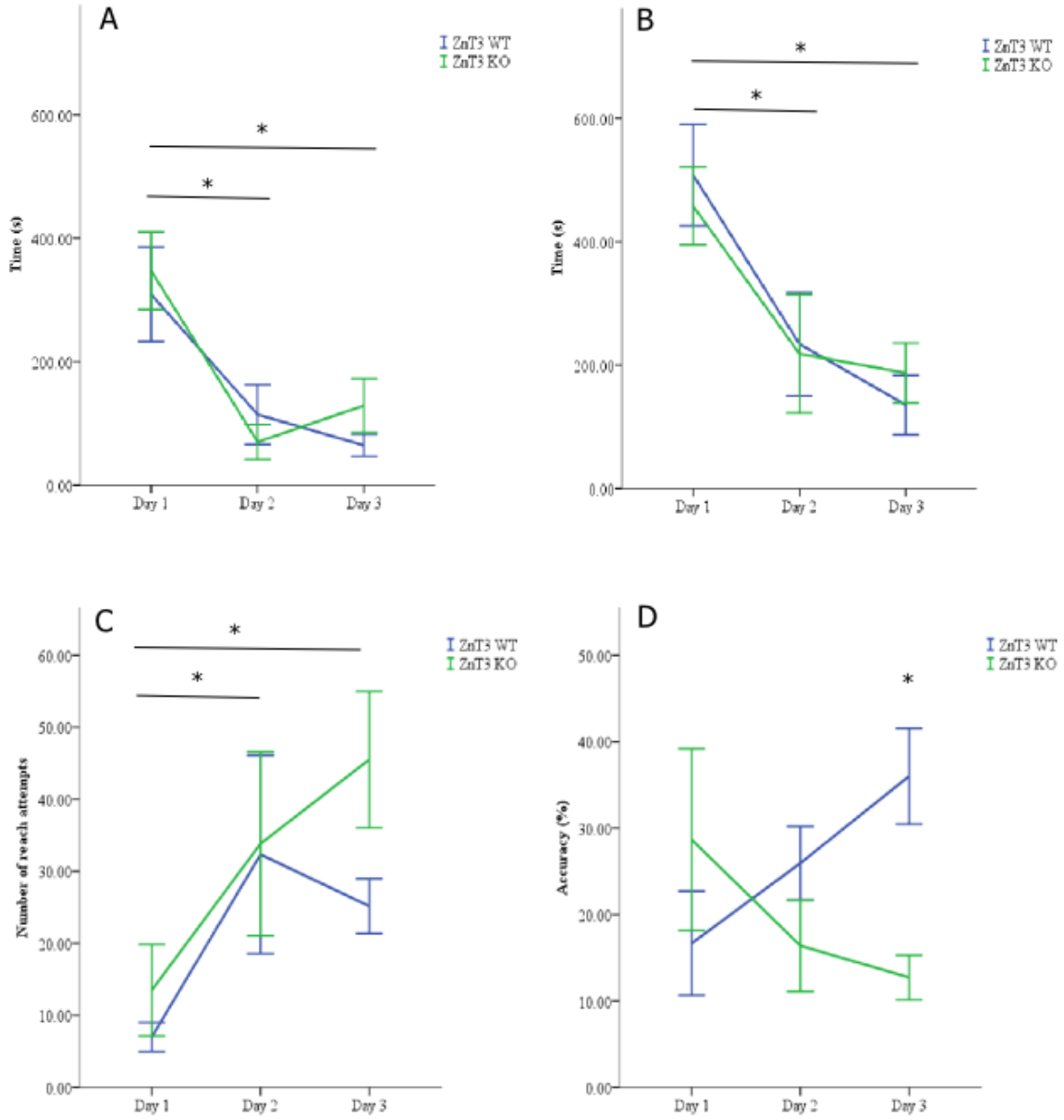

Figure 3.10. Skilled Reach. ZnT3 wildtype (WT; n=6) and knockout (KO; n=6) mice over 3 days of skilled reaching. A) Latency to first reach attempt. B) Latency to successfully retrieve first pellet. C) Number of reach attempts made each day. D) Accuracy (number of pellets retrieved / number of reach attempts x 100). Error bars represents ±SEM.

3.4 Discussion

A battery of behavioural tests including both striatal-independent and striatal-dependent motor tasks was administered to ZnT3 wildtype (WT) and knockout (ZnT3 KO) mice. ZnT3 KO mice performed at the same level as ZnT3 WT mice on most tasks. Only the pole test and skilled reach task resulted in differences between the genotypes.

The pole test examines motor coordination, as well as bradykinesia (slowness of movement). ZnT3 KO mice approached a significantly faster turn time (p=.055) and were significantly faster in total time (p=.013) than ZnT3 WT mice to descend the pole. This seems to indicate that the ZnT3 KO mice are more active than ZnT3 WT mice. It also differs from the findings of a previous study of the ZnT3 WT and KO mice which found that the ZnT3 KO mice were faster, but not significantly so (Cole et al., 2001). The Cole et al. (2001) study used both male and female mice for their analysis. However, several studies have found sex differences in many motor tasks (reviewed by Field and Pellis, 2008), so it is possible that averaging results from male and female mice would attenuate significant results for one sex or the other. This is especially likely since the differences found between males and females in turning behaviour tended to show that males took more steps than females (Field and Pellis, 2008). Though times were not included, it is probable that the extra movements made by males would cause them to turn slower. Future studies will need to examine male ZnT3 mice on striatal-dependent and independent tasks to look for sex differences.

As mentioned, rodents preforming the skilled reach task generally improve in accuracy over the course of training. Accuracy in the skilled reach task was shown to improve over the 3 days of testing for ZnT3 WT mice as expected. However, for the ZnT3 KO mice, while they started off with slightly higher accuracy than ZnT3 WT mice on day 1, they grew consistently

less accurate over the 3 days. This appeared to have nothing to do with motivation to perform the task as latency to reach and latency to successfully retrieve the first pellet paralleled ZnT3 WT mice. Also, number of reaching attempts increased steadily over the 3 days for ZnT3 KO mice, indicating that they were motivated to retrieve the pellet even if they were not often successful. This was different, although not significantly so, from the ZnT3 WT mice that increased in reach attempts from day 1 to day 2, but decreased after that. This was presumably because they were more successful and therefore did not need to reach as often to retrieve pellets.

Due to the fact that ZnT3 KO mice did not improve in accuracy as would be expected, synaptic zinc must be involved in motor learning to some degree – most likely in the corticostriatal synapses; although we cannot rule out that the changes are occurring in the cerebral cortex itself. Different regions of striatum have different functions. In general, the dorsomedial striatum (DMS) is involved in goal-directed learning; the dorsolateral striatum (DLS) is involved in habit formation; and the ventral striatum/nucleus accumbens (NAc) is involved in motivation and reward (reviewed by Liljeholm and O'Doherty, 2012). Skilled reaching, in the early stages of training, would be considered a type of goal-directed learning – the goal being to retrieve a sugar pellet. Since the ZnT3 mice were able to learn the task, albeit inefficiently, it does not seem likely that the DMS is the principle area affected. Recently, the NAc has been implicated as the interface between the limbic and motor systems and, as such, acts to make goal-directed learning more efficient. The ways it does this is by suppressing actions that are incorrect and encouraging actions that are correct for a given situation (reviewed by Floresco, 2015). Since the ZnT3 KO mice are performing the reach movement inaccurately, it is likely that the NAc is the area of striatum most affected by the lack of synaptic zinc.

The ZnT3 KO mice continually increase their reach attempts, but do not improve on the accuracy of reaching, which indicates an impulsive type of behaviour. Rather than focusing on accuracy, which will improve their chances of success, they seem to decide that the number of times they reach will determine how many pellets they retrieve. Given that the skilled reach task is not a traditional test for impulsivity, it will be critical to test these mice on those traditional tests of impulsivity; for example, the 5 choice serial reaction time task (5CSRTT). The 5CSRTT was originally designed for rats, but is now also available for mice, to test attention and can be used to test impulse control (Asinof and Paine, 2014). The task involves the rodent in an operant conditioning chamber that has 5 apertures on one wall. A light is presented over one aperture and the rodent must wait a given amount of time, then poke its nose through that aperture to receive a sugar pellet. Thus, the rodent must pay attention to all 5 lights and choose the correct one to receive its reward. However, it also must withhold inappropriate responding (such as responding too quickly), which is where impulse control comes into play. Impulsivity is a feature of many human disorders including attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), and addiction (reviewed by Pattij and Vanderschuren, 2008). Determining if dysfunction of the zincergic system is involved in impulsive behaviour will have many ramifications in terms of prevention or treatment of these disorders.

As mentioned briefly in the introduction, there are other measures that can be taken from skilled reaching tasks. A full kinematic workup could be done, where reaching behaviour is broken down into 10 different steps. Each of these steps can be compared between genotypes to examine differences in their approach to the window, as well as use of their forearm and paw during the reaching task (Whishaw, 1996). Other measures, such as trajectory of the forearm and number of non-productive reach attempts, could also be examined. Trajectory of the forearm can

be an indication of accuracy. Non-productive reach attempts would be any reach attempt a mouse made when either there was no sugar pellet present or when the sugar pellet was not in the correct location on the ledge. In this study, reach attempts were only counted when the sugar pellet was present and in the correct location; any attempts made after it had been knocked out of place or removed from the ledge were not counted. However, many mice continued to make reach attempts under these conditions. Since mice use odor to detect the presence or absence of a sugar pellet (Whishaw, 1996) and no deficits have been found in odor discrimination of ZnT3 KO mice (Cole et al., 2001), they should be able to determine that there is nothing to reach for. If they still attempted to reach for the absent pellet, this may be considered an impulsive action.

The skilled reach task, as indicated by its name, involves learning a skill. Learning takes time. Very few people, or rodents, can learn a skill in one day or even a few days. The shortest version of the skilled reach task was chosen for this study to screen mice for a deficit and because it was to be included with a battery of other motor tests. However, not all mice were able to learn the task in the short time allocated (shown in Figure 3.9 panel E). There were mice of both genotypes that did not make a successful retrieval by day 5 of training. Even those that did learn the task were not able to become proficient at it. In general, a rodent is considered successful at skilled reaching once they have reached an accuracy of at least 50%; only a few mice reached this level of accuracy. A follow up to the current study would be to extend the amount of training. Significant deficits were found during this short amount of training, but it is possible that the ZnT3 KO mouse may improve if they are given more time to learn the task.

Another aspect to consider, as mentioned earlier in relation to the pole test, is sex differences in skilled reaching. Would male mice show the same decrease in accuracy during training? Other studies have found differences in the kinematics of skilled reach for males versus

females (Field and Whishaw, 2005), as well as differences in how stress affects skilled reach performance in males and females (Jadavji and Metz, 2008). Future studies should examine male mice as well as include kinematic analyses of skilled reach movement to look for sex differences.

Other interesting follow up studies on the role of zinc in striatal-mediated motor learning would include testing other models of zinc deficiency. There are 2 receptors, GPR39 (Holst et al., 2007; Yasuda et al., 2007; Cohen et al., 2012) and GPR83 (Muller et al., 2013), which have been found to be zinc-dependent. Knockout mouse models exist for both. If synaptic zinc does play a role in goal-directed learning, these knockout mice might also show deficiencies in the skilled reach task.

In summary, it appears that synaptic zinc plays a role in learning a goal-directed task, particularly in terms of accuracy. Lack of synaptic zinc may also be linked to hyperactivity, as indicated by faster movements on the pole. However, this study just scratches the surface. Since sex differences have been found previously on many motor tasks, it is important to test male ZnT3 WT and KO mice on these tasks and potentially repeat motor tests that previously found no differences when male and female data were averaged. Also, increasing training time, as well as examining other rodent models lacking synaptic zinc will be necessary to determine the exact effect that zinc has on goal-directed learning. A critical future study will be to test ZnT3 KO mice on traditional tests of hyperactivity and impulsivity such as the 5SCRTT.

Chapter Four: **General Discussion**

4.1 Summary of Findings

Synaptic zinc is found in many areas of the brain and acts as a neuromodulator (reviewed by Nakashima and Dyck, 2009). While most research on synaptic zinc function has focused on the cerebral cortex and hippocampus, very little has examined the striatum. The striatum is involved in many different functions, both motor and cognitive, and has a large amount of synaptic zinc present. In the cortex and hippocampus, synaptic zinc has been found to be a modulator of plasticity and likely plays a similar role in the striatum. The purpose of this thesis was to examine potential anatomical and behavioural changes in ZnT3 wildtype (WT) and knockout (KO) mice in the striatum.

Chapter 2 examined the anatomical aspect by raising ZnT3 WT and KO mice in either standard cages or enriched environments, then staining their brains with Golgi-cox solution. Using the Golgi-cox stain, we looked for changes in dendritic length using Sholl analysis, as well as changes in spine density. Because of the size and complexity of the striatum, three regions were chosen: dorsolateral (DLS), dorsomedial (DMS), and ventral/nucleus accumbens (NAc). With respect to dendritic length, no differences were found between genotypes or environments. Spine density increased in all 3 regions in mice raised in enriched environments compared to those raised in standard environments. However, in the nucleus accumbens, the spine density increase was significantly greater in ZnT3 KO mice than in ZnT3 WT mice.

Chapter 3 examined the behavioural aspect by testing ZnT3 WT and KO mice on a battery of striatal-independent and dependent tasks. There were no differences between genotypes for the limb asymmetry task, the footprint analysis, the horizontal ladder task, the inverted wire hang task, or the balance beam task. For the pole task, ZnT3 KO mice were

significantly faster to descend a 50 cm vertical pole and approached significance in time taken to turn around on the pole. In the skilled reach task, ZnT3 KO mice learned the task at the same rate as the ZnT3 WT mice and performed at similar levels in terms of latency to first reach attempt, latency to successfully retrieve the first pellet, and number of reach attempts. Where the ZnT3 KO mice differed was in terms of accuracy. Most animals trained on this task start out poorly. They have very low accuracy on the first few days but improve over time. An animal is generally considered trained and successful on the task when they reach a 50% accuracy rate. While our test did not extend long enough for mice to reach 50% accuracy, the ZnT3 WT mice were headed in that direction with a steady improvement in accuracy over 3 days of training. The ZnT3 KO mice, on the other hand, did not improve over the 3 days and, in fact, seemed to get worse. The implications of these studies, as well as ideas for future directions, are discussed below.

4.2 Implications

There are several possible implications based on the findings of this thesis. Based on the results of the Golgi-cox experiment, it is clear that the ZnT3 KO mice do have plastic mechanisms taking place within the striatum as evidenced by the increase in spine density in mice raised in enriched environments compared to standard environments. This plasticity was not seen in measurements of cell survival, proliferation, and death in the hippocampi of ZnT3 KO mice raised in enrichment, as they were not significantly different from ZnT3 KO mice raised in standard cages. On the other hand, ZnT3 WT mice raised in enriched environments had increases in hippocampal cell survival and proliferation, and decreases in cell death compared to ZnT3 WT mice raised in standard environments (Chrusch, 2015). This indicates that enrichment differentially affects zincergic function based on brain region.

Since the enriched ZnT3 KO mice showed significantly greater increases in spine density in the NAc, even over enriched ZnT3 WT mice, it would seem that synaptic zinc has a very important role in the NAc. As discussed in Chapter 2, the NAc is part of the reward circuitry of the brain (reviewed by Yager et al., 2015). Rewards consist of anything that causes pleasure including, but not limited to, food, sex, drugs, and social interaction (Bevins, 2001; Trezza et al., 2010). Drugs like amphetamine and cocaine that activate the reward system, cause similar increases in spine density in the NAc under standard conditions (Robinson and Kolb, 1999b; Li et al., 2003; Lee et al., 2006). The increased social ability and novelty involved with the enriched environment could be considered types of rewards. As such, one possible interpretation of the increased spine density in enriched ZnT3 KO mice is that they are more sensitive to novelty and/or reward than their WT littermates.

Another implication from Chapter 3 is that the ZnT3 KO mice are more active than their WT littermates. This is evidenced in the pole test where ZnT3 KO mice were significantly faster to descend the pole. One of the uses of the pole test is to scan for bradykinesia, or slowness of movement. As such, it is often used to test rodent models of Parkinson's disease (Matsuura et al., 1997). Fastness of movement, or hyperactivity, is the logical opposite of bradykinesia. Therefore, it seems evident that the female ZnT3 KO mice are hyperactive.

There are a couple of implications based on the results of the skilled reach task. The first is that motor learning per se is not the problem. ZnT3 KO mice were able to learn the task as indicated by their progression through the groups – non-learner to mediocre to eager to successful - over the 5 days of training. The skilled reach task would be considered goal-directed learning – the goal being to retrieve the sugar pellet from the shelf by reaching through the slit in the front of the reaching box. Goal-directed learning is generally thought to be governed by the

dorsomedial striatum (DMS; Yin et al., 2005). However, there are also motivation and reward aspects to the task. Mice were food deprived to motivate them to reach for the sugar pellets and the sugar pellet itself is the reward. These aspects are generally thought to be governed by the nucleus accumbens (NAc; Carelli, 2002; Carlezon Jr and Thomas, 2009). Since the ZnT3 KO mice were able to learn the task and seemed to be motivated enough to reach for and enjoy the sugar pellet, it would indicate that synaptic zinc is not greatly affecting those processes.

The main issue that the ZnT3 KO mice had in the skilled reach was improving their accuracy. Rodents are usually considered successful at skilled reach only once they have reached 50% accuracy. Using this standard, the ZnT3 KO mice are not able to successfully learn the skilled reach task, at least not over 3 days of training. Although they did not reach the 50% accuracy mark, ZnT3 WT mice showed significant improvement in accuracy over 3 days. ZnT3 KO mice did not show a similar improvement over time, and actually seemed to get worse. However, they were equivalent to the ZnT3 WT mice on every other measurement taken over the course of the task. Their lack of accuracy with increasing number of reach attempts is more indicative of either a sensory deficit involving an inability to sense where their forelimb is located in space or a sense of impatience or impulsivity on their part.

Many studies, both human and animal, have been done on impulsivity. There are many definitions of impulsivity. However, the most commonly used definition is "impulsivity encompasses a range of actions which are poorly conceived, prematurely expressed, unduly risky or inappropriate to the situation and that often result in undesirable consequences" (Daruna, 1993). This seems to fit well with what is seen in the ZnT3 KO mice. They are reaching successfully but then not refining their next reach attempt to match the previous successful attempts. They seem to think that reaching more often is better than reaching more accurately.

As a result, they expend more energy, by making more reaching attempts, to retrieve a single pellet – an "undesirable consequence" for a hungry mouse.

Dysfunction of the frontostriatal system has been linked to increases in impulsivity (van den Bos et al., 2015). Neuroimaging studies have found that the prefrontal cortex has many connections to the striatum (reviewed by Elliott, 2003). Alterations of these connections are also linked to many psychological disorders, some of which have increased impulsive behaviour as a symptom (reveiwed by Bradshaw and Sheppard, 2000). Dopamine, as well as glutamate, serotonin, noradrenaline, and acetylcholine, has been linked to increases in impulsivity (Pattij and Vanderschuren, 2008). Increases in dopamine in the NAc, through the use of amphetamine, have been found to increase impulsive behaviour (Robbins, 2002). Zinc is located in both the frontal cortex and in the striatum (Brown and Dyck, 2004a). It has also been found to modulate most of the neurotransmitter systems implicated in impulsive behaviour (reviewed by Nakashima and Dyck, 2009). Therefore, the lack of synaptic zinc in the ZnT3 KO mouse is likely affecting the normal functioning of these neurotransmitter systems, particularly within the frontostriatal circuit.

These findings suggest that deficiency of synaptic zinc in the brain may be associated with an increase in impulsivity. This has implications for several disorders that involve increased impulsivity such as attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), and addiction. In fact, one study examined the effects of zinc supplementation in children with ADHD and found that zinc significantly reduced hyperactivity and impulsivity (Bilici et al., 2004). Since systemic zinc levels do not appear to alter vesicular zinc levels (Takeda et al., 2005), it would be interesting to see if zinc supplementation in people with increased impulsivity, or in rodent models of ADHD and other disorders involving impulsivity, show

similar decreased impulsive actions. It is unlikely that zinc supplementation of ZnT3 KO mice would affect their hyperactivity on the pole test or impulsivity in the skilled reach task as they lack the transporter to load zinc into vesicles.

Given what was found in Chapter 2 (differential increase in spine density in enriched ZnT3 KO mice), it seems that the ZnT3 KO mice may be more sensitive to novelty and/or reward. This fits nicely with the finding in Chapter 3 that the ZnT3 KO mice seem to be more impulsive. An animal (or human) that is highly sensitive to reward is more likely to act without thinking to get that reward – they place a higher value on speed over accuracy. To reiterate the discussion in Chapter 2, the NAc seems to act as an interface between the limbic system and the motor system and, as such, acts to increase the efficiency of goal-directed tasks. Since accuracy is one aspect that would improve efficiency and the ZnT3 KO mice were able to learn the skilled reach task in a timely matter – if not in an efficient one – it would appear that synaptic zinc has a distinct role in the NAc. Further studies will be necessary to determine how, specifically, synaptic zinc is affecting the normal function of the NAc. A few such studies are discussed below.

4.3 Future Directions

4.3.1 Behavioural Tasks

When considering the performance of the ZnT3 KO mice on the skilled reach task there are two main areas that may cause their inaccuracy – proprioceptive deficits or increased impatience or impulsiveness. Neither can be proven or ruled out using endpoint measures alone, as was done in Chapter 3. However, other methods of scoring the skilled reach task are available and could be used to determine whether the mice have a problem with proprioception or whether

they are simply more impulsive than ZnT3 WT mice. A kinematic analysis examines 10 aspects of the reaching movement and scores whether the movement was normal, impaired, or absent. A slightly different analysis examines gestures made during the reach attempt. These types of analyses could be used to determine whether ZnT3 KO mice are reaching in the same manner as ZnT3 WT mice. If they are not reaching the same way as WT mice, they likely have proprioceptive deficits. If they are reaching in the same way, they may be more impulsive than WT mice.

While the findings from these studies seem to indicate that ZnT3 KO mice are hyperactive and may be impulsive, the pole test and the skilled reach test are not traditionally used to look for these traits in rodents. It will be important to examine how the ZnT3 KO mice do in tests that are specifically designed to measure hyperactivity and impulsiveness. One of the main tests of impulsiveness in rodents is the 5 choice serial reaction time task (5CSRTT). The 5CSRTT measures different aspects of behaviour and can be setup differently in such a way as to dissociate between several issues including attention, impulsivity, and motivation. The 5CSRTT is commonly used to determine rodent models of ADHD (Winstanley et al., 2006). Other tests of impulsivity include go/no go, stop signal reaction time, and delay discounting tasks (Winstanley et al., 2006).

Since impulsivity is a common feature in drug addiction (Winstanley, 2007), it may also be interesting to examine how ZnT3 KO mice react to drugs of addiction. Self-administration and conditioned place preference are the most common tests used in rodent studies of drug addiction (Lynch et al., 2010). Self-administration of a drug involves implanting a catheter into the rodent which allows administration of the drug in response to a particular behaviour of the rodent, such as the pressing of a lever (reviewed by Lynch et al., 2010). If the rodent finds the

drug rewarding, they will press the lever more often to get a 'fix' of the drug. The number of responses a rodent has to make to get the drug can be altered to determine just how much the rodent is willing to work to receive the drug. Conditioned place preference involves administering a drug or placebo to a rodent then placing them in one of two chambers (reviewed by Lynch et al., 2010). The drug is always paired with one chamber while the placebo is always paired with the other. After a given number of sessions, the rodent is allowed to explore both chambers. If they found the drug rewarding, they will spend more time in the chamber that was paired with the drug. It is possible, given the results of this thesis, that the ZnT3 KO mice will be more sensitive to drugs of addiction and more likely than ZnT3 WT mice to become addicted as well as to act impulsively to obtain the drug.

Novelty seeking has also been linked with drug abuse (Bardo et al., 1996; Bevins, 2001). As the enrichment cages are rearranged every week, animals raised in them are exposed to a novel environment on a weekly basis. The ZnT3 KO mice had greater increases in spine density in the NAc, which may indicate a hypersensitivity to the novel environment. This is yet another reason it would be interesting to examine how ZnT3 KO mice respond to drugs of abuse.

4.3.2 Sex differences

Many behavioural studies use only one sex for testing or combine both sexes. However, for motor tasks this may not provide an accurate picture of what is going on. Sex differences have been found on several motor tasks including skilled reach and tasks involving turning around (Field and Pellis, 2008). Since initial testing of the ZnT3 KO mice averaged results from both male and females, it calls into question their findings, at least in terms of motor behaviour

(Cole et al., 2001). Re-testing the male and female mice separately on motor tasks may have different results.

One interesting study was done in rats looking at sex differences in skilled reaching before, during, and after restraint stress (Jadavji and Metz, 2008). While baseline reaching accuracy was not different, they found that males and females responded differently to stress in that females were reaching significantly better than males on certain days during the stress and females recovered (i.e. improved accuracy) faster than males after the stress had been removed. Synaptic zinc and ZnT3 have been linked to the stress response (Suh et al., 2005). Therefore, ZnT3 KO mice may respond differently to stress. Since the ZnT3 KO mice are not accurate under normal conditions, it would be interesting to see if stress affects them, for worse or for better.

In addition to the possible sex differences in motor tasks, several studies have found sex differences in brain anatomy (Cahill, 2006). Since the Golgi-cox enrichment study only examined female ZnT3 WT and KO mice, examination of male ZnT3 WT and KO mice under these same conditions is necessary to determine if males are equally affected by enrichment.

4.3.3 Mechanism of action

As mentioned in the Chapter 1, synaptic zinc can modulate many receptors and pathways which makes it difficult to determine where zinc might be acting to cause the differences in the ZnT3 KO mice. As mentioned in section 4.2, human studies have found that frontostriatal circuitry is important when studying impatience/impulsivity in adolescents and increased connectivity is associated with decreased impatience (van den Bos et al., 2015). Therefore, it seems likely that connections between frontal cortex and striatum are involved in the impatience

seen in the ZnT3 KO mouse. Since synaptic zinc is present in both of these areas, the possibility that the differences found in the ZnT3 KO mice are actually due to changes in the frontal cortex which result in altered efferent connections to the striatum cannot be ruled out. Other brain areas linked to impulsivity include basolateral amygdala (BLA), medial striatum, NAc, and orbitofrontal cortex (OFC; Winstanley et al., 2006). These areas also contain synaptic zinc and can also not be ruled out as locations in which lack of synaptic zinc may play a role in increasing impulsivity or hyperactivity. Future studies should examine how lack of synaptic zinc in specific areas affects behaviour. This would be best done through the use of a conditional knockout mouse.

Synaptic zinc has been shown to modulate glutamatergic and dopaminergic receptors. While much research has been done on zinc's effect on the glutamatergic system, its role in the dopaminergic system is not well characterized. It is also possible that how zinc modulates each system individually is different than how it modulates the interaction between the 2 systems. Further research in needed not only on how the dopaminergic and glutamatergic systems interact within the striatum, but also how zinc is affecting this interaction.

Many studies have examined long-term potentiation (LTP) and long-term depression (LTD), potential mechanisms of plasticity, in the striatum, specifically in MSNs (Kreitzer and Malenka, 2008; Lerner and Kreitzer, 2011). However, there were no studies that examined the effect that zinc has on LTP/LTD in the striatum. Since zinc has been found to modulate LTP in the hippocampus and amygdala (reviewed by Nakashima and Dyck, 2009), it is likely that it also modulates LTP in the striatum. Electrophysiology studies examining striatal LTP/LTD in the presence and absence of zinc should be carried out.

Another interesting avenue to explore would be how dopamine levels are affected in the ZnT3 KO mice versus ZnT3 WT mice. It is possible to measure dopamine levels in awake animals using fast-scan cyclic voltammetry (Heien et al., 2005). Since dopamine plays an important role in the striatum and may be linked to impulsive behaviours (van Gaalen et al., 2006; Dalley et al., 2008; Pattij and Vanderschuren, 2008), it will be critical to determine if the ZnT3 KO animals have normal levels of dopamine in the striatum; and, given the finding in Chapter 2, particularly the nucleus accumbens.

4.3.4 Human applications

As briefly mentioned in Chapter 1, zinc dysfunction and striatal dysfunction have been linked to several human disorders individually, some of which overlap. A few of these, including ADHD and ASD, have impulsivity as one possible symptom. Since one study has already found an improvement in hyperactivity and impulsiveness with zinc supplementation (Bilici et al., 2004), it might be interesting to see if zinc supplementation helps in other disorders involving impulsive behaviour, such as ASD and addiction.

4.4 Conclusion

The goal of this thesis was to expand on current knowledge of synaptic zinc in the striatum, both anatomically and behaviourally. To this end, two studies were conducted. The first examined changes in neuronal morphology of MSNs of the striatum in ZnT3 WT and KO mice raised in standard or enriched environments. The main findings from this study were that ZnT3 KO mice can benefit from enrichment as evidenced by increased spine density in all 3 regions of striatum and that the ZnT3 KO mice especially benefit in the nucleus accumbens. This may

indicate that they are more sensitive to reward or novelty. The second study was a battery of behavioural task. Main findings from these were that the ZnT3 KO mice appear to be hyperactive, as well as more impulsive than their WT littermates. Put together, these studies have interesting implications for human disorders that include hyperactivity and impulsiveness as symptoms such as ADHD, ASD, and addiction. Future studies should include a more thorough examination of ZnT3 mice, and other rodent models of zinc dysfunction, on traditional tests of impulsivity including the 5CSRT and drug addiction studies, as well as human studies using zinc supplementation in populations with increased hyperactivity and impulsivity. It will also be important to delve further into the mechanisms through which zinc is acting in the striatum in general and in the nucleus accumbens specifically.

References

Abraham WC, Logan B, Greenwood JM, Dragunow M (2002) Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. J Neurosci 22:9626-9634.

Adlard PA, Parncutt JM, Finkelstein DI, Bush AI (2010) Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? J Neurosci 30:1631-1636.

Afifi AK (1994) Basal ganglia: functional anatomy and physiology. Part 2. J Child Neurol 9:352-361.

Alexander GE (1994) Basal ganglia-thalamocortical circuits: their role in control of movements. J Clin Neurophysiol 11:420-431.

Alexander GE, Crutcher MD, DeLong MR (1990) Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 85:119-146.

Angeles-Castellanos M, Mendoza J, Escobar C (2007) Restricted feeding schedules phase shift daily rhythms of c-Fos and protein Per1 immunoreactivity in corticolimbic regions in rats. Neuroscience 144:344-355.

Antle MC, Silver R (2005) Orchestrating time: arrangements of the brain circadian clock. Trends Neurosci 28:145-151.

Artola A, von Frijtag JC, Fermont PC, Gispen WH, Schrama LH, Kamal A, Spruijt BM (2006) Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. Eur J Neurosci 23:261-272.

Asinof SK, Paine TA (2014) The 5-choice serial reaction time task: A task of attention and impulse control for rodents. J Vis Exp.

Badgaiyan RD, Fischman AJ, Alpert NM (2008) Explicit motor memory activates the striatal dopamine system. Neuroreport 19:409-412.

Bardo M, Klebaur J, Valone J, Deaton C (2001) Environmental enrichment decreases intravenous self-administration of amphetamine in female and male rats. Psychopharmacology (Berl) 155:278-284.

Bardo MT, Donohew RL, Harrington NG (1996) Psychobiology of novelty seeking and drug seeking behavior. Behav Brain Res 77:23-43.

Bevins RA (2001) Novelty seeking and reward: Implications for the study of high-risk behaviors. Cur Dir Psychol Sci 10:189-193.

Bilici M, Yildirim F, Kandil S, Bekaroglu M, Yildirmis S, Deger O, Ulgen M, Yildiran A, Aksu H (2004) Double-blind, placebo-controlled study of zinc sulfate in the treatment of attention deficit hyperactivity disorder. Prog Neuropsychopharmacol Biol Psychiatry 28:181-190.

Bitanihirwe BK, Cunningham MG (2009) Zinc: the brain's dark horse. Synapse 63:1029-1049.

Bjorklund NL, Volz TJ, Schenk JO (2007) Differential effects of Zn2+ on the kinetics and cocaine inhibition of dopamine transport by the human and rat dopamine transporters. Eur J Pharmacol 565:17-25.

Blakemore LJ, Trombley PQ (2004) Diverse modulation of olfactory bulb AMPA receptors by zinc. Neuroreport 15:919-923.

Bowling SL, Bardo MT (1994) Locomotor and rewarding effects of amphetamine in enriched, social, and isolate reared rats. Pharm Biochem Be 48:459-464.

Bowling SL, Rowlett JK, Bardo MT (1993) The effect of environmental enrichment on amphetamine-stimulated locomotor activity, dopamine synthesis and dopamine release. Neuropharmacology 32:885-893.

Bradshaw JL, Sheppard DM (2000) The neurodevelopmental frontostriatal disorders: evolutionary adaptiveness and anomalous lateralization. Brain Lang 73:297-320.

Bresink I, Ebert B, Parsons CG, Mutschler E (1996) Zinc changes AMPA receptor properties: results of binding studies and patch clamp recordings. Neuropharmacology 35:503-509.

Brown CE, Dyck RH (2002) Rapid, experience-dependent changes in levels of synaptic zinc in primary somatosensory cortex of the adult mouse. J Neurosci 22:2617-2625.

Brown CE, Dyck RH (2003) An improved method for visualizing the cell bodies of zincergic neurons. J Neurosci Methods 129:41-47.

Brown CE, Dyck RH (2004) Distribution of zincergic neurons in the mouse forebrain. J Comp Neurol 479:156-167.

Brown CE, Dyck RH (2005) Modulation of synaptic zinc in barrel cortex by whisker stimulation. Neuroscience 134:355-359.

Cahill L (2006) Why sex matters for neuroscience. Nat Rev Neurosci 7:477-484.

Cai Y, Liu S, Li N, Xu S, Zhang Y, Chan P (2009) Postnatal ontogenesis of molecular clock in mouse striatum. Brain Res 1264:33-38.

Carelli RM (2002) The nucleus accumbens and reward: Neurophysiological investigations in behaving animals. Beh Cog Neurosci Rev 1:281-296.

Carlezon Jr WA, Thomas MJ (2009) Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. Neuropharmacology 56, Supplement 1:122-132.

Castaneda TR, de Prado BM, Prieto D, Mora F (2004) Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. J Pineal Res 36:177-185.

Chasapis CT, Loutsidou AC, Spiliopoulou CA, Stefanidou ME (2012) Zinc and human health: an update. Arch Toxicol 86:521-534.

Chen CC, Gilmore A, Zuo Y (2014) Study motor skill learning by single-pellet reaching tasks in mice. J Vis Exp.

Chrusch M (2015) The role of synaptic zinc in experience-dependent plasticity. In: Neuroscience. Calgary: University of Calgary.

Cohen L, Asraf H, Sekler I, Hershfinkel M (2012) Extracellular pH regulates zinc signaling via an Asp residue of the zinc-sensing receptor (ZnR/GPR39). J Biol Chem 287:33339-33350.

Cole TB, Martyanova A, Palmiter RD (2001) Removing zinc from synaptic vesicles does not impair spatial learning, memory, or sensorimotor functions in the mouse. Brain Res 891:253-265.

Cole TB, Wenzel HJ, Kafer KE, Schwartzkroin PA, Palmiter RD (1999) Elimination of zinc from synaptic vesicles in the intact mouse brain by disruption of the ZnT3 gene. Proc Natl Acad Sci U S A 96:1716-1721.

Cole TB, Robbins CA, Wenzel HJ, Schwartzkroin PA, Palmiter RD (2000) Seizures and neuronal damage in mice lacking vesicular zinc. Epilepsy Res 39:153-169.

Comery TA, Shah R, Greenough WT (1995) Differential rearing alters spine density on medium-sized spiny neurons in the rat corpus striatum: Evidence for association of morphological plasticity with early response gene expression. Neurobio Learn Mem 63:217-219.

Comery TA, Stamoudis CX, Irwin SA, Greenough WT (1996) Increased density of multiple-head dendritic spines on medium-sized spiny neurons of the striatum in rats reared in a complex environment. Neurobio Learn Mem 66:93-96.

Costa RM (2007) Plastic corticostriatal circuits for action learning: what's dopamine got to do with it? Ann N Y Acad Sci 1104:172-191.

Dalley JW, Mar AC, Economidou D, Robbins TW (2008) Neurobehavioral mechanisms of impulsivity: Fronto-striatal systems and functional neurochemistry. Pharmacol Biochem Be 90:250-260.

Danscher G (1981) Histochemical demonstration of heavy metals. A revised version of the sulphide silver method suitable for both light and electronmicroscopy. Histochemistry 71:1-16.

Danscher G (1982) Exogenous selenium in the brain. A histochemical technique for light and electron microscopical localization of catalytic selenium bonds. Histochemistry 76:281-293.

Danscher G, Haug FM, Fredens K (1973) Effect of diethyldithiocarbamate (DEDTC) on sulphide silver stained boutons. Reversible blocking of Timm's sulphide silver stain for "heavy" metals in DEDTC treated rats (light microscopy). Exp Brain Res 16:521-532.

Daruna JH, Barnes, Patricia A. (1993) A neurodevelopmental view of impulsivity. Washington, DC, US: American Psychological Association.

Dayalu P, Albin RL (2015) Huntington disease: pathogenesis and treatment. Neurol Clin 33:101-114.

Dibner C, Schibler U, Albrecht U (2010) The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol 72:517-549.

Do J, Kim JI, Bakes J, Lee K, Kaang BK (2012) Functional roles of neurotransmitters and neuromodulators in the dorsal striatum. Learn Mem 20:21-28.

Doeppner TR, Kaltwasser B, Bähr M, Hermann DM (2014) Effects of neural progenitor cells on post-stroke neurological impairment—a detailed and comprehensive analysis of behavioral tests. Front Cell Neurosci 8:338.

Doya K (2000) Complementary roles of basal ganglia and cerebellum in learning and motor control. Curr Opin Neurobiol 10:732-739.

Dreixler JC, Leonard JP (1994) Subunit-specific enhancement of glutamate receptor responses by zinc. Brain Res Mol Brain Res 22:144-150.

Eibl JK, Abdallah Z, Ross GM (2010) Zinc-metallothionein: a potential mediator of antioxidant defence mechanisms in response to dopamine-induced stress. Can J Physiol Pharmacol 88:305-312.

Elliott R (2003) Executive functions and their disorders: Imaging in clinical neuroscience. Brit Med Bull 65:49-59.

Faherty CJ, Kerley D, Smeyne RJ (2003) A Golgi-Cox morphological analysis of neuronal changes induced by environmental enrichment. Dev Brain Res 141:55-61.

Fahrni CJ, O'Halloran TV (1999) Aqueous coordination chemistry of quinoline-based fluorescence probes for the biological chemistry of zinc. J Am Chem Soc 121:11448-11458.

Ferreira TL, Moreira KM, Ikeda DC, Bueno OF, Oliveira MG (2003) Effects of dorsal striatum lesions in tone fear conditioning and contextual fear conditioning. Brain Res 987:17-24.

Ferreira TL, Shammah-Lagnado SJ, Bueno OF, Moreira KM, Fornari RV, Oliveira MG (2008) The indirect amygdala-dorsal striatum pathway mediates conditioned freezing: insights on emotional memory networks. Neuroscience 153:84-94.

Field E, Pellis S (2008) The Brain as the Engine of Sex Differences in the Organization of Movement in Rats. Arch Sex Behav 37:30-42.

Field EF, Whishaw IQ (2005) Sexually dimorphic postural adjustments are used in a skilled reaching task in the rat. Behav Brain Res 163:237-245.

Floresco SB (2015) The nucleus accumbens: an interface between cognition, emotion, and action. Annu Rev Psychol 66:25-52.

Fone KC, Porkess MV (2008) Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neurosci Biobehav Rev 32:1087-1102.

Foster TC, Dumas TC (2001) Mechanism for increased hippocampal synaptic strength following differential experience. J Neurophysiol 85:1377-1383.

Frederickson CJ, Koh JY, Bush AI (2005) The neurobiology of zinc in health and disease. Nat Rev Neurosci 6:449-462.

Frederickson CJ, Rampy BA, Reamy-Rampy S, Howell GA (1992) Distribution of histochemically reactive zinc in the forebrain of the rat. J Chem Neuroanat 5:521-530.

Frederickson CJ, Suh SW, Silva D, Frederickson CJ, Thompson RB (2000) Importance of zinc in the central nervous system: the zinc-containing neuron. J Nutr 130:1471s-1483s.

Freeze BS, Kravitz AV, Hammack N, Berke JD, Kreitzer AC (2013) Control of basal ganglia output by direct and indirect pathway projection neurons. J Neurosci 33:18531-18539.

Furtado JC, Mazurek MF (1996) Behavioral characterization of quinolinate-induced lesions of the medial striatum: relevance for Huntington's disease. Exp Neurol 138:158-168.

Gallardo CM, Darvas M, Oviatt M, Chang CH, Michalik M, Huddy TF, Meyer EE, Shuster SA, Aguayo A, Hill EM, Kiani K, Ikpeazu J, Martinez JS, Purpura M, Smit AN, Patton DF, Mistlberger RE, Palmiter RD, Steele AD (2014) Dopamine receptor 1 neurons in the dorsal striatum regulate food anticipatory circadian activity rhythms in mice. eLife 3.

Gardoni F, Bellone C (2015) Modulation of the glutamatergic transmission by Dopamine: a focus on Parkinson, Huntington and Addiction diseases. Front Cell Neurosci 9:25.

Gauthier MA, Eibl JK, Crispo JA, Ross GM (2008) Covalent arylation of metallothionein by oxidized dopamine products: a possible mechanism for zinc-mediated enhancement of dopaminergic neuron survival. Neurotox Res 14:317-328.

Grabrucker S, Jannetti L, Eckert M, Gaub S, Chhabra R, Pfaender S, Mangus K, Reddy PP, Rankovic V, Schmeisser MJ, Kreutz MR, Ehret G, Boeckers TM, Grabrucker AM (2014) Zinc deficiency dysregulates the synaptic ProSAP/Shank scaffold and might contribute to autism spectrum disorders. Brain 137:137-152.

Graybiel AM (1984) Correspondence between the dopamine islands and striosomes of the mammalian striatum. Neuroscience 13:1157-1187.

Graybiel AM, Ragsdale CW, Jr. (1978) Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. Proc Natl Acad Sci U S A 75:5723-5726.

Graybiel AM, Chesselet MF (1984) Compartmental distribution of striatal cell bodies expressing [Met]enkephalin-like immunoreactivity. Proc Natl Acad Sci U S A 81:7980-7984.

Graybiel AM, Baughman RW, Eckenstein F (1986) Cholinergic neuropil of the striatum observes striosomal boundaries. Nature 323:625-627.

Haber SN (2014) The place of dopamine in the cortico-basal ganglia circuit. Neuroscience 282c:248-257.

Harbour VL, Weigl Y, Robinson B, Amir S (2013) Comprehensive mapping of regional expression of the clock protein PERIOD2 in rat forebrain across the 24-h day. PLoS ONE 8:e76391.

Hart G, Leung BK, Balleine BW (2013) Dorsal and ventral streams: The distinct role of striatal subregions in the acquisition and performance of goal-directed actions. Neurobiology of Learning and Memory.

Heien MLAV, Khan AS, Ariansen JL, Cheer JF, Phillips PEM, Wassum KM, Wightman RM (2005) Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. Proc Natl Acad Sci U S A 102:10023-10028.

Hering H, Sheng M (2001) Dendritic spines: structure, dynamics and regulation. Nat Rev Neurosci 2:880-888.

Hirano T, Kikuchi K, Urano Y, Nagano T (2002) Improvement and Biological Applications of Fluorescent Probes for Zinc, ZnAFs. J Am Chem Soc 124:6555-6562.

Holst B, Holliday ND, Bach A, Elling CE, Cox HM, Schwartz TW (2004) Common structural basis for constitutive activity of the ghrelin receptor family. J Biol Chem 279:53806-53817.

Holst B, Egerod KL, Schild E, Vickers SP, Cheetham S, Gerlach LO, Storjohann L, Stidsen CE, Jones R, Beck-Sickinger AG, Schwartz TW (2007) GPR39 signaling is stimulated by zinc ions but not by obestatin. Endocrinology 148:13-20.

Hood S, Cassidy P, Cossette MP, Weigl Y, Verwey M, Robinson B, Stewart J, Amir S (2010) Endogenous dopamine regulates the rhythm of expression of the clock protein PER2 in the rat dorsal striatum via daily activation of D2 dopamine receptors. J Neurosci 30:14046-14058.

Hornykiewicz O (1992) Mechanisms of neuronal loss in Parkinson's disease: a neuroanatomical-biochemical perspective. Clin Neurol Neurosurg 94 Suppl:S9-11.

Huang YZ, Pan E, Xiong ZQ, McNamara JO (2008) Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. Neuron 57:546-558.

Hussain S, Ali SF (2002) Zinc potentiates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced dopamine depletion in caudate nucleus of mice brain. Neurosci Lett 335:25-28.

Hwang JJ, Park MH, Choi SY, Koh JY (2005) Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. J Biol Chem 280:11995-12001.

Iijima M, Nikaido T, Akiyama M, Moriya T, Shibata S (2002) Methamphetamine-induced, suprachiasmatic nucleus-independent circadian rhythms of activity and mPer gene expression in the striatum of the mouse. Eur J Neurosci 16:921-929.

Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Rev 31:6-41.

Imbesi M, Yildiz S, Dirim Arslan A, Sharma R, Manev H, Uz T (2009) Dopamine receptor-mediated regulation of neuronal "clock" gene expression. Neuroscience 158:537-544.

Ingham CA, Hood SH, Arbuthnott GW (1989) Spine density on neostriatal neurones changes with 6-hydroxydopamine lesions and with age. Brain Res 503:334-338.

Irvine GI, Logan B, Eckert M, Abraham WC (2006) Enriched environment exposure regulates excitability, synaptic transmission, and LTP in the dentate gyrus of freely moving rats. Hippocampus 16:149-160.

Jadavji NM, Metz GA (2008) Sex differences in skilled movement in response to restraint stress and recovery from stress. Behav Brain Res 195:251-259.

Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493-495.

Kishioka A, Uemura T, Fukushima F, Mishina M (2013) Consolidation of auditory fear memories formed by weak unconditioned stimuli requires NMDA receptor activation and de novo protein synthesis in the striatum. Mol Brain 6:17.

Ko CH, Takahashi JS (2006) Molecular components of the mammalian circadian clock. Hum Mol Genet 15:R271-R277.

Koizumi S, Ikeda M, Inoue K, Nakazawa K, Inoue K (1995) Enhancement by zinc of ATP-evoked dopamine release from rat pheochromocytoma PC12 cells. Brain Res 673:75-82.

Kolb B, Gorny G, Söderpalm AHV, Robinson TE (2003) Environmental complexity has different effects on the structure of neurons in the prefrontal cortex versus the parietal cortex or nucleus accumbens. Synapse 48:149-153.

Kononen J, Koistinaho J, Alho H (1990) Circadian rhythm in c-fos-like immunoreactivity in the rat brain. Neurosci Lett 120:105-108.

Kreitzer AC, Malenka RC (2008) Striatal plasticity and basal ganglia circuit function. Neuron 60:543-554.

Lee JY, Son HJ, Choi JH, Cho E, Kim J, Chung SJ, Hwang O, Koh JY (2009) Cytosolic labile zinc accumulation in degenerating dopaminergic neurons of mouse brain after MPTP treatment. Brain Res 1286:208-214.

Lee K-W, Kim Y, Kim AM, Helmin K, Nairn AC, Greengard P (2006) Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. Proc Natl Acad Sci U S A 103:3399-3404.

Leggio MG, Mandolesi L, Federico F, Spirito F, Ricci B, Gelfo F, Petrosini L (2005) Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. Behav Brain Res 163:78-90.

Lerner TN, Kreitzer AC (2011) Neuromodulatory control of striatal plasticity and behavior. Curr Opin Neurobiol 21:322-327.

Lewis MH (2004) Environmental complexity and central nervous system development and function. Ment Retard Dev Disabil Res Rev 10:91-95.

Li Y, Kolb B, Robinson TE (2003) The location of persistent amphetamine-induced changes in the density of dendritic spines on medium spiny neurons in the nucleus accumbens and caudate-putamen. Neuropsychopharmacology 28:1082-1085.

Liljeholm M, O'Doherty JP (2012) Contributions of the striatum to learning, motivation, and performance: an associative account. Trends Cogn Sci 16:467-475.

Lim NC, Freake HC, Brückner C (2005) Illuminating Zinc in Biological Systems. Chem–Eur J 11:38-49.

Lin AM (2001) Coexistence of zinc and iron augmented oxidative injuries in the nigrostriatal dopaminergic system of SD rats. Free Radic Biol Med 30:225-231.

Lin DD, Cohen AS, Coulter DA (2001) Zinc-induced augmentation of excitatory synaptic currents and glutamate receptor responses in hippocampal CA3 neurons. J Neurophysiol 85:1185-1196.

Linden AM, Sandu C, Aller MI, Vekovischeva OY, Rosenberg PH, Wisden W, Korpi ER (2007) TASK-3 knockout mice exhibit exaggerated nocturnal activity, impairments in cognitive functions, and reduced sensitivity to inhalation anesthetics. J Pharmacol Exp Ther 323:924-934.

Liu Y, Teeter MM, DuRand CJ, Neve KA (2006) Identification of a Zn2+-binding site on the dopamine D2 receptor. Biochem Biophys Res Commun 339:873-879.

Liu Y, Wong TP, Aarts M, Rooyakkers A, Liu L, Lai TW, Wu DC, Lu J, Tymianski M, Craig AM, Wang YT (2007) NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. J Neurosci 27:2846-2857.

Lo HS, Chiang HC, Lin AM, Chiang HY, Chu YC, Kao LS (2004) Synergistic effects of dopamine and Zn2+ on the induction of PC12 cell death and dopamine depletion in the striatum: possible implication in the pathogenesis of Parkinson's disease. Neurobiol Dis 17:54-61.

Luong TN, Carlisle HJ, Southwell A, Patterson PH (2011) Assessment of motor balance and coordination in mice using the balance beam. J Vis Exp.

Lynch WJ, Girgenti MJ, Breslin FJ, Newton SS, Taylor JR (2008) Gene profiling the response to repeated cocaine self-administration in dorsal striatum: a focus on circadian genes. Brain Res 1213:166-177.

Lynch WJ, Nicholson KL, Dance ME, Morgan RW, Foley PL (2010) Animal Models of Substance Abuse and Addiction: Implications for Science, Animal Welfare, and Society. Comp Med 60:177-188.

Maloney SE, Rieger MA, Dougherty JD (2013) Identifying essential cell types and circuits in autism spectrum disorders. Int Rev Neurobiol 113:61-96.

Marques JM, Olsson IA (2010) Performance of juvenile mice in a reach-to-grasp task. J Neurosci Methods 193:82-85.

Martel G, Hevi C, Kane-Goldsmith N, Shumyatsky GP (2011) Zinc transporter ZnT3 is involved in memory dependent on the hippocampus and perirhinal cortex. Behav Brain Res 223:233-238.

Martel G, Hevi C, Friebely O, Baybutt T, Shumyatsky GP (2010) Zinc transporter 3 is involved in learned fear and extinction, but not in innate fear. Learn Mem 17:582-590.

Massey PV, Johnson BE, Moult PR, Auberson YP, Brown MW, Molnar E, Collingridge GL, Bashir ZI (2004) Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. J Neurosci 24:7821-7828.

Matamales M, Bertran-Gonzalez J, Salomon L, Degos B, Deniau JM, Valjent E, Herve D, Girault JA (2009) Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. PLoS ONE 4:e4770.

Matsuura K, Kabuto H, Makino H, Ogawa N (1997) Pole test is a useful method for evaluating the mouse movement disorder caused by striatal dopamine depletion. J Neurosci Methods 73:45-48.

McAllister AK, Katz LC, Lo DC (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. Neuron 18:767-778.

McDonald CG, Dailey VK, Bergstrom HC, Wheeler TL, Eppolito AK, Smith LN, Smith RF (2005) Periadolescent nicotine administration produces enduring changes in dendritic morphology of medium spiny neurons from nucleus accumbens. Neurosci Lett 385:163-167.

McDonald RJ, White NM (1994) Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. Behav Neural Biol 61:260-270.

McGeorge AJ, Faull RL (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 29:503-537.

McNeill TH, Brown SA, Rafols JA, Shoulson I (1988) Atrophy of medium spiny I striatal dendrites in advanced Parkinson's disease. Brain Res 455:148-152.

Mengual E, Casanovas-Aguilar C, Perez-Clausell J, Gimenez-Amaya JM (1995) Heterogeneous and compartmental distribution of zinc in the striatum and globus pallidus of the rat. Neuroscience 66:523-537.

Middleton FA, Strick PL (2000) Basal ganglia and cerebellar loops: motor and cognitive circuits. Brain Res Rev 31:236-250.

Mistlberger RE (1994) Circadian food-anticipatory activity: formal models and physiological mechanisms. Neurosci Biobehav Rev 18:171-195.

Mistlberger RE (2011) Neurobiology of food anticipatory circadian rhythms. Physiol Behav 104:535-545.

Muller A, Kleinau G, Piechowski CL, Muller TD, Finan B, Pratzka J, Gruters A, Krude H, Tschop M, Biebermann H (2013) G-protein coupled receptor 83 (GPR83) signaling determined by constitutive and zinc(II)-induced activity. PLoS ONE 8:e53347.

Nakashima AS, Dyck RH (2008) Enhanced plasticity in zincergic, cortical circuits after exposure to enriched environments. J Neurosci 28:13995-13999.

Nakashima AS, Dyck RH (2009) Zinc and cortical plasticity. Brain research reviews 59:347-373.

Nakashima AS, Dyck RH (2010) Dynamic, experience-dependent modulation of synaptic zinc within the excitatory synapses of the mouse barrel cortex. Neuroscience 170:1015-1019.

Nakashima AS, Butt RH, Dyck RH (2011) Alterations in protein and gene expression within the barrel cortices of ZnT3 knockout mice: experience-independent and dependent changes. Neurochem Int 59:860-870.

Nelson AB, Kreitzer AC (2014) Reassessing models of basal ganglia function and dysfunction. Annu Rev Neurosci 37:117-135.

Norregaard L, Frederiksen D, Nielsen EO, Gether U (1998) Delineation of an endogenous zinc-binding site in the human dopamine transporter. EMBO J 17:4266-4273.

Ogura T, Ogata M, Akita H, Jitsuki S, Akiba L, Noda K, Hoka S, Saji M (2005) Impaired acquisition of skilled behavior in rotarod task by moderate depletion of striatal dopamine in a pre-symptomatic stage model of Parkinson's disease. Neurosci Res 51:299-308.

Packard MG, Knowlton BJ (2002) Learning and memory functions of the basal ganglia. Annu Rev Neurosci 25:563-593.

Paoletti P, Neyton J (2007) NMDA receptor subunits: function and pharmacology. Curr Opin Pharmacol 7:39-47.

Pappas SS, Leventhal DK, Albin RL, Dauer WT (2014) Mouse models of neurodevelopmental disease of the basal ganglia and associated circuits. Curr Top Dev Biol 109:97-169.

Pattij T, Vanderschuren LJMJ (2008) The neuropharmacology of impulsive behaviour. Trends Pharmacol Sci 29:192-199.

Perez-Clausell J, Danscher G (1985) Intravesicular localization of zinc in rat telencephalic boutons. A histochemical study. Brain Res 337:91-98.

Rachline J, Perin-Dureau F, Le Goff A, Neyton J, Paoletti P (2005) The micromolar zinc-binding domain on the NMDA receptor subunit NR2B. J Neurosci 25:308-317.

Richfield EK (1993) Zinc modulation of drug binding, cocaine affinity states, and dopamine uptake on the dopamine uptake complex. Mol Pharmacol 43:100-108.

Robbins TW (2002) The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology (Berl) 163:362-380.

Robinson TE, Kolb B (1997) Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. J Neurosci 17:8491-8497.

Robinson TE, Kolb B (1999a) Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. Synapse 33:160-162.

Robinson TE, Kolb B (1999b) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11:1598-1604.

Robinson TE, Gorny G, Mitton E, Kolb B (2001) Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. Synapse 39:257-266.

Rojas P, Franco-Perez JE, Rojas C, Rojas-Castaneda J, Ebadi M, Fernandez-Valverde F, Serrano-Garcia N (2005) Reduction of zinc-positive terminal fields in striatum of mouse after 1-methyl-4-phenylpyridinium neurotoxicity. Neurotoxicology 26:959-968.

Sayyah M, Olapour A, Saeedabad Y, Yazdan Parast R, Malayeri A (2012) Evaluation of oral zinc sulfate effect on obsessive-compulsive disorder: a randomized placebo-controlled clinical trial. Nutrition 28:892-895.

Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST (2000) CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology 39:777-787.

Schetz JA, Sibley DR (1997) Zinc allosterically modulates antagonist binding to cloned D1 and D2 dopamine receptors. J Neurochem 68:1990-1997.

Schetz JA, Sibley DR (2001) The binding-site crevice of the D4 dopamine receptor is coupled to three distinct sites of allosteric modulation. J Pharmacol Exp Ther 296:359-363.

Schetz JA, Chu A, Sibley DR (1999) Zinc modulates antagonist interactions with D2-like dopamine receptors through distinct molecular mechanisms. J Pharmacol Exp Ther 289:956-964.

Sedelis M, Hofele K, Auburger GW, Morgan S, Huston JP, Schwarting RK (2000) MPTP susceptibility in the mouse: behavioral, neurochemical, and histological analysis of gender and strain differences. Behav Genet 30:171-182.

Segovia G, Del Arco A, De Blas M, Garrido P, Mora F (2010) Environmental enrichment increases the in vivo extracellular concentration of dopamine in the nucleus accumbens: a microdialysis study. J Neural Transm 117:1123-1130.

Sheline CT, Zhu J, Zhang W, Shi C, Cai AL (2013) Mitochondrial inhibitor models of Huntington's disease and Parkinson's disease induce zinc accumulation and are attenuated by inhibition of zinc neurotoxicity in vitro or in vivo. Neurodegener Dis 11:49-58.

Shohag H, Ullah A, Qusar S, Rahman M, Hasnat A (2012) Alterations of serum zinc, copper, manganese, iron, calcium, and magnesium concentrations and the complexity of interelement relations in patients with obsessive-compulsive disorder. Biol Trace Elem Res 148:275-280.

Silberberg G, Bolam JP (2015) Local and afferent synaptic pathways in the striatal microcircuitry. Curr Opin Neurobiol 33:182-187.

Sindreu C, Palmiter RD, Storm DR (2011) Zinc transporter ZnT-3 regulates presynaptic Erk1/2 signaling and hippocampus-dependent memory. Proc Natl Acad Sci U S A 108:3366-3370.

Sirevaag AM, Black JE, Shafron D, Greenough WT (1988) Direct evidence that complex experience increases capillary branching and surface area in visual cortex of young rats. Brain Res 471:299-304.

Slomianka L, Danscher G, Frederickson CJ (1990) Labeling of the neurons of origin of zinc-containing pathways by intraperitoneal injections of sodium selenite. Neuroscience 38:843-854.

Sorensen JC, Slomianka L, Christensen J, Zimmer J (1995) Zinc-containing telencephalic connections to the rat striatum: a combined Fluoro-Gold tracing and histochemical study. Exp Brain Res 105:370-382.

Spires TL, Grote HE, Garry S, Cordery PM, Van Dellen A, Blakemore C, Hannan AJ (2004) Dendritic spine pathology and deficits in experience-dependent dendritic plasticity in R6/1 Huntington's disease transgenic mice. Eur J Neurosci 19:2799-2807.

Stanley JL, Lincoln RJ, Brown TA, McDonald LM, Dawson GR, Reynolds DS (2005) The mouse beam walking assay offers improved sensitivity over the mouse rotarod in

determining motor coordination deficits induced by benzodiazepines. J Psychopharmacol 19:221-227.

Suh SW, Jo SM, Vajda Z, Danscher G (2005) Adrenalectomy-induced ZnT3 downregulation in mouse hippocampus is followed by vesicular zinc depletion. Neurosci Lett 377:164-169.

Takeda A, Tamano H, Tochigi M, Oku N (2005) Zinc homeostasis in the hippocampus of zinc-deficient young adult rats. Neurochem Int 46:221-225.

Tarohda T, Ishida Y, Kawai K, Yamamoto M, Amano R (2005) Regional distributions of manganese, iron, copper, and zinc in the brains of 6-hydroxydopamine-induced parkinsonian rats. Anal Bioanal Chem 383:224-234.

Tillerson JL, Miller GW (2003) Grid performance test to measure behavioral impairment in the MPTP-treated-mouse model of parkinsonism. J Neurosci Methods 123:189-200.

Travaglia A, La Mendola D, Magrì A, Pietropaolo A, Nicoletti VG, Grasso G, Malgieri G, Fattorusso R, Isernia C, Rizzarelli E (2013) Zinc(II) Interactions with brain-derived neurotrophic factor N-terminal peptide fragments: Inorganic features and biological perspectives. Inorg Chem 52:11075-11083.

Trezza V, Baarendse PJJ, Vanderschuren LJMJ (2010) The pleasures of play: pharmacological insights into social reward mechanisms. Trends Pharmacol Sci 31:463-469.

Uz T, Akhisaroglu M, Ahmed R, Manev H (2003) The pineal gland is critical for circadian Period1 expression in the striatum and for circadian cocaine sensitization in mice. Neuropsychopharmacology 28:2117-2123.

van den Bos W, Rodriguez CA, Schweitzer JB, McClure SM (2015) Adolescent impatience decreases with increased frontostriatal connectivity. P Natl Acad Sci 112:E3765-E3774.

van Gaalen MM, van Koten R, Schoffelmeer ANM, Vanderschuren LJMJ (2006) Critical Involvement of Dopaminergic Neurotransmission in Impulsive Decision Making. Biol Psychiatry 60:66-73.

Venable N, Pinto-Hamuy T, Arraztoa JA, Contador MT, Chellew A, Peran C, Valenzuela X (1988) Greater efficacy of preweaning than postweaning environmental enrichment on maze learning in adult rats. Behav Brain Res 31:89-92.

Wall MJ (2005) A role for zinc in cerebellar synaptic transmission? Cerebellum 4:224-229.

Whishaw IQ (1996) An endpoint, descriptive, and kinematic comparison of skilled reaching in mice (Mus musculus) with rats (Rattus norvegicus). Behav Brain Res 78:101-111.

Williams K (1996) Separating dual effects of zinc at recombinant N-methyl-D-aspartate receptors. Neurosci Lett 215:9-12.

Winstanley CA (2007) The orbitofrontal cortex, impulsivity, and addiction. Ann N Y Acad Sci 1121:639-655.

Winstanley CA, Eagle DM, Robbins TW (2006) Behavioral models of impulsivity in relation to ADHD: Translation between clinical and preclinical studies. Clin Psychol Rev 26:379-395.

Yager LM, Garcia AF, Wunsch AM, Ferguson SM (2015) The ins and outs of the striatum: Role in drug addiction. Neuroscience 301:529-541.

Yasuda S, Miyazaki T, Munechika K, Yamashita M, Ikeda Y, Kamizono A (2007) Isolation of Zn2+ as an endogenous agonist of GPR39 from fetal bovine serum. J Recept Signal Transduct Res 27:235-246.

Yin HH, Ostlund SB, Knowlton BJ, Balleine BW (2005) The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 22:513-523.

Zaja-Milatovic S, Milatovic D, Schantz AM, Zhang J, Montine KS, Samii A, Deutch AY, Montine TJ (2005) Dendritic degeneration in neostriatal medium spiny neurons in Parkinson disease. Neurology 64:545-547.

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APPENDIX A: APPENDIX

A.1. Introduction

Most, if not all, organisms exhibit a daily rhythm of activity – behaviourally and physiologically. This daily rhythm, or oscillation, is controlled by many oscillators in both the brain and the body, but the master oscillator is the suprachiasmatic nucleus (SCN) located in the hypothalamus. It receives input from the eyes and causes daily rhythms to occur that tend to follow periods of light and dark. The SCN is divided into an outer shell and inner core which have slightly different functions. However, they work together to produce circadian rhythms which involves autoregulatory feedback loops of transcription and translation. Many genes – known as clock genes - are involved in these loops; these include Clock, Bmal1, Period (Per), Cryptochrome (Cry), Rev-erb α, and Rorα (reviewed by Antle and Silver, 2005; Ko and Takahashi, 2006). While the SCN is thought to be the central oscillator controlling the brain and the rest of the body, there are other areas of the brain that have been found to exhibit circadian rhythmicity of the clock genes as well. One of these areas is the striatum.

As the main input center for the basal ganglia, the striatum plays a role in voluntary motor control, eye movements, procedural learning, habitual learning, and cognition including motivation and reward (reviewed by Liljeholm and O'Doherty, 2012). It consists of the caudate nucleus, putamen, nucleus accumbens, and olfactory tubercle (McGeorge and Faull, 1989). In humans, the caudate and putamen are separated by the internal capsule and can thus be distinguished from one another. However, in rodents the internal capsule is more spread out making the caudate and putamen indistinguishable. Therefore, it is generally referred to as the caudate-putamen (CPu). Regardless of species, the caudate and putamen are generally considered to be the dorsal striatum while the nucleus accumbens is termed the ventral striatum.

The main afferent connection to the striatum is glutamatergic input from the cortex. However, the striatum also receives input from the thalamus (glutamatergic) and the substantia nigra (dopaminergic; Alexander et al., 1990). The role of dopamine in the striatum is to modulate synaptic plasticity and thus plays a role in acquisition of skilled behaviours (Ogura et al., 2005), motor learning (Costa, 2007), and the formation of motor memories (Badgaiyan et al., 2008). Lack of nigrostriatal dopaminergic input leads to Parkinson's disease (Hornykiewicz, 1992).

Previous studies have found that circadian rhythms exist within the striatum for various neurotransmitters, immediate-early genes (cFos), as well as clock genes. Glutamate, GABA, and dopamine all exhibit circadian rhythms in the striatum and nucleus accumbens – some of which are light dependent, some are light independent – and this differs between the 2 sub-regions (Castaneda et al., 2004). Expression of cFos in the caudate-putamen of rats is lowest at circadian time 12 (CT12; designated as activity onset by convention) and highest at CT0 (Kononen et al., 1990). In the adult mouse striatum, clock genes were found to exhibit rhythmicity – with Bmal1, Clock, Npas2, and Cry1 peaking in the late dark or early light time, Rev-erb α at mid-light, and Per1 at the transition from light to dark (Cai et al., 2009). Another study found that the Per1 gene and protein show circadian rhythmicity in the striatum with the protein peaking during the day. *Per1* mRNA peaked 16 hours earlier (Uz et al., 2003). In 2013, Harbour et al. found that Per2 rhythms in the striatum have opposite rhythms to those found in the SCN (Harbour et al., 2013) and altering the Per2 rhythm in a dopamine-depleted striatum using activation of dopamine 2 (D2) receptors had no effect on Per2 rhythms in the SCN (Hood et al., 2010). In addition to the discovery that clock genes in the striatum exhibit circadian rhythms, it has also been shown that dopamine can drive these rhythms. Dopamine receptor agonists have differing effects on striatal

clock gene expression depending on receptor class (D1-class versus D2-class) both in cultured striatal cells and *in vivo,* and are able to shift rhythms of Per1 protein (Imbesi et al., 2009).

In addition, there have been studies linking circadian rhythmicity in the striatum to reward-seeking behaviours, for example, food and drug seeking (reviewed by Dibner et al., 2010). In particular, food anticipatory activity (FAA) – involving increases in locomotion, core body temperature and hormones – still occurs and follows a circadian rhythm in SCN-lesioned animals, suggesting there is a food entrainable oscillator (FEO) that operates independent of the SCN (reviewed by Mistlberger, 1994; Mistlberger, 2011). There is some evidence that the striatum may be involved. When rats were fed ad libitum, Per1 showed rhythmic expression, the peak of which could be shifted in the nucleus accumbens when the rats were switched to a restricted feeding schedule (Angeles-Castellanos et al., 2007). Dopamine 1 receptors (D1R) in the dorsal striatum have also been linked to FAA and D1R knockout mice have suppressed Per2 gene expression (Gallardo et al., 2014).

There has also been much research into drug seeking behaviour. Many aspects of drug addiction (e.g. sensitivity to the drug, conditioned place preference, self-administration, etc.) appear to follow a specific rhythm. Much research on drug-related rhythms has been done using methamphetamine, a drug that increases the amount of dopamine in the brain, some of which was performed in SCN-lesioned animals and led to the proposal of a methamphetamine-sensitive circadian oscillator (MASCO; reviewed by Dibner et al., 2010). Injections of methamphetamine into mice can shift rhythms of clock gene expression (Per1 and Per2) in the striatum without have any effect on rhythms in the SCN (Iijima et al., 2002). Changes in the expression of clock genes (Per2, Clock, Bmal1, Cry1) in the striatum has been found with cocaine self-

administration as well (Lynch et al., 2008). Overall, clock gene expression in the striatum seems to play an important role in food and drug seeking behaviours.

Another potential modulator of the striatum is the bivalent metal zinc, which is an essential component to all cells (reviewed by Chasapis et al., 2012). A proportion of the zinc found in the brain gets localized into synaptic vesicles of neurons (called zincergic neurons) and can be released alongside glutamate to affect post-synaptic cells (Frederickson et al., 2000). Zinc can act on receptors (including glutamatergic, GABAergic, glycinergic, serotonergic, purinergic, cholinergic, and dopaminergic, as well as voltage-gated ion channels) or enter the cell through calcium-permeable ion channels or through the sodium-zinc exchanger and exert effects from inside the cell. It affects signal transduction pathways, including cyclic adenosine monophosphate (cAMP), protein kinase C (PKC), and calcium/calmodulin-dependent protein kinase II (CaMKII) pathways (reviewed by Nakashima and Dyck, 2009). Recently, a g-protein coupled receptor, GPR39, was found to be activated by zinc (Holst et al., 2007; Yasuda et al., 2007; Cohen et al., 2012) and may play a role in plasticity (Holst et al., 2004). In addition, zinc has been shown to indirectly activate the tropomyosin-related kinase (Trk) pathway, specifically TrkB which is normally activated by brain derived neurotrophic factor (BDNF; Hwang et al., 2005; Huang et al., 2008).

In terms of function of vesicular zinc in the brain, it is thought to be involved in plasticity. This has been shown in the somatosensory (barrel) cortex with response to whisker plucking (Nakashima and Dyck, 2010), as well as in the hippocampus in terms of long-term potentiation and depression (reviewed by Nakashima and Dyck, 2009).

In the forebrain, vesicular zinc is located almost everywhere including in the cerebral cortex, hippocampus, amygdala, and, of interest to this paper, the striatum (see Figure 2 in

Brown and Dyck, 2003). While there has been a great deal of research into the role of vesicular zinc in the hippocampus and somatosensory cortex, very few studies have examined its role in the striatum. The two studies that have looked at zinc in the striatum compared zinc staining to stains for other histochemical markers of the striatum (Mengual et al., 1995) and performed retrograde tracing to determine zincergic afferent projections to the striatum (Sorensen et al., 1995).

Due to the importance of dopamine in the striatum, the effect zinc has on the dopaminergic system will be examined more closely. Although not much research has been done on zinc and the dopaminergic system, zinc binding sites have been found on dopamine receptors (Schetz and Sibley, 2001; Liu et al., 2006) as well as on the dopamine uptake transporter (DAT; Norregaard et al., 1998). At what is arguably considered physiological concentration (3-300μM), zinc potentiates dopamine release, while inhibiting release at higher (>1mM) concentrations (Koizumi et al., 1995). Binding of zinc to these sites has been found to modulate binding of dopamine receptor antagonists in a manner that is dose-dependent, allosteric, and reversible (Schetz and Sibley, 1997; Schetz et al., 1999). In addition, Bjorklund et al. (2007) found that zinc inhibits dopamine transport through the dopamine transporter. Also when examining drugs that affect the dopaminergic system, such as cocaine or cocaine analogues, zinc was found to potentiate the drugs' action (Richfield, 1993; Bjorklund et al., 2007). It follows then that if zinc were depleted in the brain, the dopaminergic system would likely be affected.

To facilitate research on the role of vesicular zinc, in 1999 a mouse knockout model was created (Cole et al., 1999). These mice lack zinc transporter 3 (ZnT3) – the protein required to package zinc into vesicles to be released from the cell. Upon first inspection of these mice, they appeared behaviourally normal with only an increased susceptibility to kainate-induced seizures

(Cole et al., 2000; Cole et al., 2001). More recently, however, deficits have been found, specifically that the ZnT3 knockout (KO) mice seem to lack neuroplasticity. This is evidenced by changes in gene and protein expression (Nakashima et al., 2011), as well as by deficits in behavioural tasks that examine ability to change, such as the t-alternation maze (Sindreu et al., 2011) and a version of the Morris water task that involves learning an initial platform location and then learning a new platform location (Martel et al., 2011).

Since ZnT3 KO mice lack synaptic zinc and synaptic zinc is involved in many different signalling pathways including dopaminergic modulation, it is possible that the rhythm of clock gene expression in the striatum may be affected and may lead to changes in reward-related behaviour (none of which has been examined in these mice). The purpose of the current study is to examine whether changes caused by lack of zincergic signaling (in ZnT3 knockout mice) affects the circadian oscillation of gene expression in the striatum using cFos as a marker of changes in gene expression.

A.2. Methods

A.2.1. Animals

Mice that were heterozygous for the ZnT3 allele were bred to produce litters consisting of wildtype, heterozygous, and knockout mice. Adult male ZnT3 wildtype (WT) and knockout (KO) mice produced from this colony were used for this study. They were raised in a 12 hour light/dark cycle (lights on at 6am and off at 6pm) with ad libitum access to food and water. All procedures were approved by the Animal Care Committee of the University of Calgary and conformed to the guidelines set out by the Canadian Council for Animal Care.

A.2.2. Procedure

Mice (3 per genotype per time point) were perfused at 4 time points (ZT0, ZT6, ZT12, and ZT18) with 50 ml of cold phosphate-buffered saline (PBS) followed by 50 ml of cold 4% paraformaldehyde (PFA). Brains were removed and post-fixed in 4% PFA overnight. They were then cryoprotected in 20% sucrose for 24 hours and placed in a -80˚C freezer until staining was completed. Brains were cut at 35 μm on a sliding microtome. Six series were collected. *cFos immunohistochemistry.* Staining procedures began by rinsing the brain slices in 0.5% hydrogen peroxide (H2O2) in 0.3% Triton X-100 in PBS (PBSx) for 30 minutes. Slices were then rinsed in PBSx (3 x 10 min) before incubation in 1% normal goat serum (Vector Laboratories Inc., Burlingame, CA, USA). After 90 min, slices were incubated for 48 hours in the primary antibody (anti cfos; Santa Cruz, Dallas, TX, USA). Sections were rinsed in PBSx (3 x 10 min) and incubated in the secondary antibody (goat anti-Rabbit 1:200; Vector Laboratories Inc., Burlingame, CA, USA) for 60 min. Sections were again rinsed in PBSx (3 x 10 min) and incubated in an avidin-biotin complex (ABC kit; Vector Laboratories Inc., Burlingame, CA, USA) for 60 min. After another rinse with PBSx (3 x 10 min), sections were developed using diaminobenzidine (DAB) and 8% nickel chloride until cells bodies could be seen under a dissecting microscope (approximately 5 min). The reaction was terminated with a series of PBS washes. Slices were mounted on 1.5% gelatin-coated slides, dehydrated in an ascending series of alcohol (70%, 95%, 100% for 10 min each) then cleared with xylenes for 10 min. Slides were coverslipped with Permount.

A.2.3. Quantification

Pictures of each slice were taken at 100X magnification. Areas of dorsal striatum were determined. Labelled nuclei in each area were counted on 4 consecutive slices in both hemispheres for each brain using the ImageJ software (ImageJ 1.48; National Institutes of Health, Bethesda, MD, USA) automated counting function.

A.2.4. Statistics

A factorial analysis-of-variance (ANOVA) was used to compare between time points as well as between ZnT3 WT and KO mice at each time point. Tukey's HSD was used for post hoc follow-up comparisons. A paired t-test was used to compare labelled nuclei between left and right striata.

A.3. Results

In general, labelled cell bodies were located on the medial portion of the striatum as opposed to the lateral portion. Four mice were removed from analysis due to poor staining. There was no significant difference between number of labelled nuclei in the left (M=118.05, SD=41.42) and right (M=126.54, SD=53.10) striata, $t(19)=1.14$, $p=.270$. A main effect of circadian time was found, $F(3,12)=4.88$, $p=.019$. Post hoc testing indicated significant differences between ZT0 and ZT12, $t(9)=2.98$, $p=.049$, and between ZT6 and ZT12, $t(10)=3.52$, $p=.019$. However, no significant differences were found for genotype and there was no interaction between genotype and circadian time. That being said, ZnT3 WT mice had peak cFos expression at ZT0, while ZnT3 KO mice had peak expression at ZT6. Both had trough cFos expression at ZT12 as shown in *Figure 1*. *Figure 2* shows representative examples of cFos labelled nuclei for ZnT3 WT and KO mice at all 4 time points.

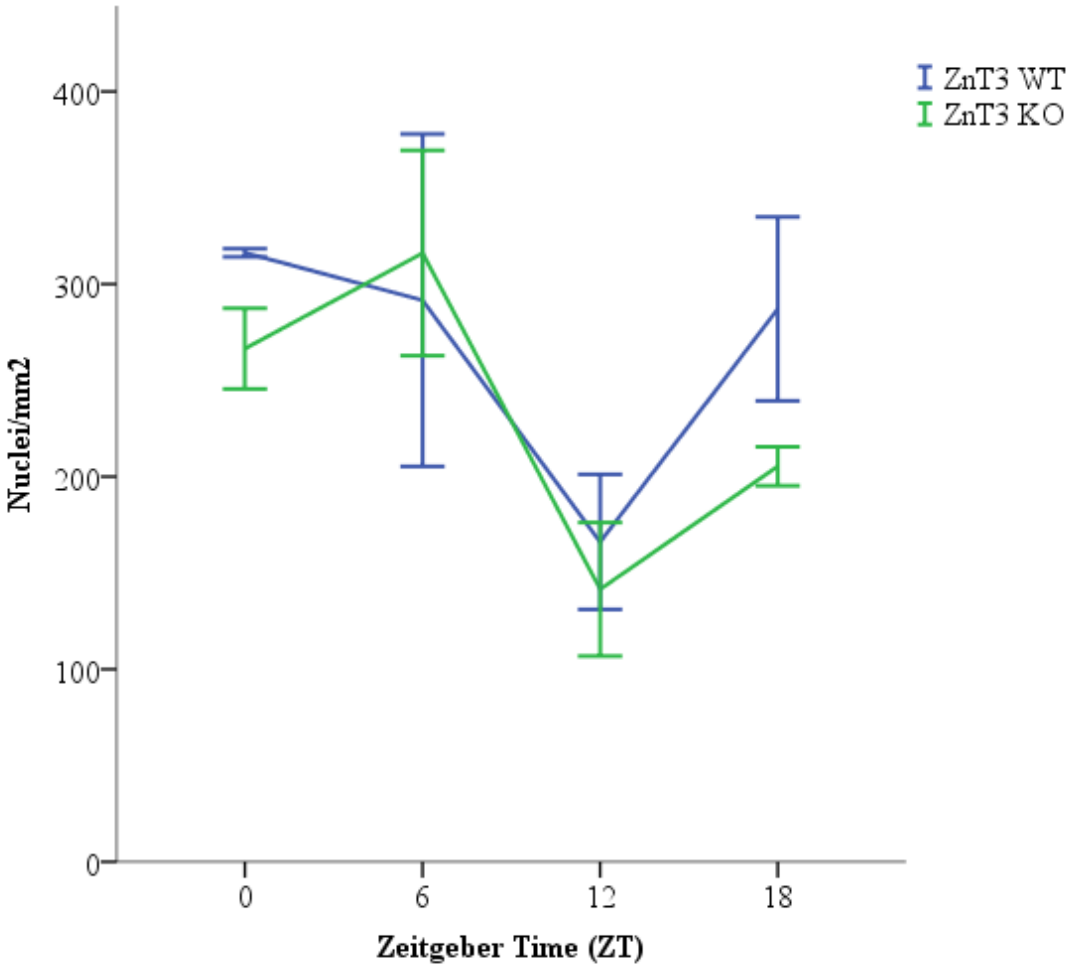

Figure A.4.1. Mean labelled cFos nuclei/mm2 at 4 time points (ZT0, ZT6, ZT12, ZT18) for ZnT3 wildtype and knockout mice. Error bar represent ± SEM.

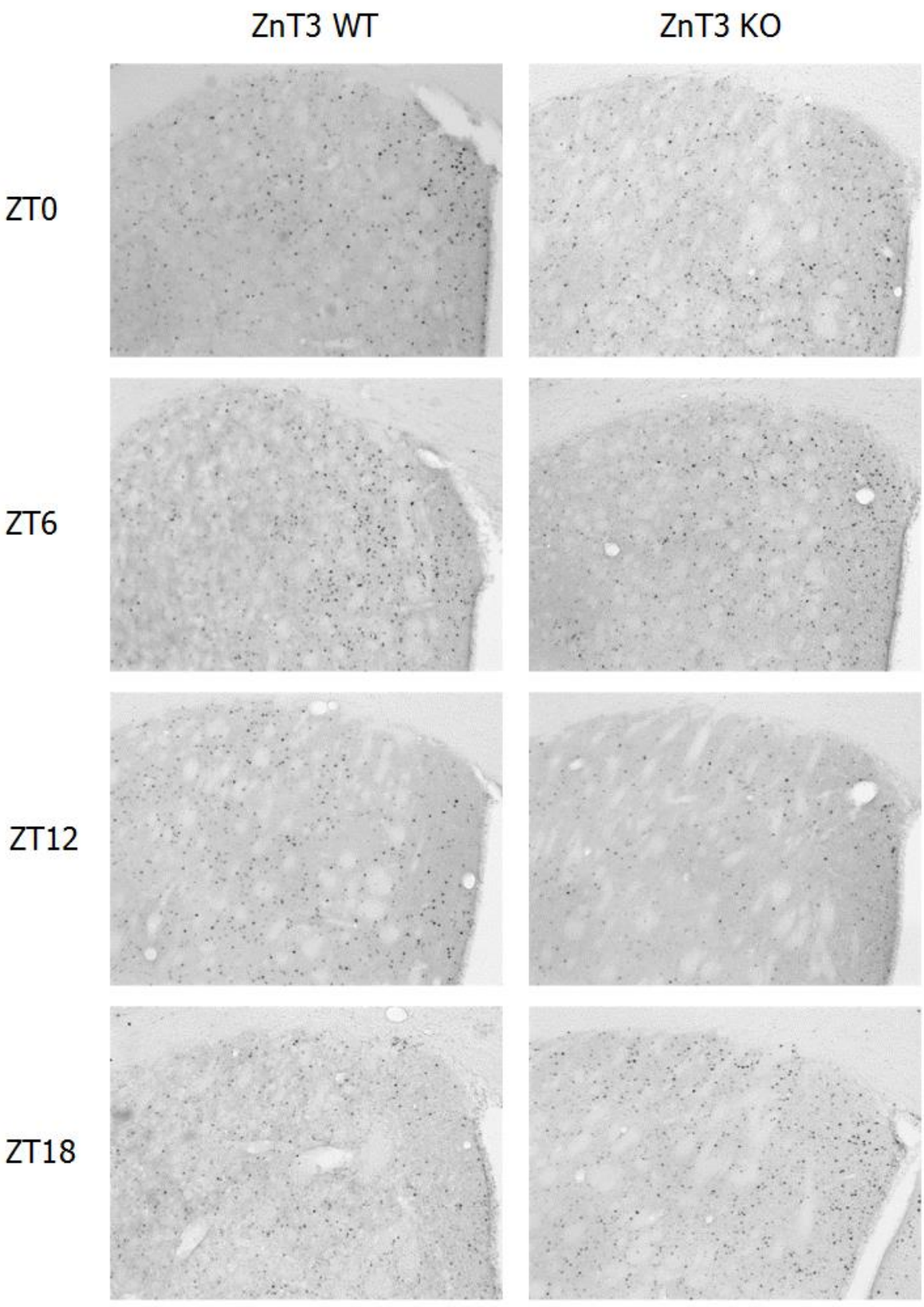
ZnT3 WT
ZnT3 KO
ZT0
ZT6
ZT12
ZT18

Figure A.4.2. cFos labelled nuclei at 4 time points (zeitgeber time (ZT) 0, ZT6, ZT12, ZT18) for ZnT3 wildtype (WT) and knockout (KO) animals.

A.4. Discussion

When examining cFos expression in the striata of ZnT3 wildtype (WT) and knockout (KO) mice, cFos expression was found to be rhythmic, with highest expression around ZT0-ZT6 and lowest expression at ZT12. This is consistent with what was found in rats (Kononen et al., 1990). However, ZnT3 KO mice exhibit essentially the same rhythm as ZnT3 WT mice, indicating that the effect caused by a lack of zinc in the striatum does not affect circadian expression of cFos in that structure.

The exact function of synaptic zinc in the striatum is still unknown, but zinc in general has been found to affect both glutamatergic and dopaminergic systems – both important transporter systems within the striatum. Zinc likely plays a modulatory role in these systems. As mentioned earlier, zinc binding domains have been found on both the dopamine receptors and the dopamine transporter (Norregaard et al., 1998; Schetz and Sibley, 2001; Liu et al., 2006). The effect of zinc on the receptors and transporter seems to be to increase efflux and block uptake of dopamine, presumably leading to more dopamine in the synaptic cleft (Koizumi et al., 1995; Norregaard et al., 1998). However, most of these studies use *in vitro* methods rather than *in vivo*, so the actual effect of zinc on the dopaminergic system may be different in live animals than what has been found so far in slice preparations.

Another set of studies has examined the role of zinc on the survival of dopaminergic neurons with contradictory results. Using PC12 cells from a rat, Lo et al. (2004) found that zinc and dopamine applied together could induce cell death. However, Eibl et al. (2010) and Gauthier et al. (2008) found that zinc, through metallothionein, enhanced survival of dopaminergic neurons in oxidative environments where dopamine normally forms toxic products. This hints at

a complicated relationship between zinc and dopamine that may depend on the presence or absence of other molecules (such as metallothioneins).

Because of the role of the nigrostriatal dopaminergic system in Parkinson's disease, several studies have examined the interaction between zinc and dopamine with the substantia nigra (SN) or through the use of drugs that mimic Parkinson's disease in animals. One study found that infusing zinc into the SN led to degeneration of the nigrostriatal dopamine system (Lin, 2001). Another study found that zinc accumulation in the SN indicated degeneration of dopaminergic neurons (Lee et al., 2009). This leads to a chicken or egg conundrum. Is the increased zinc leading to the death of dopaminergic neurons or does death of the dopaminergic neurons cause the increase in zinc? And how do these changes within the substantia nigra affect its main target, the striatum?

Drugs such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 1-methyl-4-phenylpyridinium (MPP+), and 6-hydroxydopamine **(**6-OHDA) are considered dopaminergic neurotoxins. They cause depletion of dopamine in the brain and, as such, are frequently used in research on Parkinson's disease. One study found that zinc could potentiate dopamine depletion in MPTP treated animals, but caused no changes in dopamine levels when administered alone (Hussain and Ali, 2002). Other studies looked at the effects of the drug on zinc levels in the brain rather than dopamine levels. Rojas et al. (2005) found that MPP+ caused decreases of zinc in the striatum, while Sheline et al. (2013) found increases in zinc in the presence of MPP+. 6-OHDA was found to increase zinc levels in the substantia nigra, globus pallidus, putamen, and amygdala (Tarohda et al., 2005).

The results from all of these studies point to a complex relationship between zinc and dopamine, the understanding of which is further complicated by the fact that some studies were

done *in vitro* while others were done *in vivo*. Clearly more research needs to be done to determine the relationship between zinc and dopamine *in vivo* in order to make sense of the role of zinc within the striatum. In addition to the lack of knowledge of the normal role of zinc in the striatum, we are also unaware of the effects that lack of zinc (as seen in the ZnT3 KO mice) might have on the striatum. Since few deficits have been found in the ZnT3 KOs, it is possible that there are compensatory mechanisms involved, but it is also uncertain as to what those mechanisms might be.

Since the ZnT3 KO mice do not differ from ZnT3 WT in terms of circadian cFos expression in the striatum, it is possible that they will not show differences in activities linked to circadian functions involving the striatum, such as food seeking and drug seeking. This is not to suggest that the ZnT3 KO mice will not show deficiencies on those tasks at all, but perhaps not in terms of aspects that pertain to time of day. For example, they may be more or less sensitive to a drug or not have a conditioned place preference, but exhibit drug-seeking behaviours at the same time as wildtype. That being said, cFos is only a general indicator of gene expression. We might have found differences in expression between ZnT3 WT and KO animals if we had used a more specific indicator such as dopamine itself, dopamine receptors, the dopamine transporter, or even clock gene or protein expression. For example, it might be of interest to stain for the Period gene/protein considering its rhythmicity in the striatum has been shown to be attenuated without dopamine present (Iijima et al., 2002; Lynch et al., 2008; Hood et al., 2010; Gallardo et al., 2014). Also, this study examined the entire dorsal striatum, but did not examine cell counts in the dorsolateral versus dorsomedial striatum or in the ventral striatum (nucleus accumbens). Since these regions are functionally distinct within the striatum, it is possible that looking at

these regions independently may show a difference in cFos-labelled nuclei even though the striatum as a whole does not.

In summary, while the SCN in the hypothalamus is thought to be the central rhythm generator for the body, other areas of the brain also exhibit circadian rhythmicity that, in some cases, appear to be independent of the SCN. One of these areas is the striatum. Expression of immediate-early genes, as well as some clock genes and neurotransmitters, have been found to follow a circadian rhythm. Zinc is an important signaling molecule in the brain and can affect many signaling pathways, including those within the striatum. However, lack of vesicular zinc, due to removal of the ZnT3 transporter in ZnT3 KO mice, does not appear to affect the circadian rhythm of cFos expression in the striatum.

A.5. References

Abraham WC, Logan B, Greenwood JM, Dragunow M (2002) Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. J Neurosci 22:9626-9634.

Adlard PA, Parncutt JM, Finkelstein DI, Bush AI (2010) Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? J Neurosci 30:1631-1636.

Afifi AK (1994) Basal ganglia: functional anatomy and physiology. Part 2. J Child Neurol 9:352-361.

Alexander GE (1994) Basal ganglia-thalamocortical circuits: their role in control of movements. J Clin Neurophysiol 11:420-431.

Alexander GE, DeLong MR, Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357-381.

Alexander GE, Crutcher MD, DeLong MR (1990) Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 85:119-146.

Angeles-Castellanos M, Mendoza J, Escobar C (2007) Restricted feeding schedules phase shift daily rhythms of c-Fos and protein Per1 immunoreactivity in corticolimbic regions in rats. Neuroscience 144:344-355.

Antle MC, Silver R (2005) Orchestrating time: arrangements of the brain circadian clock. Trends Neurosci 28:145-151.

Artola A, von Frijtag JC, Fermont PC, Gispen WH, Schrama LH, Kamal A, Spruijt BM (2006) Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. Eur J Neurosci 23:261-272.

Asinof SK, Paine TA (2014) The 5-Choice Serial Reaction Time Task: A Task of Attention and Impulse Control for Rodents. JoVE:e51574.

Badgaiyan RD, Fischman AJ, Alpert NM (2008) Explicit motor memory activates the striatal dopamine system. Neuroreport 19:409-412.

Balkaya M, Kröber JM, Rex A, Endres M (2013) Assessing post-stroke behavior in mouse models of focal ischemia. J Cereb Blood Flow Metab 33:330-338.

Bardo M, Klebaur J, Valone J, Deaton C (2001) Environmental enrichment decreases intravenous self-administration of amphetamine in female and male rats. Psychopharmacology (Berl) 155:278-284.

Bardo MT, Donohew RL, Harrington NG (1996) Psychobiology of novelty seeking and drug seeking behavior. Behav Brain Res 77:23-43.

Bevins RA (2001) Novelty Seeking and Reward: Implications for the Study of High-Risk Behaviors. Cur Dir Psychol Sci 10:189-193.

Bilici M, Yildirim F, Kandil S, Bekaroglu M, Yildirmis S, Deger O, Ulgen M, Yildiran A, Aksu H (2004) Double-blind, placebo-controlled study of zinc sulfate in the treatment of attention deficit hyperactivity disorder. Prog Neuropsychopharmacol Biol Psychiatry 28:181-190.

Bitanihirwe BK, Cunningham MG (2009) Zinc: the brain's dark horse. Synapse 63:1029-1049.

Bjorklund NL, Volz TJ, Schenk JO (2007) Differential effects of Zn2+ on the kinetics and cocaine inhibition of dopamine transport by the human and rat dopamine transporters. Eur J Pharmacol 565:17-25.

Blakemore LJ, Trombley PQ (2004) Diverse modulation of olfactory bulb AMPA receptors by zinc. Neuroreport 15:919-923.

Bowling SL, Bardo MT (1994) Locomotor and rewarding effects of amphetamine in enriched, social, and isolate reared rats. Pharmacol Biochem Be 48:459-464.

Bowling SL, Rowlett JK, Bardo MT (1993) The effect of environmental enrichment on amphetamine-stimulated locomotor activity, dopamine synthesis and dopamine release. Neuropharmacology 32:885-893.

Bradshaw JL, Sheppard DM (2000) The neurodevelopmental frontostriatal disorders: evolutionary adaptiveness and anomalous lateralization. Brain Lang 73:297-320.

Bresink I, Ebert B, Parsons CG, Mutschler E (1996) Zinc changes AMPA receptor properties: results of binding studies and patch clamp recordings. Neuropharmacology 35:503-509.

Brooks SP, Dunnett SB (2009) Tests to assess motor phenotype in mice: a user's guide. Nat Rev Neurosci 10:519-529.

Brown CE, Dyck RH (2002) Rapid, experience-dependent changes in levels of synaptic zinc in primary somatosensory cortex of the adult mouse. J Neurosci 22:2617-2625.

Brown CE, Dyck RH (2003) An improved method for visualizing the cell bodies of zincergic neurons. J Neurosci Methods 129:41-47.

Brown CE, Dyck RH (2004a) Distribution of zincergic neurons in the mouse forebrain. J Comp Neurol 479:156-167.

Brown CE, Dyck RH (2004b) Distribution of zincergic neurons in the mouse forebrain. J Comp Neurol 479:156-167.

Brown CE, Dyck RH (2005) Modulation of synaptic zinc in barrel cortex by whisker stimulation. Neuroscience 134:355-359.

Cahill L (2006) Why sex matters for neuroscience. Nat Rev Neurosci 7:477-484.

Cai Y, Liu S, Li N, Xu S, Zhang Y, Chan P (2009) Postnatal ontogenesis of molecular clock in mouse striatum. Brain Res 1264:33-38.

Carelli RM (2002) The nucleus accumbens and reward: Neurophysiological investigations in behaving animals. Beh Cog Neurosci Rev 1:281-296.

Carlezon Jr WA, Thomas MJ (2009) Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. Neuropharmacology 56, Supplement 1:122-132.

Castaneda TR, de Prado BM, Prieto D, Mora F (2004) Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. J Pineal Res 36:177-185.

Chasapis CT, Loutsidou AC, Spiliopoulou CA, Stefanidou ME (2012) Zinc and human health: an update. Archives of toxicology 86:521-534.

Chen CC, Gilmore A, Zuo Y (2014) Study motor skill learning by single-pellet reaching tasks in mice. Journal of visualized experiments : JoVE.

Chrusch M (2015) The role of synaptic zinc in experience-dependent plasticity. In: Neuroscience. Calgary: University of Calgary.

Cohen L, Asraf H, Sekler I, Hershfinkel M (2012) Extracellular pH regulates zinc signaling via an Asp residue of the zinc-sensing receptor (ZnR/GPR39). J Biol Chem 287:33339-33350.

Cole TB, Martyanova A, Palmiter RD (2001) Removing zinc from synaptic vesicles does not impair spatial learning, memory, or sensorimotor functions in the mouse. Brain Res 891:253-265.

Cole TB, Wenzel HJ, Kafer KE, Schwartzkroin PA, Palmiter RD (1999) Elimination of zinc from synaptic vesicles in the intact mouse brain by disruption of the ZnT3 gene. Proc Natl Acad Sci U S A 96:1716-1721.

Cole TB, Robbins CA, Wenzel HJ, Schwartzkroin PA, Palmiter RD (2000) Seizures and neuronal damage in mice lacking vesicular zinc. Epilepsy Res 39:153-169.

Comery TA, Shah R, Greenough WT (1995) Differential rearing alters spine density on medium-sized spiny neurons in the rat corpus striatum: Evidence for association of morphological plasticity with early response gene expression. Neurobiol Learn Mem 63:217-219.

Comery TA, Stamoudis CX, Irwin SA, Greenough WT (1996) Increased density of multiple-head dendritic spines on medium-sized spiny neurons of the striatum in rats reared in a complex environment. Neurobiol Learn Mem 66:93-96.

Costa RM (2007) Plastic corticostriatal circuits for action learning: what's dopamine got to do with it? Ann N Y Acad Sci 1104:172-191.

Dalley JW, Mar AC, Economidou D, Robbins TW (2008) Neurobehavioral mechanisms of impulsivity: Fronto-striatal systems and functional neurochemistry. Pharmacol Biochem Be 90:250-260.

Danscher G (1981) Histochemical demonstration of heavy metals. A revised version of the sulphide silver method suitable for both light and electronmicroscopy. Histochemistry 71:1-16.

Danscher G (1982) Exogenous selenium in the brain. A histochemical technique for light and electron microscopical localization of catalytic selenium bonds. Histochemistry 76:281-293.

Danscher G, Haug FM, Fredens K (1973) Effect of diethyldithiocarbamate (DEDTC) on sulphide silver stained boutons. Reversible blocking of Timm's sulphide silver stain for "heavy" metals in DEDTC treated rats (light microscopy). Exp Brain Res 16:521-532.

Daruna JH, Barnes, Patricia A. (1993) A neurodevelopmental view of impulsivity. Washington, DC, US: American Psychological Association.

Dayalu P, Albin RL (2015) Huntington disease: pathogenesis and treatment. Neurol Clin 33:101-114.

Dibner C, Schibler U, Albrecht U (2010) The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annual review of physiology 72:517-549.

Do J, Kim JI, Bakes J, Lee K, Kaang BK (2012) Functional roles of neurotransmitters and neuromodulators in the dorsal striatum. Learn Mem 20:21-28.

Doeppner TR, Kaltwasser B, Bähr M, Hermann DM (2014) Effects of neural progenitor cells on post-stroke neurological impairment—a detailed and comprehensive analysis of behavioral tests. Frontiers in cellular neuroscience 8:338.

Doya K (2000) Complementary roles of basal ganglia and cerebellum in learning and motor control. Curr Opin Neurobiol 10:732-739.

Dreixler JC, Leonard JP (1994) Subunit-specific enhancement of glutamate receptor responses by zinc. Brain Res Mol Brain Res 22:144-150.

Eibl JK, Abdallah Z, Ross GM (2010) Zinc-metallothionein: a potential mediator of antioxidant defence mechanisms in response to dopamine-induced stress. Can J Physiol Pharmacol 88:305-312.

Elliott R (2003) Executive functions and their disorders: Imaging in clinical neuroscience. Brit Med Bull 65:49-59.

Faherty CJ, Kerley D, Smeyne RJ (2003) A Golgi-Cox morphological analysis of neuronal changes induced by environmental enrichment. Developmental Brain Research 141:55-61.

Fahrni CJ, O'Halloran TV (1999) Aqueous coordination chemistry of quinoline-based fluorescence probes for the biological chemistry of zinc. J Am Chem Soc 121:11448-11458.

Ferreira TL, Moreira KM, Ikeda DC, Bueno OF, Oliveira MG (2003) Effects of dorsal striatum lesions in tone fear conditioning and contextual fear conditioning. Brain Res 987:17-24.

Ferreira TL, Shammah-Lagnado SJ, Bueno OF, Moreira KM, Fornari RV, Oliveira MG (2008) The indirect amygdala-dorsal striatum pathway mediates conditioned freezing: insights on emotional memory networks. Neuroscience 153:84-94.

Field E, Pellis S (2008) The Brain as the Engine of Sex Differences in the Organization of Movement in Rats. Arch Sex Behav 37:30-42.

Field EF, Whishaw IQ (2005) Sexually dimorphic postural adjustments are used in a skilled reaching task in the rat. Behav Brain Res 163:237-245.

Floresco SB (2015) The nucleus accumbens: an interface between cognition, emotion, and action. Annu Rev Psychol 66:25-52.

Fone KC, Porkess MV (2008) Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neurosci Biobehav Rev 32:1087-1102.

Foster TC, Dumas TC (2001) Mechanism for increased hippocampal synaptic strength following differential experience. J Neurophysiol 85:1377-1383.

Frederickson CJ, Koh JY, Bush AI (2005) The neurobiology of zinc in health and disease. Nat Rev Neurosci 6:449-462.

Frederickson CJ, Rampy BA, Reamy-Rampy S, Howell GA (1992) Distribution of histochemically reactive zinc in the forebrain of the rat. J Chem Neuroanat 5:521-530.

Frederickson CJ, Suh SW, Silva D, Frederickson CJ, Thompson RB (2000) Importance of zinc in the central nervous system: the zinc-containing neuron. J Nutr 130:1471s-1483s.

Freeze BS, Kravitz AV, Hammack N, Berke JD, Kreitzer AC (2013) Control of basal ganglia output by direct and indirect pathway projection neurons. J Neurosci 33:18531-18539.

Furtado JC, Mazurek MF (1996) Behavioral characterization of quinolinate-induced lesions of the medial striatum: relevance for Huntington's disease. Exp Neurol 138:158-168.

Gallardo CM, Darvas M, Oviatt M, Chang CH, Michalik M, Huddy TF, Meyer EE, Shuster SA, Aguayo A, Hill EM, Kiani K, Ikpeazu J, Martinez JS, Purpura M, Smit AN, Patton DF, Mistlberger RE, Palmiter RD, Steele AD (2014) Dopamine receptor 1 neurons in the dorsal striatum regulate food anticipatory circadian activity rhythms in mice. eLife 3.

Gardoni F, Bellone C (2015) Modulation of the glutamatergic transmission by Dopamine: a focus on Parkinson, Huntington and Addiction diseases. Frontiers in cellular neuroscience 9:25.

Gauthier MA, Eibl JK, Crispo JA, Ross GM (2008) Covalent arylation of metallothionein by oxidized dopamine products: a possible mechanism for zinc-mediated enhancement of dopaminergic neuron survival. Neurotox Res 14:317-328.

Gholamrezaei G, Whishaw IQ (2009) Individual differences in skilled reaching for food related to increased number of gestures: evidence for goal and habit learning of skilled reaching. Behav Neurosci 123:863-874.

Grabrucker S, Jannetti L, Eckert M, Gaub S, Chhabra R, Pfaender S, Mangus K, Reddy PP, Rankovic V, Schmeisser MJ, Kreutz MR, Ehret G, Boeckers TM, Grabrucker AM (2014) Zinc deficiency dysregulates the synaptic ProSAP/Shank scaffold and might contribute to autism spectrum disorders. Brain 137:137-152.

Graybiel AM (1984) Correspondence between the dopamine islands and striosomes of the mammalian striatum. Neuroscience 13:1157-1187.

Graybiel AM, Ragsdale CW, Jr. (1978) Histochemically distinct compartments in the striatum of human, monkeys, and cat demonstrated by acetylthiocholinesterase staining. Proc Natl Acad Sci U S A 75:5723-5726.

Graybiel AM, Chesselet MF (1984) Compartmental distribution of striatal cell bodies expressing [Met]enkephalin-like immunoreactivity. Proc Natl Acad Sci U S A 81:7980-7984.

Graybiel AM, Baughman RW, Eckenstein F (1986) Cholinergic neuropil of the striatum observes striosomal boundaries. Nature 323:625-627.

Haber SN (2014) The place of dopamine in the cortico-basal ganglia circuit. Neuroscience 282c:248-257.

Harbour VL, Weigl Y, Robinson B, Amir S (2013) Comprehensive mapping of regional expression of the clock protein PERIOD2 in rat forebrain across the 24-h day. PLoS ONE 8:e76391.

Hart G, Leung BK, Balleine BW (2013) Dorsal and ventral streams: The distinct role of striatal subregions in the acquisition and performance of goal-directed actions. Neurobiology of Learning and Memory.

Heien MLAV, Khan AS, Ariansen JL, Cheer JF, Phillips PEM, Wassum KM, Wightman RM (2005) Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. Proc Natl Acad Sci U S A 102:10023-10028.

Hering H, Sheng M (2001) Dendritic spines: structure, dynamics and regulation. Nat Rev Neurosci 2:880-888.

Hirano T, Kikuchi K, Urano Y, Nagano T (2002) Improvement and Biological Applications of Fluorescent Probes for Zinc, ZnAFs. J Am Chem Soc 124:6555-6562.

Holst B, Holliday ND, Bach A, Elling CE, Cox HM, Schwartz TW (2004) Common structural basis for constitutive activity of the ghrelin receptor family. J Biol Chem 279:53806-53817.

Holst B, Egerod KL, Schild E, Vickers SP, Cheetham S, Gerlach LO, Storjohann L, Stidsen CE, Jones R, Beck-Sickinger AG, Schwartz TW (2007) GPR39 signaling is stimulated by zinc ions but not by obestatin. Endocrinology 148:13-20.

Hood S, Cassidy P, Cossette MP, Weigl Y, Verwey M, Robinson B, Stewart J, Amir S (2010) Endogenous dopamine regulates the rhythm of expression of the clock protein PER2 in the rat dorsal striatum via daily activation of D2 dopamine receptors. J Neurosci 30:14046-14058.

Hornykiewicz O (1992) Mechanisms of neuronal loss in Parkinson's disease: a neuroanatomical-biochemical perspective. Clin Neurol Neurosurg 94 Suppl:S9-11.

Huang YZ, Pan E, Xiong ZQ, McNamara JO (2008) Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. Neuron 57:546-558.

Hussain S, Ali SF (2002) Zinc potentiates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced dopamine depletion in caudate nucleus of mice brain. Neurosci Lett 335:25-28.

Hwang JJ, Park MH, Choi SY, Koh JY (2005) Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. J Biol Chem 280:11995-12001.

Iijima M, Nikaido T, Akiyama M, Moriya T, Shibata S (2002) Methamphetamine-induced, suprachiasmatic nucleus-independent circadian rhythms of activity and mPer gene expression in the striatum of the mouse. Eur J Neurosci 16:921-929.

Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain research reviews 31:6-41.

Imbesi M, Yildiz S, Dirim Arslan A, Sharma R, Manev H, Uz T (2009) Dopamine receptor-mediated regulation of neuronal "clock" gene expression. Neuroscience 158:537-544.

Ingham CA, Hood SH, Arbuthnott GW (1989) Spine density on neostriatal neurones changes with 6-hydroxydopamine lesions and with age. Brain Res 503:334-338.

Irvine GI, Logan B, Eckert M, Abraham WC (2006) Enriched environment exposure regulates excitability, synaptic transmission, and LTP in the dentate gyrus of freely moving rats. Hippocampus 16:149-160.

Jadavji NM, Metz GA (2008) Sex differences in skilled movement in response to restraint stress and recovery from stress. Behav Brain Res 195:251-259.

Kempermann G, Kuhn HG, Gage FH (1997) More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493-495.

Kishioka A, Uemura T, Fukushima F, Mishina M (2013) Consolidation of auditory fear memories formed by weak unconditioned stimuli requires NMDA receptor activation and de novo protein synthesis in the striatum. Mol Brain 6:17.

Ko CH, Takahashi JS (2006) Molecular components of the mammalian circadian clock. Hum Mol Genet 15:R271-R277.

Koizumi S, Ikeda M, Inoue K, Nakazawa K, Inoue K (1995) Enhancement by zinc of ATP-evoked dopamine release from rat pheochromocytoma PC12 cells. Brain Res 673:75-82.

Kolb B, Gorny G, Söderpalm AHV, Robinson TE (2003) Environmental complexity has different effects on the structure of neurons in the prefrontal cortex versus the parietal cortex or nucleus accumbens. Synapse 48:149-153.

Kononen J, Koistinaho J, Alho H (1990) Circadian rhythm in c-fos-like immunoreactivity in the rat brain. Neurosci Lett 120:105-108.

Kreitzer AC, Malenka RC (2008) Striatal plasticity and basal ganglia circuit function. Neuron 60:543-554.

Lee JY, Son HJ, Choi JH, Cho E, Kim J, Chung SJ, Hwang O, Koh JY (2009) Cytosolic labile zinc accumulation in degenerating dopaminergic neurons of mouse brain after MPTP treatment. Brain Res 1286:208-214.

Lee K-W, Kim Y, Kim AM, Helmin K, Nairn AC, Greengard P (2006) Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. Proc Natl Acad Sci U S A 103:3399-3404.

Leggio MG, Mandolesi L, Federico F, Spirito F, Ricci B, Gelfo F, Petrosini L (2005) Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. Behav Brain Res 163:78-90.

Lerner TN, Kreitzer AC (2011) Neuromodulatory control of striatal plasticity and behavior. Curr Opin Neurobiol 21:322-327.

Lewis MH (2004) Environmental complexity and central nervous system development and function. Ment Retard Dev Disabil Res Rev 10:91-95.

Li Y, Kolb B, Robinson TE (2003) The location of persistent amphetamine-induced changes in the density of dendritic spines on medium spiny neurons in the nucleus accumbens and caudate-putamen. Neuropsychopharmacology 28:1082-1085.

Liljeholm M, O'Doherty JP (2012) Contributions of the striatum to learning, motivation, and performance: an associative account. Trends Cogn Sci 16:467-475.

Lim NC, Freake HC, Brückner C (2005) Illuminating Zinc in Biological Systems. Chem-Eur J 11:38-49.

Lin AM (2001) Coexistence of zinc and iron augmented oxidative injuries in the nigrostriatal dopaminergic system of SD rats. Free Radic Biol Med 30:225-231.

Lin DD, Cohen AS, Coulter DA (2001) Zinc-induced augmentation of excitatory synaptic currents and glutamate receptor responses in hippocampal CA3 neurons. J Neurophysiol 85:1185-1196.

Linden AM, Sandu C, Aller MI, Vekovischeva OY, Rosenberg PH, Wisden W, Korpi ER (2007) TASK-3 knockout mice exhibit exaggerated nocturnal activity, impairments in cognitive functions, and reduced sensitivity to inhalation anesthetics. J Pharmacol Exp Ther 323:924-934.

Liu Y, Teeter MM, DuRand CJ, Neve KA (2006) Identification of a Zn2+-binding site on the dopamine D2 receptor. Biochem Biophys Res Commun 339:873-879.

Liu Y, Wong TP, Aarts M, Rooyakkers A, Liu L, Lai TW, Wu DC, Lu J, Tymianski M, Craig AM, Wang YT (2007) NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. J Neurosci 27:2846-2857.

Lo HS, Chiang HC, Lin AM, Chiang HY, Chu YC, Kao LS (2004) Synergistic effects of dopamine and Zn2+ on the induction of PC12 cell death and dopamine depletion in the striatum: possible implication in the pathogenesis of Parkinson's disease. Neurobiol Dis 17:54-61.

Luong TN, Carlisle HJ, Southwell A, Patterson PH (2011) Assessment of motor balance and coordination in mice using the balance beam. Journal of visualized experiments : JoVE.

Lynch WJ, Girgenti MJ, Breslin FJ, Newton SS, Taylor JR (2008) Gene profiling the response to repeated cocaine self-administration in dorsal striatum: a focus on circadian genes. Brain Res 1213:166-177.

Lynch WJ, Nicholson KL, Dance ME, Morgan RW, Foley PL (2010) Animal Models of Substance Abuse and Addiction: Implications for Science, Animal Welfare, and Society. Comp Med 60:177-188.

Maloney SE, Rieger MA, Dougherty JD (2013) Identifying essential cell types and circuits in autism spectrum disorders. Int Rev Neurobiol 113:61-96.

Marques JM, Olsson IA (2010) Performance of juvenile mice in a reach-to-grasp task. J Neurosci Methods 193:82-85.

Martel G, Hevi C, Kane-Goldsmith N, Shumyatsky GP (2011) Zinc transporter ZnT3 is involved in memory dependent on the hippocampus and perirhinal cortex. Behav Brain Res 223:233-238.

Martel G, Hevi C, Friebely O, Baybutt T, Shumyatsky GP (2010) Zinc transporter 3 is involved in learned fear and extinction, but not in innate fear. Learn Mem 17:582-590.

Massey PV, Johnson BE, Moult PR, Auberson YP, Brown MW, Molnar E, Collingridge GL, Bashir ZI (2004) Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. J Neurosci 24:7821-7828.

Matamales M, Bertran-Gonzalez J, Salomon L, Degos B, Deniau JM, Valjent E, Herve D, Girault JA (2009) Striatal medium-sized spiny neurons: identification by nuclear staining and study of neuronal subpopulations in BAC transgenic mice. PLoS ONE 4:e4770.

Matsuura K, Kabuto H, Makino H, Ogawa N (1997) Pole test is a useful method for evaluating the mouse movement disorder caused by striatal dopamine depletion. J Neurosci Methods 73:45-48.

McAllister AK, Katz LC, Lo DC (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. Neuron 18:767-778.

McDonald CG, Dailey VK, Bergstrom HC, Wheeler TL, Eppolito AK, Smith LN, Smith RF (2005) Periadolescent nicotine administration produces enduring changes in dendritic morphology of medium spiny neurons from nucleus accumbens. Neurosci Lett 385:163-167.

McDonald RJ, White NM (1994) Parallel information processing in the water maze: evidence for independent memory systems involving dorsal striatum and hippocampus. Behav Neural Biol 61:260-270.

McGeorge AJ, Faull RL (1989) The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 29:503-537.

McNeill TH, Brown SA, Rafols JA, Shoulson I (1988) Atrophy of medium spiny I striatal dendrites in advanced Parkinson's disease. Brain Res 455:148-152.

Mengual E, Casanovas-Aguilar C, Perez-Clausell J, Gimenez-Amaya JM (1995) Heterogeneous and compartmental distribution of zinc in the striatum and globus pallidus of the rat. Neuroscience 66:523-537.

Metz GA, Whishaw IQ (2009) The Ladder Rung Walking Task: A Scoring System and its Practical Application. Journal of visualized experiments : JoVE:1204.

Middleton FA, Strick PL (2000) Basal ganglia and cerebellar loops: motor and cognitive circuits. Brain research reviews 31:236-250.

Mistlberger RE (1994) Circadian food-anticipatory activity: formal models and physiological mechanisms. Neurosci Biobehav Rev 18:171-195.

Mistlberger RE (2011) Neurobiology of food anticipatory circadian rhythms. Physiol Behav 104:535-545.

Muller A, Kleinau G, Piechowski CL, Muller TD, Finan B, Pratzka J, Gruters A, Krude H, Tschop M, Biebermann H (2013) G-protein coupled receptor 83 (GPR83) signaling determined by constitutive and zinc(II)-induced activity. PLoS ONE 8:e53347.

Nakashima AS, Dyck RH (2008) Enhanced plasticity in zincergic, cortical circuits after exposure to enriched environments. J Neurosci 28:13995-13999.

Nakashima AS, Dyck RH (2009) Zinc and cortical plasticity. Brain research reviews 59:347-373.

Nakashima AS, Dyck RH (2010) Dynamic, experience-dependent modulation of synaptic zinc within the excitatory synapses of the mouse barrel cortex. Neuroscience 170:1015-1019.

Nakashima AS, Butt RH, Dyck RH (2011) Alterations in protein and gene expression within the barrel cortices of ZnT3 knockout mice: experience-independent and dependent changes. Neurochem Int 59:860-870.

Nelson AB, Kreitzer AC (2014) Reassessing models of basal ganglia function and dysfunction. Annu Rev Neurosci 37:117-135.

Norregaard L, Frederiksen D, Nielsen EO, Gether U (1998) Delineation of an endogenous zinc-binding site in the human dopamine transporter. Embo J 17:4266-4273.

Ogura T, Ogata M, Akita H, Jitsuki S, Akiba L, Noda K, Hoka S, Saji M (2005) Impaired acquisition of skilled behavior in rotarod task by moderate depletion of striatal dopamine in a pre-symptomatic stage model of Parkinson's disease. Neurosci Res 51:299-308.

Packard MG, Knowlton BJ (2002) Learning and memory functions of the basal ganglia. Annu Rev Neurosci 25:563-593.

Paoletti P, Neyton J (2007) NMDA receptor subunits: function and pharmacology. Curr Opin Pharmacol 7:39-47.

Pappas SS, Leventhal DK, Albin RL, Dauer WT (2014) Mouse models of neurodevelopmental disease of the basal ganglia and associated circuits. Current topics in developmental biology 109:97-169.

Pattij T, Vanderschuren LJMJ (2008) The neuropharmacology of impulsive behaviour. Trends Pharmacol Sci 29:192-199.

Perez-Clausell J, Danscher G (1985) Intravesicular localization of zinc in rat telencephalic boutons. A histochemical study. Brain Res 337:91-98.

Rachline J, Perin-Dureau F, Le Goff A, Neyton J, Paoletti P (2005) The micromolar zinc-binding domain on the NMDA receptor subunit NR2B. J Neurosci 25:308-317.

Richfield EK (1993) Zinc modulation of drug binding, cocaine affinity states, and dopamine uptake on the dopamine uptake complex. Mol Pharmacol 43:100-108.

Robbins TW (2002) The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology (Berl) 163:362-380.

Robinson TE, Kolb B (1997) Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. J Neurosci 17:8491-8497.

Robinson TE, Kolb B (1999a) Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. Synapse 33:160-162.

Robinson TE, Kolb B (1999b) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11:1598-1604.

Robinson TE, Gorny G, Mitton E, Kolb B (2001) Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. Synapse 39:257-266.

Rojas P, Franco-Perez JE, Rojas C, Rojas-Castaneda J, Ebadi M, Fernandez-Valverde F, Serrano-Garcia N (2005) Reduction of zinc-positive terminal fields in striatum of mouse after 1-methyl-4-phenylpyridinium neurotoxicity. Neurotoxicology 26:959-968.

Sayyah M, Olapour A, Saeedabad Y, Yazdan Parast R, Malayeri A (2012) Evaluation of oral zinc sulfate effect on obsessive-compulsive disorder: a randomized placebo-controlled clinical trial. Nutrition 28:892-895.

Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST (2000) CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology 39:777-787.

Schetz JA, Sibley DR (1997) Zinc allosterically modulates antagonist binding to cloned D1 and D2 dopamine receptors. J Neurochem 68:1990-1997.

Schetz JA, Sibley DR (2001) The binding-site crevice of the D4 dopamine receptor is coupled to three distinct sites of allosteric modulation. J Pharmacol Exp Ther 296:359-363.

Schetz JA, Chu A, Sibley DR (1999) Zinc modulates antagonist interactions with D2-like dopamine receptors through distinct molecular mechanisms. J Pharmacol Exp Ther 289:956-964.

Sedelis M, Hofele K, Auburger GW, Morgan S, Huston JP, Schwarting RK (2000) MPTP susceptibility in the mouse: behavioral, neurochemical, and histological analysis of gender and strain differences. Behav Genet 30:171-182.

Segovia G, Del Arco A, De Blas M, Garrido P, Mora F (2010) Environmental enrichment increases the in vivo extracellular concentration of dopamine in the nucleus accumbens: a microdialysis study. J Neural Transm 117:1123-1130.

Sheline CT, Zhu J, Zhang W, Shi C, Cai AL (2013) Mitochondrial inhibitor models of Huntington's disease and Parkinson's disease induce zinc accumulation and are attenuated by inhibition of zinc neurotoxicity in vitro or in vivo. Neuro-degenerative diseases 11:49-58.

Shohag H, Ullah A, Qusar S, Rahman M, Hasnat A (2012) Alterations of serum zinc, copper, manganese, iron, calcium, and magnesium concentrations and the complexity of

interelement relations in patients with obsessive-compulsive disorder. Biol Trace Elem Res 148:275-280.

Silberberg G, Bolam JP (2015) Local and afferent synaptic pathways in the striatal microcircuitry. Curr Opin Neurobiol 33:182-187.

Sindreu C, Palmiter RD, Storm DR (2011) Zinc transporter ZnT-3 regulates presynaptic Erk1/2 signaling and hippocampus-dependent memory. Proc Natl Acad Sci U S A 108:3366-3370.

Sirevaag AM, Black JE, Shafron D, Greenough WT (1988) Direct evidence that complex experience increases capillary branching and surface area in visual cortex of young rats. Brain Res 471:299-304.

Slomianka L, Danscher G, Frederickson CJ (1990) Labeling of the neurons of origin of zinc-containing pathways by intraperitoneal injections of sodium selenite. Neuroscience 38:843-854.

Sorensen JC, Slomianka L, Christensen J, Zimmer J (1995) Zinc-containing telencephalic connections to the rat striatum: a combined Fluoro-Gold tracing and histochemical study. Exp Brain Res 105:370-382.

Spires TL, Grote HE, Garry S, Cordery PM, Van Dellen A, Blakemore C, Hannan AJ (2004) Dendritic spine pathology and deficits in experience-dependent dendritic plasticity in R6/1 Huntington's disease transgenic mice. Eur J Neurosci 19:2799-2807.

Stanley JL, Lincoln RJ, Brown TA, McDonald LM, Dawson GR, Reynolds DS (2005) The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. J Psychopharmacol 19:221-227.

Suh SW, Jo SM, Vajda Z, Danscher G (2005) Adrenalectomy-induced ZnT3 downregulation in mouse hippocampus is followed by vesicular zinc depletion. Neurosci Lett 377:164-169.

Takeda A, Tamano H, Tochigi M, Oku N (2005) Zinc homeostasis in the hippocampus of zinc-deficient young adult rats. Neurochem Int 46:221-225.

Tarohda T, Ishida Y, Kawai K, Yamamoto M, Amano R (2005) Regional distributions of manganese, iron, copper, and zinc in the brains of 6-hydroxydopamine-induced parkinsonian rats. Anal Bioanal Chem 383:224-234.

Tillerson JL, Miller GW (2003) Grid performance test to measure behavioral impairment in the MPTP-treated-mouse model of parkinsonism. J Neurosci Methods 123:189-200.

Travaglia A, La Mendola D, Magrì A, Pietropaolo A, Nicoletti VG, Grasso G, Malgieri G, Fattorusso R, Isernia C, Rizzarelli E (2013) Zinc(II) interactions with brain-derived neurotrophic factor N-terminal peptide fragments: Inorganic features and biological perspectives. Inorg Chem 52:11075-11083.

Trezza V, Baarendse PJJ, Vanderschuren LJMJ (2010) The pleasures of play: pharmacological insights into social reward mechanisms. Trends Pharmacol Sci 31:463-469.

Uz T, Akhisaroglu M, Ahmed R, Manev H (2003) The pineal gland is critical for circadian Period1 expression in the striatum and for circadian cocaine sensitization in mice. Neuropsychopharmacology 28:2117-2123.

van den Bos W, Rodriguez CA, Schweitzer JB, McClure SM (2015) Adolescent impatience decreases with increased frontostriatal connectivity. P Natl Acad Sci 112:E3765-E3774.

van Gaalen MM, van Koten R, Schoffelmeer ANM, Vanderschuren LJMJ (2006) Critical involvement of dopaminergic neurotransmission in impulsive decision making. Biol Psychiatry 60:66-73.

Venable N, Pinto-Hamuy T, Arraztoa JA, Contador MT, Chellew A, Peran C, Valenzuela X (1988) Greater efficacy of preweaning than postweaning environmental enrichment on maze learning in adult rats. Behav Brain Res 31:89-92.

Wall MJ (2005) A role for zinc in cerebellar synaptic transmission? Cerebellum 4:224-229.

Whishaw IQ (1996) An endpoint, descriptive, and kinematic comparison of skilled reaching in mice (Mus musculus) with rats (Rattus norvegicus). Behav Brain Res 78:101-111.

Williams K (1996) Separating dual effects of zinc at recombinant N-methyl-D-aspartate receptors. Neurosci Lett 215:9-12.

Winstanley CA (2007) The orbitofrontal cortex, impulsivity, and addiction. Ann N Y Acad Sci 1121:639-655.

Winstanley CA, Eagle DM, Robbins TW (2006) Behavioral models of impulsivity in relation to ADHD: Translation between clinical and preclinical studies. Clin Psychol Rev 26:379-395.

Yager LM, Garcia AF, Wunsch AM, Ferguson SM (2015) The ins and outs of the striatum: Role in drug addiction. Neuroscience 301:529-541.

Yasuda S, Miyazaki T, Munechika K, Yamashita M, Ikeda Y, Kamizono A (2007) Isolation of Zn2+ as an endogenous agonist of GPR39 from fetal bovine serum. Journal of receptor and signal transduction research 27:235-246.

Yin HH, Ostlund SB, Knowlton BJ, Balleine BW (2005) The role of the dorsomedial striatum in instrumental conditioning. Eur J Neurosci 22:513-523.

Zaja-Milatovic S, Milatovic D, Schantz AM, Zhang J, Montine KS, Samii A, Deutch AY, Montine TJ (2005) Dendritic degeneration in neostriatal medium spiny neurons in Parkinson disease. Neurology 64:545-547.

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