Behavioural Changes in Bilateral Vestibular Deafferented Rats and the Effects of D_2 Dopamine Receptor Antagonism

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Abstract

Increased locomotor activities as well as circling behaviours in animals with bilateral vestibular loss are well documented in the literature. However, the cause of these behavioural changes are still unknown. Dysfunction of the striatal dopaminergic system is responsible for a number of known movement disorders. The D_2 dopamine receptor is known to be involved in the regulation of behaviour. The aim of this study was to investigate the effects of the D_2 antagonist, eticlopride, in rats 2 months following bilateral vestibular deafferentation surgery, using an open field maze to test locomotor behaviours and the 5-choice serial reaction time task to measure impulsivity. The levels of the D_2 receptor in the striatum and frontal cortex were then measured using western blotting. BVD rats were found to show behaviours already reported in animals with vestibular loss. Treatment with eticlopride was found not to inhibit these behaviours. There were no changes in the amount of the D_2 receptor in the striatum or frontal cortex at one or six months post surgery. The main effect of D_2 receptor inhibition in this study was a decreased response to the drug in BVD rats compared to shams at the 0.02 mg/kg dose. Blockade of the D_2 receptor did not inhibit the cause of the behavioural changes in BVD rats. However, the drug did produce a surgery dependent effect. This suggests that while these behaviours are not due to the D_2 receptor, there is a change in the dopaminergic pathways in BVD rats.

Table of Contents

Acknowledgements I

Abstract..... III

Table of Contents..... IV

List of Figures and Tables VI

List of Abbreviations VII

CHAPTER 1: INTRODUCTION 1

1.1 The Vestibular System 2

1.2 Symptoms of Bilateral Vestibular Loss in Humans 3

1.3 Animal Modelling of Vestibular Dysfunction: Bilateral Vestibular Deafferentation (BVD) 5

1.4 Symptoms of Bilateral Vestibular Loss in Rodent Models 5

1.4 The Striatal Vestibular Pathway 8

1.6 Dopamine in the Striatum and Changes in the Dopaminergic System Following Vestibulopathy 11

1.7 Effects of the D_2 Dopamine Receptor on Behaviours Related to Vestibular Dysfunction 14

1.8 Aims 15

CHAPTER 2: METHODS 16

2.1 Animals 17

2.2 Bilateral Vestibular Deafferentation (BVD) and Sham Surgery 17

2.2.1 Confirmation of Bilateral Vestibular Deafferentation 18

2.3 Drug 18

2.4 Behavioural Assessment: Open Field Maze 20

2.4.1 Equipment 20

2.4.2 Procedure 20

2.4.3 Measured Variables 21

2.5 Behavioural Assessment: Five-Choice Serial Reaction Time Task (5-CSRTT) 21
2.5.1 Apparatus 21
2.5.2 Behavioural Task 23
2.5.3 Drug Testing 25
2.5.4 Performance measures 25

2.6 Brain Removal and Tissue Preparation 26
2.6.1 Tissue Collection 26
2.6.2 Tissue Preparation 27

2.7 Western Blotting 28
2.7.1 Antibodies 28
2.7.2 Protocol 28

2.8 Statistical Analysis 29

CHAPTER 3: RESULTS 31

3.1 Open Field Maze 32

3.2 5-Choice Serial Reaction Time Task 41
3.2.1 5 second ITI 41
3.2.2 Variable ITI 44

3.3 Western Blotting 50

CHAPTER 4: DISCUSSION 54

4.1 Behavioural Changes Following BVD Surgery 55

4.2 D_2 Dopamine Antagonism in BVD Rats 57

4.3 Neurochemical Measures 59

4.4 Overall conclusions 61

REFERENCES 65

List of Figures and Tables

Figures

1.1: The Vestibular Labyrinth……..…..2

1.2: The basic anatomy of the basal ganglia…….....…8

1.3: Diagram of the Direct and Indirect pathways from the striatum to the output nuclei…….....……12

2.1: Latin square design for eticlopride drug treatment…….....19

2.2: Diagram of the 5-CSRTT chamber……...……...…..22

3.1: Total distance travelled per minute in the open field maze……..……....…33

3.2: Number of rotations per minute in the open field maze……..……....….34

3.3: Velocity of movement per minute in the open field maze…..……...……..35

3.4: Duration of movement per minute in the open field maze…...……...…….36

3.5: Duration of mobility per minute in the open field maze……...……...…….37

3.6: Frequency of mobility per minute in the open field maze……...……...……..38

3.7: Amount of time spent in the inner, middle or outer zone per minute in the open field maze….....39

3.8: Percentage of correct responses recorded in variable ITI 5-CSRTT……..……..45

3.9: Percentage of omissions recorded in variable ITI 5-CSRTT……..……..46

3.10: Number of premature responses recorded in variable ITI 5-CSRTT……..……47

3.11: Total number of perseverative responses recorded in all periods of variable ITI 5-CSRTT….....48

3.12: Latency to collect reward recorded in all periods of variable ITI 5-CSRTT.....48

3.13: Latency to make an incorrect response recorded in all periods of variable ITI 5-CSRTT….....49

3.14: Latency to make a correct response recorded in all periods of variable ITI 5-CSRT….49

3.15: Changes seen in the amount of the D_2 dopamine receptor isoforms following BVD or sham surgery at 1 month and 6 months post surgery in the striatum ……...52

3.16: Changes seen in the amount of the D_2 dopamine receptor isoforms following BVD or sham surgery at 1 month and 6 months post surgery in the frontal cortex……...53

Table

3.1 Effect of eticlopride on 5 Second ITI 5CSRTT …….....…….43

List of Abbreviations

5- CSRTT – Five Choice Serial Reaction Time Task

6-OH-DA - 6-Hydroxydopamine

BSA- Bovine Serum Albumin

BVD- Bilateral Vestibular Deafferentation

eGP- External Globus Palidus

HRP- Horseradish Peroxidise

GABA-γ-aminobutyric acid

iGP- Internal Globus Palidus

ITI- Inter Trial Interval

LMM- Linear Mixed Model

kDa- Kilodaltons

MRI- Magnetic Resonance Imaging

SDS- Sodium Dodecyl Sulfate

SEM- Standard Error of the Mean

SN - Substantia Nigra.

Veh- Vehicle

VOR- Vestibuloocular Reflex

VSR- Vestibulospinal Reflex

VNC- Vestibular Nucleus Complex

Chapter 1: Introduction

1.1 The Vestibular System

The vestibular system is a three dimensional sensory system that plays a vital role in the processing of the sensory information pertaining to the location and locomotion of an animal in space (Buttener-Ennever, 1992). Versions of this system are found even in complex organisms that have simplistic nervous systems, including crustaceans (Sandeman *et al.*, 1972) and jelly fish (Singla, 1975). The peripheral mammalian vestibular system is made up of two labyrinths, each of which contains three semicircular canals and the two otolith organs: the saccule and the utricle (see Figure 1.1). This system is able to detect head acceleration via receptor hair cells in the semicircular canals (angular acceleration) and in the otoliths (linear acceleration). The system initiates compensatory eye and limb movements in response to head movements, and functions to maintain posture under the influence of gravity.

Sensory information from the vestibular system leaves the labyrinths via the vestibular (VIIIth) nerve, which projects into the ipsilateral vestibular nuclei and the cerebellum (Carleton *et al.*, 1984). The vestibular nucleus complexes (VNCs) are two sets of bilaterally located subnuclei in the brainstem that integrate and distribute vestibular and other sensory information to other parts of the brain. They also relay information to the muscles responsible for the compensatory eye movements to maintain gaze stability in response to body movements, known as vestibulo-ocular reflexes (VORs).

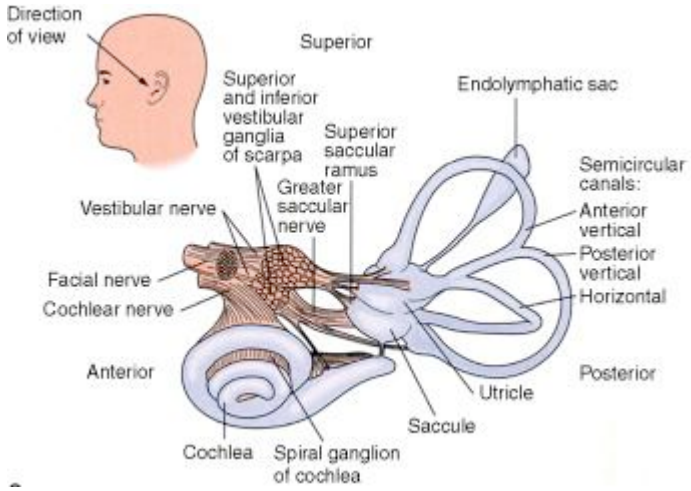


Figure 1.1: The Vestibular Labyrinth. Reproduced with from Glover (2004) with copyright permission from Elsevier

Neurons in different subnuclei of the VNC are responsible for relaying synaptic input from different parts of the labyrinth (Gacek *et al.*, 1974). The medial and superior vestibular nuclei receive input mainly from the horizontal and vertical semicircular canals (Gacek *et al.*, 1974). These neurons in the VNC then project to the ocular motor neurons and are therefore involved in the VORs (Ito *et al.*, 1976). The medial vestibular nucleus also projects to spinal motor neurons at the cervical and high thoracic levels and plays a role in the vestibulo-spinal reflexes (VSRs) which use vestibular information to control body posture. The lateral and descending vestibular nuclei receive input from the otolith organs, which are mainly involved in the vestibulo-spinal responses that generate compensatory movement of the limbs during body movement (Xerri *et al.*, 1988). Damage to any part of the vestibular pathways can result in a number of symptoms.

1.2 Symptoms of Bilateral Vestibular Loss in Humans

In humans, bilateral vestibular dysfunction can vary both in type and in severity. Bilateral vestibular loss can occur due to a number of causes, the most common of which are ototoxicity due to aminoglycoside antibiotics and Ménière's Disease (Rinne *et al.*, 1998; Zingler *et al.*, 2007). While in the acute stages of loss the symptoms can be severe, these symptoms lessen over time due to a process known as "vestibular compensation" (Precht *et al.*, 1966; Rinne *et al.*, 1998; Smith *et al.*, 1988). As a result of this compensation, if the vestibular damage develops slowly, then few symptoms may be experienced. However such slow vestibular damage is not common, and vestibular loss usually occurs suddenly and rapidly (Rinne *et al.*, 1998).

The most common symptoms of bilateral vestibular loss in humans occur due to the loss of the VORs and VSRs. The loss of VORs result in oscillopsia, where the perception of the

visual world is blurred due to the lack of eye movement compensation for head movements (Ito *et al.*, 1976). The loss of the VSRs can result in gait ataxia, where there is disruption of normal walking due to postural imbalance. A 1995 study found that many common complaints from patients with bilateral vestibulopathy include a sense of abnormal movement, dizziness and vertigo (Krebs *et al.*, 1995). Patients have also been shown to have unsteadiness while performing transient movements such as moving from sitting to standing, as well as walking tentatively, and at a relatively slow pace (Mamoto *et al.*, 2002).

While it could be assumed from the postural imbalance and gait dysfunction observed in patients with vestibular dysfunction, that slow rather than fast movements would result in more controlled movements following vestibular loss, it has been shown that fast movements such as running actually result in a stabilisation of balance and ability to stay on an intended path (Brandt, 2000; Brandt *et al.*, 1999). Brandt *et al* (1999) compared the differences in balance when running or walking in four patients (all women) with unilateral vestibular neuritis, a condition caused by inflammation of the vestibular nerve which results in decreased vestibular function. The patients were asked either to walk slowly or to run down a ten metre corridor with their eyes closed. While walking, the patients deviated significantly from their intended direction and often touched the wall for support. While running, however, the patients were able to stay on the intended path for greater than 10 metres and reported feeling steadier than while walking.

It has been hypothesised that the differences in the ease of movement between walking and running are due to the different requirements of vestibular input (Brandt, 2000) depending on which movement the subject is doing. While walking, the vestibular system is required for the maintenance of balance to a greater extent than when running. Jahn *et al*

(2004) identified, using functional MRI scanning, that running required the activation of fewer brain regions than walking, including those involved in navigation and balance. This suggests that running and fast movement involve responses which are independent of vestibular input, and therefore are easier for patients with vestibular deficits to perform. The deficits in humans with vestibular dysfunction are recreated in a number of animal models in order to study the changes in more detail.

1.3 Animal Modelling of Vestibular Dysfunction: Bilateral Vestibular Deafferentation (BVD)

For the purposes of vestibular research, a number of animal models of vestibular loss have been developed, the most common of which use rodents. Surgical bilateral deafferentation involves the complete inactivation of both vestibular labyrinths, resulting in a loss of the sensory hair cells in the labyrinth (Zheng *et al.*, 2006) and due to this a decrease in the information sent to the VNC . This method requires manually opening the semicircular canals and otolith organs, and removing the hair cells by using aspiration. As surgical labyrinthectomy is performed under microscopic control, this method eliminates the risk of an incomplete deafferentation, or of damaging the vestibular nerve and the brainstem. This model allows for the study of the changes seen in sudden onset vestibular loss.

1.4 Symptoms of Bilateral Vestibular Loss in Rodent Models

Similar to human symptoms, changes in locomotor behaviour following bilateral vestibular loss are also seen in rats. Indeed, the most notable behavioural symptoms following bilateral vestibular deafferentation (BVD) in animals involve changes in locomotion patterns (Goddard *et al.*, 2008). Symptoms observed immediately following BVD surgery include gait

ataxia, hyperactivity, head weaving and circling, as well as a lack of a righting reflex (Goddard *et al.*, 2008; Russell *et al.*, 2003; Zheng *et al.*, 2006). These symptoms are similar to those seen in animals that have undergone chemical labyrinthectomy as well as in vestibular deficient genetic models (Alleva *et al.*, 1978; Fedrowitz *et al.*, 2003; Kaiser *et al.*, 2001; Seth *et al.*, 1982). Due to vestibular compensation some lesion symptoms, including head weaving and defective righting reflex, either decrease in severity or completely disappear over time (Goddard *et al.*, 2008). However, locomotor hyperactivity and circling behaviours have been observed in BVD animals immediately on the recovery of mobility post-surgery (Goddard *et al.*, 2008), and in rats up to 14 months following deafferentation surgery, indicating that they are probably permanent (Baek *et al.*, 2010).

While in humans the loss of bilateral vestibular function results in a reduction in the velocity of movement, with patients moving more slowly than healthy subjects (Mamoto *et al.*, 2002), the opposite is seen in rodents. Hyperactivity in BVD rats has been observed at 3 weeks, 3 months, 5 months (Goddard *et al.*, 2008) and as long as 14 months post-surgery (Baek *et al.*, 2010). In a study involving rats with streptomycin-induced bilateral vestibular loss, rats treated with the drug travelled 45% further than the untreated controls during a 10 minute open field trial (Basile *et al.*, 1999). While it is still unknown why there is a difference in the behaviours seen in humans and rodents it is possible that it is due to a conscious decision in humans to move more slowly even though it has been shown to be more difficult (Brandt, 2000; Brandt *et al.*, 1999), while rodents move more on instinct and therefore more quickly.

Bidirectional circling is another locomotor behaviour shown consistently in all animal models of bilateral vestibular loss (Basile *et al.*, 1999; Cryns *et al.*, 2004; Fedrowitz *et al.*,

2003; Fedrowitz *et al.*, 2000; Goddard *et al.*, 2008; Ishiguro *et al.*, 2007; Russell *et al.*, 2003; Vidal *et al.*, 2004; Zheng *et al.*, 2006). $Isk^{-/-}$ mutant mice have a null mutation of the IsK gene (Vetter *et al.*, 1996) and are born with functioning vestibular labyrinths that degrade over time but have functioning central vestibular pathways. The loss of function is due to a reduction of transepithelial potassium secretions in the inner ear which causes degradation of the hair cells and transitional epithelium (Nicolas *et al.*, 2001). These mice show turning in either direction randomly, and have been shown to have a mean angular velocity of 780° s^{-1} over five consecutive 360° turns (Vidal *et al.*, 2004). Circling behaviour like this is often seen in animals with striatal dysfunction (Schwarting *et al.*, 1996) and it is possible that a loss of vestibular input to the brain could be responsible for changes in the striatum, and therefore for the development of the symptoms.

Some early studies investigating behavioural changes in rats with vestibular loss were carried out using genetic models. One such study concluded that differences in the response of vestibular deficient Stargazer rats and their unaffected littermates to the D_2 receptor antagonist haloperidol, were due solely to genetic changes to the central dopaminergic system, and were unrelated to the vestibular disorder (Brock *et al.*, 1996). However, this does not take into account that changes in the dopaminergic system between the two groups may be a secondary effect of the vestibular dysfunction.

Some research into the effects of altered vestibular function using genetic models, as well as chemical deafferentation, have shown results that suggest that vestibular function must be lost early in development in order to result in behavioural changes attributed to the basal ganglia (Alleva *et al.*, 1978; Kaiser *et al.*, 2001). Following chronic treatment with streptomycin in rats, it was found that the animals displayed only the behavioural effects

typical of vestibular loss, including locomotor hyperactivity and circling, when treated from 2 to 22 days of age (Alleva *et al.*, 1978), but not when older weaned rats were treated (Vernier *et al.*, 1968). On comparing the behaviours seen in the chemically deafferented rats with those seen in circling *ci2/ci2* mutant rats, animals that have autosomal recessive mutations in the circling (*Ci*) gene exhibit intense asymmetric circling (Richter *et al.*, 1999), Kaiser *et al* (2001) suggested the possibility that changes in behaviour are due to secondary changes in the basal ganglia during development resulting from a lack of vestibular input. However, none of these results accounts for the sudden onset of locomotor behaviours at the completion of surgical deafferentation that have been observed (Baek *et al.*, 2010; Goddard *et al.*, 2008).

1.4 The Striatal Vestibular Pathway

It has been documented (Muskens, 1914) that animals with lesions to the vestibular system show behavioural changes similar to those seen in animals with striatal lesions. It is therefore possible that changes in vestibular function result in changes in the activity of the striatum due to connecting pathways.

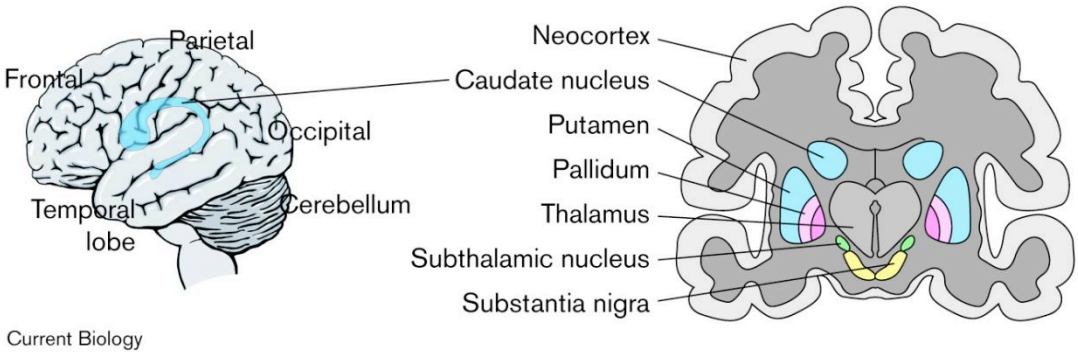


Figure 1.2: The basic anatomy of the basal ganglia. Reproduced with from Day et al., (2005) with copyright permission from Elsevier

The striatum is made up of two regions, the caudate nucleus and the putamen, which are the major input stations of the basal ganglia (see Figure 1.2). The caudate nucleus and the putamen are responsible for the initiation and the modulation of voluntary movement, as well as for movement that is performed at a subconscious level (Hassler, 1978; Patestas *et al.*, 2006).

In 1922, Muskens conducted a number of experiments in which a pathway was identified between the striatum and the VNC. Following the unilateral lesion of the VNC in cats via the manipulation of a needle, there was degeneration of the neuron tracts from the VNC to the putamen of the striatum, as well as degradation of the neurons in the globus pallidus, a region of the basal ganglia that receives information from the striatum (Muskens, 1922). It was hypothesised that this pathway might be directed through the thalamus, as lesions to all of these regions, the VNC, striatum and thalamus, result in symptoms characteristic of vestibular damage, including circling movements and hyperactivity (Muskens, 1922).

Despite Muskens' hypothesis, the presence of a direct vestibulo-thalamo-striatal pathway was not confirmed until a number of decades later, as until this point, other researchers believed that vestibular input to the striatum travelled via the thalamus and the cortex. Potegal *et al* (1971) unilaterally stimulated the vestibular nerve branches in the inner ear of anaesthetised cats, which resulted in a bilateral response recorded in the head of the caudate nucleus. This result was produced even following the inactivation of the vestibular projections to the cortex, and thus suggested that vestibular information received by the thalamus was not all directed through the cortex to the striatum as previously thought. Instead it appeared that vestibular information received by the thalamus in part went directly to the striatum.

The vestibulo-thalamo-striatal pathway was shown definitively in rats following labelling of neurons in the three regions: the VNC, the thalamus and the striatum (Lai *et al.*, 2000). Using anterograde and retrograde labelling techniques in rats, Lai *et al* (2000) demonstrated a neuronal pathway from the medial vestibular nucleus to the parafasicular nucleus of the thalamus, and then on to the dorsolateral striatum. When biotinylated dextranamine was injected into the medial vestibular nucleus, fifty percent of labelled neurons were found to make contact with neurons in the striatum that were labelled with Cholera toxin B (Lai *et al.*, 2000). However, it is still to be determined which neuron populations in the striatum are receiving the vestibular input.

There is evidence to support the notion that the striatal vestibular pathway is present not only in rats but in humans. Following cold water caloric vestibular stimulation in 6 healthy patients, there was an increase in activity in the putamen as seen by increased blood flow in PET scans (Bottini *et al.*, 1994). Bottini *et al* (1994) hypothesised that the putamen, together with the vestibular system, has a role in directing movement in space. By showing that information from the vestibular system can have an effect in the striatum in healthy patients, it can be assumed that the loss of vestibular input would also have an effect and could therefore explain the locomotor behaviours seen in rodents following vestibular loss.

The striatum is considered to be the major input site for the basal ganglia, which is the region of the brain responsible for a number of psychomotor behaviours. The most common example of striatal changes producing symptoms like those seen in vestibular deficient animals is seen in the extensive use of 6-hydroxydopamine (6-OH-DA) in the study of behavioural function controlled by the basal ganglia. Injections of 6-OH-DA into the substantia nigra and ventral tegmental area result in cell death in multiple regions of the brain

(Blum *et al.*, 2001; Kostrzewa *et al.*, 1974), of which the striatum has been found to be the region that is most affected (Schwarting *et al.*, 1996). After entering neurons via dopamine reuptake transporters, 6-OH-DA then causes damage via interruption of the mitochondrial electron transport chain as well as the creation of reactive oxygen species (Blum *et al.*, 2001; Kostrzewa *et al.*, 1974). Animals with 6-OH-DA lesions are found to display circling behaviour, which can be activated by the handling of the animal. Research has shown that asymmetric turning is usually in the direction of the lesioned side, and requires significant depletion of striatal dopamine to occur (Schwarting *et al.*, 1996).

1.6 Dopamine in the Striatum and Changes in the Dopaminergic System Following Vestibulopathy

Dopamine is a monoamine neurotransmitter produced by neurons in the substantia nigra and the ventral tegmental area. Dopamine neurons project down two pathways: from the ventral tegmental area to the prefrontal cortex and also the nucleus accumbens and from the substantia nigra to the striatum. The prefrontal cortex is separate from the striatum in both location and function and is partly responsible for cognitive control in the brain (Koechlin *et al.*, 2003; Miller *et al.*, 2001). In vertebrates there are 5 recognised dopamine receptors, which are divided into two classes named after the two main types of dopamine receptor. The D_1-like class includes the D_1 and D_5 receptors, which are G_s G-protein coupled receptors, while the D_2-like class is made up of the D_2, D_3 and D_4 receptors that are G_i G-protein coupled receptors (Reviewed by Missale *et al.*, 1998). Separate from the other proteins in the D_2-like class of receptors, the D_2 dopamine receptor has been shown to have two isoforms due to differences in the splicing of RNA (Giros *et al.*, 1989; Monsma *et al.*, 1989). The two forms of the protein are designated the long form (D_2L) and the short form (D_2S) due to the difference in the number of amino acids in the proteins, with the isoforms having 444 and 415

amino acids, respectively (Giros *et al.*, 1989; Monsma *et al.*, 1989). In the striatal regions the D_1 and D_2 receptors are the receptors found in the highest concentrations (Missale *et al.*, 1998), and are therefore the most commonly studied.

Dopamine is responsible for the control of one of the main circuits in the striatum, the motor loop. The motor loop is divided into two sub-loops: the direct loop, which is excitatory, and the indirect loop (see Figure 1.3), which is inhibitory (Alexander *et al.*, 1990; Alexander *et al.*, 1986; Delong, 2000). Both pathways begin and end in the cortex as well as sending outputs to the spinal cord. The direct loop relays signals from the cortex to the putamen and then to the internal globus pallidus, where signals are redirected to the spinal cord as well as the thalamus, which then loops back to the motor cortex. The indirect loop sends signals from the motor cortex through the putamen to the external globus pallidus, then to the internal globus pallidus and finally out through the thalamus and the spinal cord (Alexander *et al.*, 1990). Both the direct loop and the indirect loop can be influenced by dopamine released from the substantia nigra (Alexander *et al.*, 1990), with the direct loop neurons of the putamen containing a high number of D_1 receptors (Delong, 2000), while the indirect loop neurons of the putamen contains more D_2 receptors (Delong, 2000).

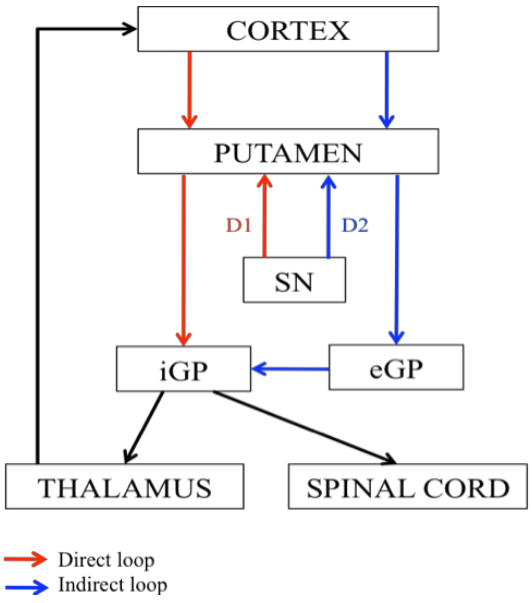


Figure 1.3: Diagram of the Direct and Indirect pathways from the striatum (putamen) to the output nuclei. iGP, internal globus palidus; eGP, external globus pallidus; SN, substantia nigra. Adapted from Alexander et al., 1990, Alexander et al., 1986 and Delong, 2000

Disruption of the motor circuit can provide an explanation of a large number of movement disorders. Normal motor behaviours require a critical balance between the direct and indirect loops (Alexander *et al.*, 1990; Delong, 2000). A decrease in substantia nigra function results in an increase in the activity of the indirect pathway, and results in the hypokinetic symptoms seen in illnesses like Parkinson's disease (Egan *et al.*, 1994). Conversely, a decrease in activity in the indirect pathway results in hyperkinetic symptoms such as those seen in Huntington's Chorea (Delong, 2000; Egan *et al.*, 1994). The effect that D_2 dopamine receptors have on the indirect loop suggests that there may be a connection between the D_2 receptors and some motor-related behavioural disorders. It is therefore possible that a change in vestibular signalling into the putamen would result in a change in the signalling pathway of the motor loop. If this change favours one pathway over the other then the imbalance formed could result in the development of the motor behaviours seen in BVD rats.

Research involving animals with disrupted vestibular function, either from genetic disorders or from chemical or surgical procedures, has demonstrated that the loss of vestibular function may affect the expression of dopamine receptors in the striatum (Schirmer *et al.*, 2007a; Seth *et al.*, 1982). This is important because Richter *et al* (1999) found that in *ci2/ci2* rats there are no morphological changes in the striatum following vestibular deafferentation, and therefore suggested that the changes in locomotor behaviour seen in these animals must be due to changes in neurochemical functions. However, changes in receptor levels vary depending on the type of vestibular dysfunction the animal experiences. Rats treated with streptomycin have been shown to have significant increases in expression levels of the D_2 dopamine receptor protein in the striatum (Schirmer *et al.*, 2007a; Seth *et al.*, 1982) but show no change in expression levels of the D_1 receptor (Schirmer *et al.*, 2007a). In contrast, the

opposite has been observed in *ci2/ci2* rats, with an increase in the level of the D_1 receptor and no change in D_2 receptor levels (Richter *et al.*, 1999).

Only one dopamine-related study has been completed in rats following BVD surgery, and the results of this study add more confusion to the question of the effects of BVD on the striatum and dopamine receptor expression levels. Autoradiological analysis of the striatum showed a decrease in the levels of the D_2 dopamine receptor, and no change in D_1 receptor levels (Giardino *et al.*, 1996). These results suggest that there may be a compensatory mechanism active here as by decreasing D_2 receptor number there will be increased activation of the indirect loop, which might restore the balance between the direct and indirect loop.

1.7 Effects of the D_2 Dopamine Receptor on Behaviours Related to Vestibular Dysfunction

The increase in circling and velocity of general locomotor behaviours seen in animals with bilateral vestibular dysfunction, suggests that there is a change in striatal function due to a hyperkinetic disorder (Richter *et al.*, 1999). This would therefore suggest that reduction in input to the striatum could result from a decrease in the activation of the indirect loop in the motor circuit. From this it can be predicted that inhibiting D_2 dopamine receptors in animals with reduced striatal input could bring the two loops of the motor circuit back into balance, and therefore decrease the pathological behaviours. Indeed, evidence supporting this hypothesis has been found in both stargazer and *ci2/ci2* rats (Brock *et al.*, 1996), in a study in which haloperidol was shown to decrease stereotypic head movements as well as locomotor activity. When *ci2/ci2* rats were treated with the specific D_2 receptor antagonist raclopride, there was a significant decrease in both locomotor hyperactivity and circling behaviour

(Schirmer *et al.*, 2007b). These results support the idea that D_2 dopamine receptors can regulate the behaviours exhibited by rats with vestibular disorders.

Behavioural disinhibition is the generation of impulsive behaviour, which Dickman (1993) defined as the failure to withhold behaviour leading to the omission of reward. Behavioural disinhibition has been shown in studies of attention deficit hyperactivity disorder to be associated with dopamine imbalances in the striatum and other associated brain regions including the substantia nigra and the nucleus acumbens (Evenden, 1999). Disruption of behavioural disinhibition is another condition like locomotor hyperactivity and circling behaviour that has been shown to be affected by the D_2 receptor, but has not been tested in vestibular-deficient rats. However, following treatment with the D_2 selective antagonist eticlopride, healthy male Wistar rats were shown to display a decrease in impulsive behaviour following treatments with amphetamines and cocaine, drugs known to cause changes in dopamine release (van Gaalen *et al.*, 2006a). It is therefore possible that changes in the activity of D_2 receptors can affect the regulation of behavioural disinhibition in rats and that treatment with a D_2 receptor antagonist may change behaviour in BVD rats.

1.8 Aims

The aim of this study was to investigate the effects of the D_2 antagonist, eticlopride, in rats with bilateral vestibular loss, using behavioural paradigms used to test locomotor behaviours and impulsivity. In addition, this study aimed to investigate the changes, if any, in the levels of the D_2 dopamine receptor in the striatum at one month and six months following surgical bilateral vestibular deafferentation.

Chapter 2: Methods

2.1 Animals

For behavioural testing, 16 male Wistar rats were randomly allocated into either BVD or sham groups (n = 8 in each group), and underwent the appropriate surgery for each condition. Four animals (3 BVD and 1 sham) were lost in the month following surgery for reasons unrelated to the surgery. In addition, 12 other Wistar rats underwent surgery (n = 6 in each BVD and sham group) to be used for neurochemical analysis. The animals weighed 250 - 300 g at the time of surgery. Following surgery animals were individually housed and maintained on a twelve hour light-dark cycle. All procedures were approved by the University of Otago Animal Ethics Committee (05/10).

2.2 Bilateral Vestibular Deafferentation (BVD) and Sham Surgery

All surgeries were conducted under a general anaesthetic of fentanyl citrate (0.2 mg/kg, s.c.), medetomidine hydrochloride (Domitor, Novartis. 0.5 mg/kg, s.c.) and atropine sulfate (50 μg/kg, s.c.). The wound margin was anaesthetised locally with 1% xylocaine (containing 1:100,000 adrenaline).

For BVD surgeries, the tympanic membrane was exposed under microscopic control using a retro-auricular approach, and the tympanic membrane, malleus, and incus were removed. The stapedial artery was cauterised and the horizontal and anterior semicircular canal ampullae drilled open. The contents of the canal ampullae and the utricle and saccule were then aspirated and the temporal bone was sealed with dental cement. The animals were then monitored during recovery. Carprofen (5 mg/kg, s.c.) was used for post-operative analgesia immediately and 24 hours after surgery. Strepsin (0.1 ml per rat, s.c) was administered once daily for 3 days after surgery. Previous temporal bone histology studies in

our research group have shown that the BVD surgery produces a complete and permanent lesion of the vestibular labyrinth with no damage beyond the temporal bone (Zheng *et al.*, 2006).

Sham surgery consisted of exposing the temporal bone and removing the tympanic membrane without producing a vestibular lesion. This procedure provided a partial auditory control that involved damage to the tympanic membrane only, with no other surgical trauma. All other procedures such as anaesthesia and recovery were the same as for the BVD animals.

2.2.1 Confirmation of Bilateral Vestibular Deafferentation

The success of the BVD surgery was confirmed when the rats in the BVD group displayed a number of symptoms typical of bilateral vestibular deafferentation (Goddard *et al.*, 2008; Russell *et al.*, 2003; Zheng *et al.*, 2006). These symptoms included the loss of the righting reflex demonstrated by suspending themselves from the top of the cage, as well as head dorsiflexion and circling. Also when suspended off the ground by their tails, the rats would curl upwards, instead of reaching for the ground, which is the behaviour seen in normal animals. The sham rats demonstrated none of these behaviours.

2.3 Drug

The D_2 DA receptor antagonist, eticlopride (Sigma), was prepared in a 1 mg/ml stock solution with saline (0.9% NaCl) and frozen at -20° C in aliquots for later use. On testing days the required aliquots were thawed and made up in saline to the final concentration of 0.05 mg/ml. Eticlopride, has been reported to cause potent blockade of D_2 receptors in the striatum following systemic administration (Kohler *et al.*, 1986) and has a significantly higher affinity

for the D_2 receptor (K_i = 0.09 nM) compared to the D_1 receptor (> 10,000 nM) (Seeman *et al.*, 1988). Eticlopride was given at 3 doses (0.06, 0.04 and 0.02 mg/kg, s.c) based on a study published using the same rat strain in the 5-Choice Serial Reaction Time Task (van Gaalen *et al.*, 2006a). To decrease the variation of responses and reduce the confounding effects of treatment order, a 4 x 4 Latin square design was used (see Figure 2.1). On each day the rats were assigned a different treatment so that on each day at least one rat received each dose and over the 4 test days all rats receive all of the doses. Separate squares were used for BVD and sham rats to ensure that doses were evenly divided between the surgical groups. This had the advantage of allowing for the minimum possible number of animals to be used, as well as controlling for possible order effects of each treatment.

		DAYS			
		1	2	3	4
BVD ANIMALS	A	veh	0.06	0.04	0.02
	B	0.06	veh	0.02	0.04
	C	0.04	0.02	veh	0.06
	D	0.02	0.04	0.06	veh

		DAYS			
		1	2	3	4
SHAM ANIMALS	A	veh	0.06	0.04	0.02
	B	0.06	veh	0.02	0.04
	C	0.04	0.02	veh	0.06
	D	0.02	0.04	0.06	veh

Figure 2.1: Latin square design for eticlopride drug treatment. Designed to allow all drug doses to be received by at least one animal on a specific testing day while having each animal receive each dose over the four testing days.

2.4 Behavioural Assessment: Open Field Maze

Five months post surgery the animals were individually tested in an open field maze in order to assess their locomotor activity.

2.4.1 Equipment

A painted wooden open field maze 56 cm (w) x 56 cm (l) x 20 cm (h) was used with a 10 cm high clear plastic sleeve flush around the top of the maze to give the environment extra height, in order to prevent escape. The environment was divided into three zones (inner, middle and outer). The movements of each rat were tracked in the maze in real time using Ethovision XT (ver. 6.1) tracking software (Noldus Information Technology), in addition to videos being recorded using both Ethovision and on a DVD recorder. Tracked data were then compared to data obtained by the videos, to ensure that the tracking program worked correctly, with the real time tracking data being used for analysis.

2.4.2 Procedure

Thirty minutes before testing animals were treated with either the D_2 dopamine receptor antagonist, eticlopride at one of 3 doses, or vehicle control. At the beginning of the task the live tracking was initiated, and the rat was put into a random corner of the maze and allowed to roam freely for 10 minutes. The rat was then removed, and the maze was cleaned using a bleach solution to remove all scent cues before the next rat was put in. The rats were each exposed to the open field for four tests to allow each drug dose to be given once, with a washout period of two days between each test.

2.4.3 Measured Variables

Measures were made per minute of the time spent in the maze. Total distance travelled (cm), mean velocity (cm/s) and mobility and movement measures were recorded to show the difference in movement between all of the groups. Mobility is a measure of the movement or the change in position of the animal. The tracking software measures it as a change in the pixels identified as the animal between sampling frames. The frequency and duration of mobility were measured and the results divided into highly mobile, mobile and immobile depending on the level of movement. Movement differs from mobility, in that it is a measure of the movement of the centre point of the rat. When the velocity of the rat centre point was above 2 cm/s, the rat was classed as moving and then classed as not moving when the velocity decreased below this point. The duration of movement was recorded for each animal. Duration in zone was used to measure the time spent in each of the zones in the environment. This was used as a measure of anxiety behaviour (Prut *et al.*, 2003).

Circling behaviour was measured using the number of body axis rotations in either a clockwise or anticlockwise direction (each 360° turn equals 1 rotation)

2.5 Behavioural Assessment: Five-Choice Serial Reaction Time Task (5-CSRTT)

2.5.1 *Apparatus*

The set-up consisted of a 25 cm (w) by 25 cm (l) by 25 cm (h) chamber (Med Associates Inc.) with the front and rear walls made of aluminium and the side walls and roof made of clear plastic. The floor consisted of aluminium bars spaced 1.5 cm apart. The chamber was housed within a wooden cabinet in order to keep the chamber dark unless the house-light was

on. The rear wall of the chamber was curved outwards and contained five apertures evenly spaced 2.5 cm apart along the wall. These apertures had 2.5 cm^2 openings and were 2.5 cm deep and 2 cm off the ground. In the centre of the opposite wall situated 2 cm off the ground was a food tray with an opening of 5 cm^2. On the ceiling above the magazine was a 3 W house-light. Standard 3 W bulbs were situated inside the 5 apertures as well as in the food tray (see Figure 2.2).

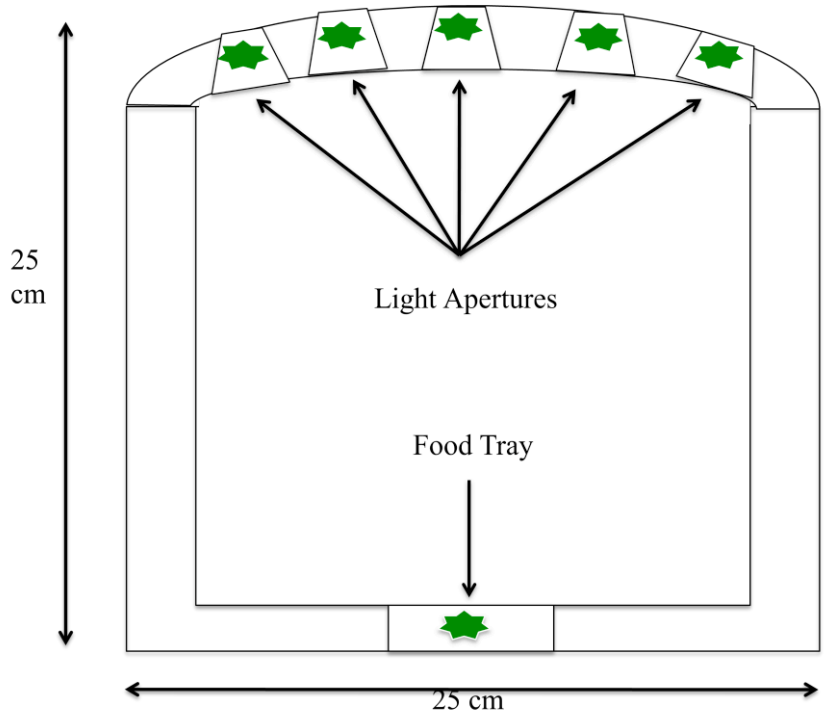


Figure 2.2: Diagram of the 5-CSRTT chamber.

Infra-red photocell beams were located in the entrance to each aperture as well as in the entrance to the food tray to detect the nose pokes. The apparatus and data collection were controlled by a computer using Med-PC IV, which controlled the timing of the programs as well as the release of sucrose tablets (Sucrose tablet 45 mg, Test Diet, USA) into the food tray by a pill dispenser at the appropriate times.

2.5.2 Behavioural Task

Animals began training 2 months post surgery. This time allowed for complete post surgical recovery including recovery from acute symptoms of BVD. Immediately prior to and during behavioural training, rats were food-deprived to 90% of their original body weight, and thereafter maintained at between 85 and 90 % of their original body weight. Rats were trained to identify a brief visual stimulus of a light turning on, which was randomly presented in one of the five apertures. Rats identified this stimulus by poking their nose into the correct aperture. The training of the rats was divided into periods of acclimation, training and drug testing.

Acclimation

At the beginning of the first acclimation phase, before the rat was put into the chamber, all of the apertures had three sucrose tablets placed in them as well as 10 tablets placed in the food tray. The house light as well as all of the lights in the aperture and the food tray were on for acclimation. The rat was placed in the chamber twice daily for familiarisation with the apparatus for 15 minutes, or until all of the tablets had been eaten. The second phase of acclimation began the day after the rat had successfully eaten all of the tablets in a single session. This second phase involved placing the rat in the chamber with all of the apertures as well as the food tray baited with sugar pellets. At the beginning of the session the house-light was on. The light in the food tray was illuminated and the session was started when the rat poked its nose into the tray, which was registered by the photocell beam. Thereafter, the light in a randomly selected aperture was illuminated and the rat was required to poke its nose into the correct hole in order to receive a food reward in the food tray. Once the food reward was collected from the food tray another of the apertures was illuminated. This process was repeated for 30 minutes. The session was run for two days with baited holes. In the third

phase of acclimation, the same programme was run as for the second phase, but without baiting the response apertures. The magazine released food into the food tray at the beginning of the session. The rats were trained without baiting of the response apertures for 2 sessions.

Training

At the beginning of each training session, the chamber was illuminated and free delivery of a single food pellet to the food tray was made. Once the collection of this tablet had been detected by the photocell beam, the light in the food tray went off and after an inter trial interval (ITI) of 5 seconds, one of the apertures was randomly illuminated for a specified stimulus duration (beginning at 60 seconds and decreasing to 5 seconds when the target parameters were reached). Nose-poke responses in the correct aperture either during or in the 5 seconds immediately after illumination (the limited hold period), resulted in a reward being delivered into the food tray, and a correct response being recorded by the computer. A nose entry into a non-illuminated aperture (an incorrect response) or no response during the response period (omission) was punished with a 5 second period of darkness (time out) during which no food was delivered and no action could be performed. Nose entries into any of the apertures after the initial response were recorded as a perseverative response. A nose entry into an aperture during the ITI was recorded as a premature response and was punished with a 5 second time out. The time out periods were used to suppress the inappropriate behaviour. Following a time out the house light was reilluminated and the next trial began with the ITI period, except for following a premature response, when the same trial was repeated. During any one session, the light stimulus was presented an equal number of times in each of the five apertures in a random order. A daily session consisted of 100 trials, or was terminated after 30 minutes of testing. The target parameters of training were a stimulus duration of 5 seconds with an omission rate of less than 20%. The rats were considered to have reached criterion

when four consecutive sessions with >85% responses were recorded, or the animal showed no improvement or decline in performance for five consecutive days. Once all of the rats had reached the criterion, drug testing commenced.

2.5.3 Drug Testing

Eticlopride and vehicle dosing, administered 30 minutes before testing, followed the same procedure as for the open field test (see 2.3) using the Latin square design. The animals were tested in the behavioural task using the 5 second stimulus duration program identical to that used in training, and also in a program of long variable ITIs. Use of the long ITI variables allowed easier examination of premature responses because the animals tended towards more premature responses at longer ITI intervals. The long variable ITI program consisted of the same parameters as the training program, except that in the single program there were four different ITI lengths (4.5s, 6s, 7.5s and 9s) that were each presented the same number of times (four) in the program in a random order. As it has been suggested that variable ITI programs should not be repeated within 4 days of each other (Bari *et al.*, 2008), the testing days were alternated between the normal ITI test and the long variable ITI tests. Drug washout days occurred between each testing day with a minimum of two days of washout. The animals were retrained until they reached the original baseline criterion before the next testing day occurred.

2.5.4 Performance measures

Accuracy of performance was measured as a percentage of total responses (correct responses/[correct + incorrect response] x100). In the case where no correct or incorrect responses were made, the result was set to zero. Two measures of inhibitory control were also calculated by the premature responses (the number of responses made in the apertures during

the ITI) and perseverative responses (repeated responses in the apertures following a correct response). Speed and decision-making were assessed according to two different latencies. The first was the latency to respond correctly, defined as the time between the onset of the visual stimulus and the point at which the animal's nose broke the beam of the illuminated hole. The second was reward latency, defined as the time between performance of a correct response and collection of the food pellet from the food tray.

2.6 Brain Removal and Tissue Preparation

Twenty-four animals were sacrificed to collect tissue for western blotting. These included 12 animals (five BVD and seven sham) from the previous behavioural experiments at 6 months post surgery, as well as 12 additional naive animals (six BVD and six sham) sacrificed at 1 month post surgery. The left and right striatum and frontal cortices were collected to measure the levels of D_2 dopamine receptors in these regions. The frontal cortices were removed in order to detect if there was any change in receptor levels in the prefrontal cortex, which has no responsibility for movement control.

2.6.1 Tissue Collection

The animals were sacrificed by decapitation and the whole brain was rapidly removed from the skull and placed in ice-cold 0.9% saline solution. The striatum and frontal cortices were dissected out of the brain and placed on a porcelain 12-well plate that was chilled on dry ice. Once the tissue was frozen on the plate it was then placed into 1.5 ml plastic tubes and stored at -80°C until it was prepared for Western blotting.

2.6.2 Tissue Preparation

The tissue was homogenised into membrane preparations, chosen in order to assess only the receptors that were membrane bound, and not those that were sequestered into the cytosol. The tissue was homogenised in a 0.32 M sucrose solution using a hand-held glass homogeniser on ice. The resulting homogenate was then centrifuged at 1000g to remove unbroken cells and blood vessels. The supernatant was then collected and centrifuged at 20000g. After the pellet was re-suspended in ice-cold distilled water, it was centrifuged again at 8000g. The resulting supernatant and buffy coat were then collected and centrifuged at 48000g. The pellet was then re-suspended in Tris HCl buffer (50 mM, pH 7.4), and centrifuged at 48000g. The supernatant was then discarded, and the pellet snap frozen in dry ice and then re-suspended in Tris HCl buffer and centrifuged again. This last step was repeated once more, and the resulting supernatant was removed and discarded. The remaining pellet was frozen at -80°C until a Bradford assay was performed to determine protein concentration.

The tissue was then equalised to 1.5 mg/ml for the striatum and 2.5 mg/ml for the frontal cortex using tissue buffer. The samples were then mixed with gel loading buffer (50 mM Tris-HCl, 10% SDS, 10% glycerol, 10% 2- mercaptoethanol, 2 mg/ml bromophenol blue) at a volume ratio of 1:1 and boiled for 5 minutes. The samples were then frozen at -20°C until used for Western blotting.

2.7 Western Blotting

2.7.1 Antibodies

The D_2 dopamine receptor was labelled using a monocolonal mouse-derived D_2 dopamine receptor (B-10, SC-5303: Santa Cruz biotechnology, 1:1000) primary antibody, and a goat anti-mouse IgG horseradish peroxidase (HRP) conjugated (Sigma, 1:1000) secondary antibody. β-actin was used to standardise samples within gels and was labelled using a goat derived antibody (Santa Cruz Biotechnology, SC-69879, 1:5000) and a donkey anti-goat IgG HRP conjugated (Sigma, 1:5000) secondary antibody.

2.7.2 Protocol

Ten μg of samples were loaded into the wells of 10% SDS-polyacrylamide mini gels. Also loaded were pre-stained protein markers (10–250 kDa; Bio-Rad, Precision Plus: Dual color) to use for molecular weight determination. An internal standard was loaded into each of the gels in order to allow for comparison between gels when performing analysis. The samples were run through the gel at room temperature at 90 V until the protein met the interface between the stacking and resolving gels, and thereafter at 180 V (Bio-Rad PowerPack 3000) until the loading dye reached the bottom of the gel. The proteins were transferred to polyvinylidene-difluoride membranes using a transblotting apparatus (Bio-Rad) overnight, using transfer buffer (25% methanol, 1.5% glycine and 0.3% Tris base) at a 10 V variable current (Bio-Rad PowerPack 3000) for 18 hours at room temperature. Following transfer, non specific IgG binding was blocked by incubation of the membrane in Tris-Tween buffer solution (0.1% tween-20) containing 0.5% skim milk powder and 0.1% bovine serum albumin (BSA) (Sigma) for 6 hours at 4° C. The primary antibody was then placed on the membrane in a Tris-Tween buffer solution containing 0.5% milk powder and incubated overnight at 4° C. The membrane was then washed in Tris-Tween buffer and incubated with

the secondary antibody in Tris-Tween Buffer with 0.5% milk powder for 4 hours at 4° C. Detection was performed using the enhanced chemiluminescence (ECL) system (Amersham Biosciences, NZ). Hyperfilm (Amersham Biosciences, NZ) was exposed to the membrane for 1 minute for the D_2 antibody, and for 10 seconds for the β-actin antibody.

The results of the western blotting were analysed using densitometry to determine protein concentration. Imaging was performed using a calibrated imaging densitometer and analysed using Quality One software. Results were collected as volume (optical density^2 × area) as this takes into account the variation in the size of the bands not just the darkness. The long (51 kDa band) and short (48 kDa band) isoforms of the D_2 receptor were analysed separately as well as combined to test for total changes in the amount of receptor. The resulting data were expressed as a percentage of the density of actin and then as a ratio of the internal standard to allow comparison between membranes. Normalising the protein volume to the internal standard of their individual band results in changes in the ratios of proteins and therefore does not allow for comparison between the two isoforms.

2.8 Statistical Analysis

The data from the open field maze, the 5-CSRTT and western blots were analysed using a Linear Mixed Model (LMM) analysis using a restricted maximum likelihood procedure in SPSS 17 (Gurka *et al.*, 2011; Kutner *et al.*, 2005; Norusis, 2010) .This was selected as the best choice of analysis as it allows for a number of problems with the data that other statistical tests do not address including the assumption of the independence of data, which is violated by the repeated measures structure of this experimental design (Norusis, 2010). The LMM also allows for inclusion of incomplete data sets, which was required at some points in the analysis (Norusis, 2010).

All data were tested for normality and transformed when necessary to ensure normality before analysis. The model that best fit the data was decided by comparing the Akaike's information criteria and selecting the model with the lowest value. This information criterion was used as it appears to be the best at allowing for small sample sizes (Norusis, 2010). This model was then run to decide the significance of the variables followed by Bonferroni post-hoc tests. LMM analysis does not perform pairwise comparisons on interactions involving random and fixed variables. All factors (surgery, drug, ITI etc.) were considered to be fixed variables except in the case of the minute variable in the open field maze, which was set as a random factor. As it was set as a random factor, the LMM did not perform comparative analysis on this variable.

Chapter 3: Results

3.1 Open Field Maze

Surgery alone was shown to have a significant effect on both the total distance travelled ($F(1,69.5) = 5.2$, $p = 0.025$; see Figure 3.1) as well as the number of rotations ($F(1,33.5) = 4.7$, $p = 0.037$; see Figure 3.2) with BVD rats travelling further and circling more than the sham group. However, BVD rats were found not to have a significantly different velocity compared with sham rats (see Figure 3.3). There was no significant rotational direction × surgery interaction, meaning that neither BVD nor sham rats favoured one direction over the other (see Figure 3.2). When analysed individually, no animal in either the BVD or sham groups favoured one rotational direction over the other (results not shown).

Significant drug effects were seen for all variables except mobility duration, and movement duration. Velocity showed a significant difference between the speed travelled following treatment with the vehicle and 0.02 mg/kg doses compared with the 0.04 and 0.06 mg/kg doses ($F(3,108.4) = 30.9$, $p < 0.001$; see Figure 3.3). Rats treated with the vehicle and 0.02 mg/kg doses travelled faster than those at the higher doses (pairwise comparison = $p < 0.001$ for all). A significant difference was seen in total distance between the vehicle groups and all drug doses ($F(3,76.2) = 68.6$, $p < 0.001$; see Figure 3.1) with rats treated with eticlopride travelling significantly further than when treated with the vehicle (pairwise comparison = $p < 0.05$ for all). A significant decrease in the number of rotations was seen between the vehicle and all drug doses ($F(3,128.1) = 10.0$, $p < 0.001$; pairwise comparison = $p < 0.001$ for all; see Figure 3.2). There was also a significant interaction between movement duration and drug, with movement duration decreasing as the drug dose increased ($F(3,209.8) = 81.7$, $p < 0.001$; see Figure 3.4).

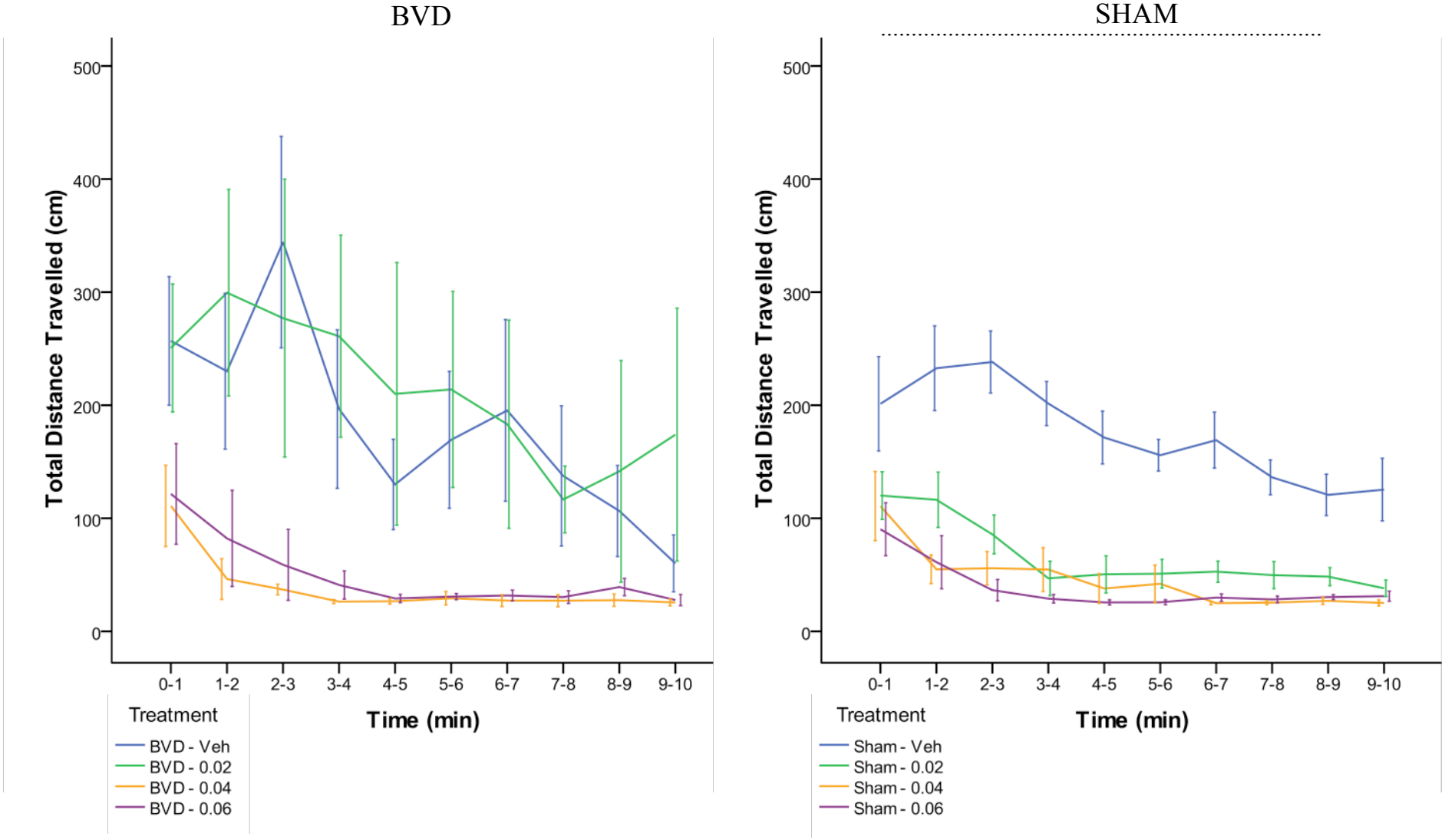


Figure 3.1: Total distance travelled per minute in the open field maze showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

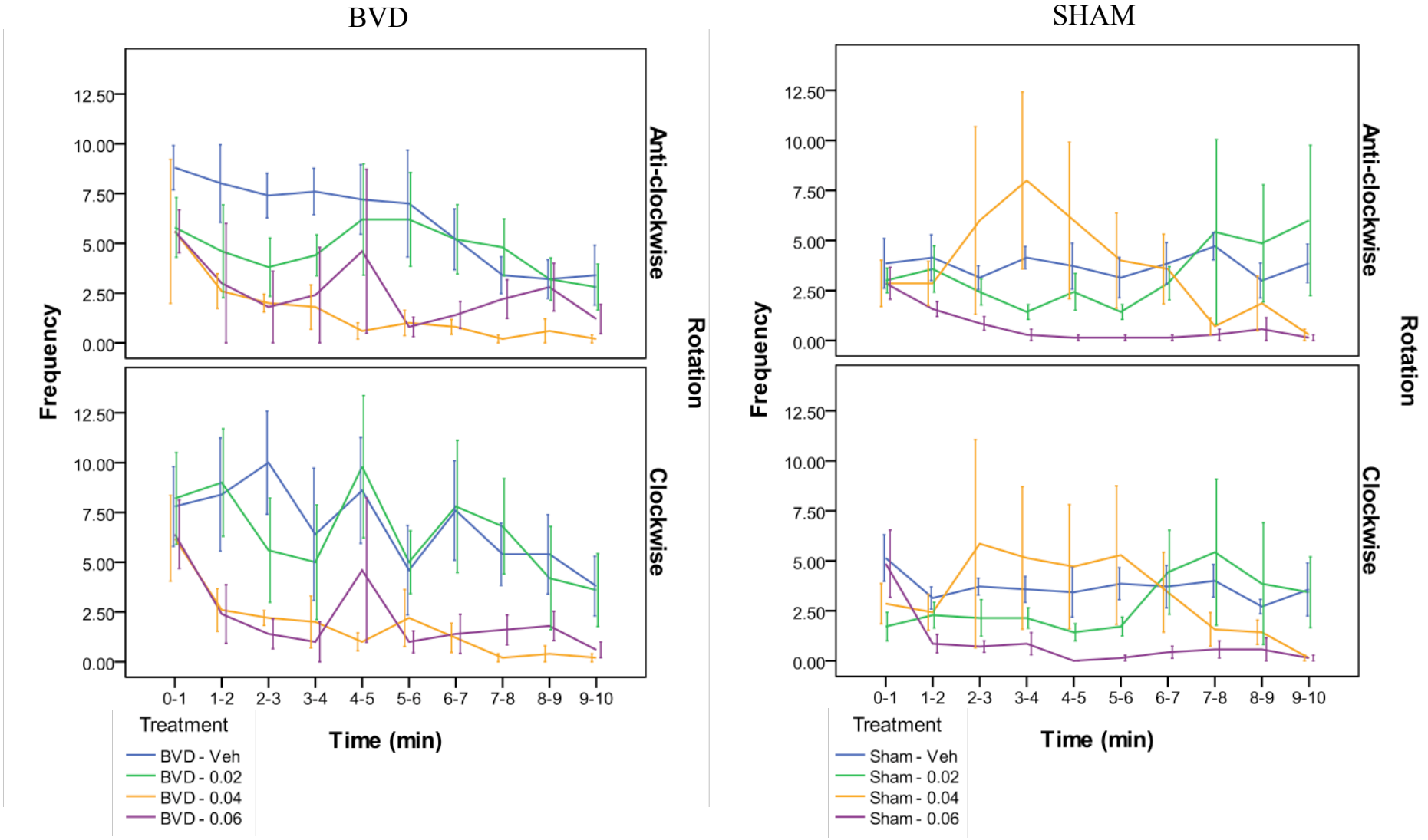


Figure 3.2: Number of rotations per minute in the open field maze in either a clockwise or anti-clockwise direction showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

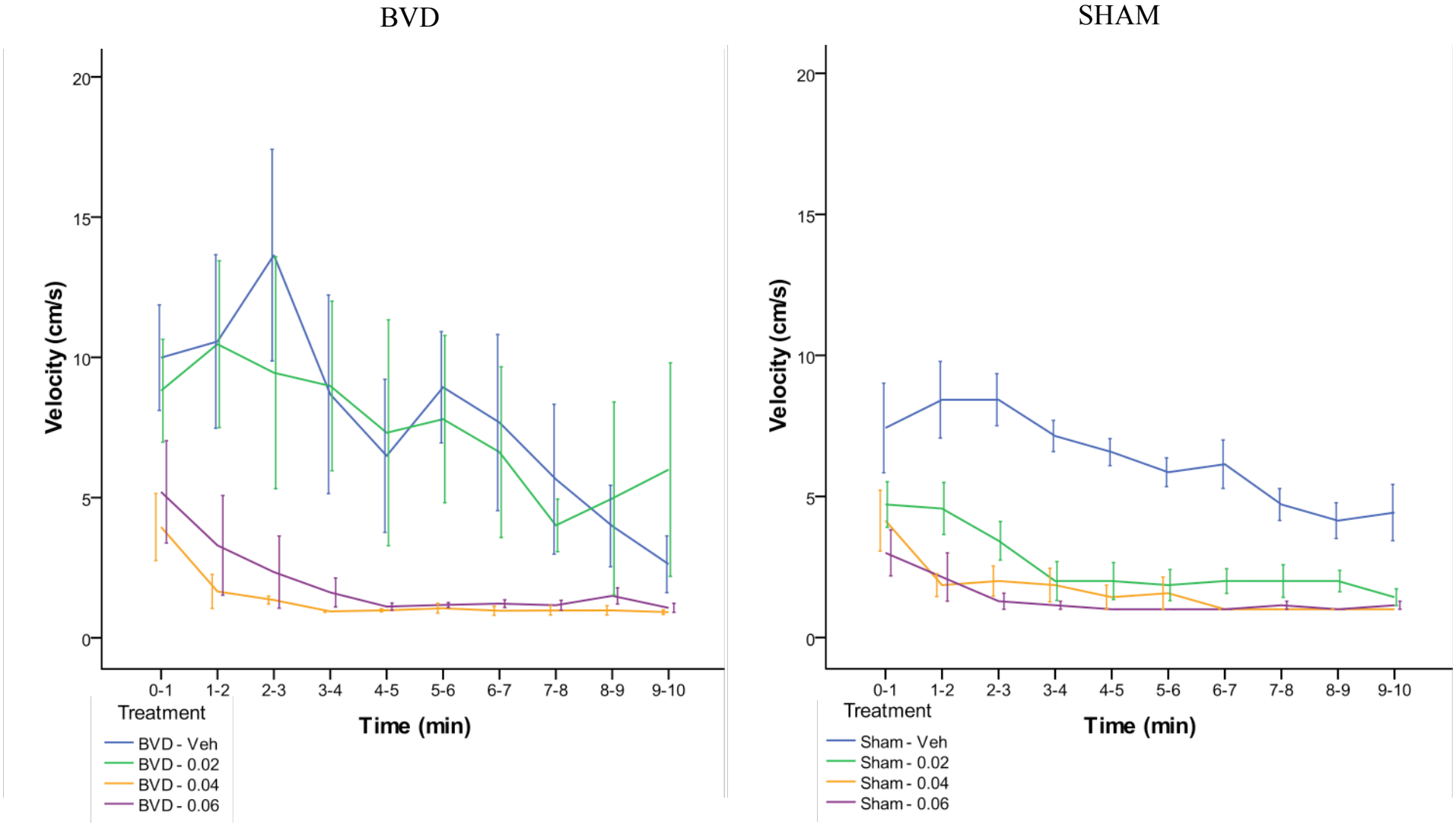


Figure 3.3: Velocity of movement per minute in the open field maze showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

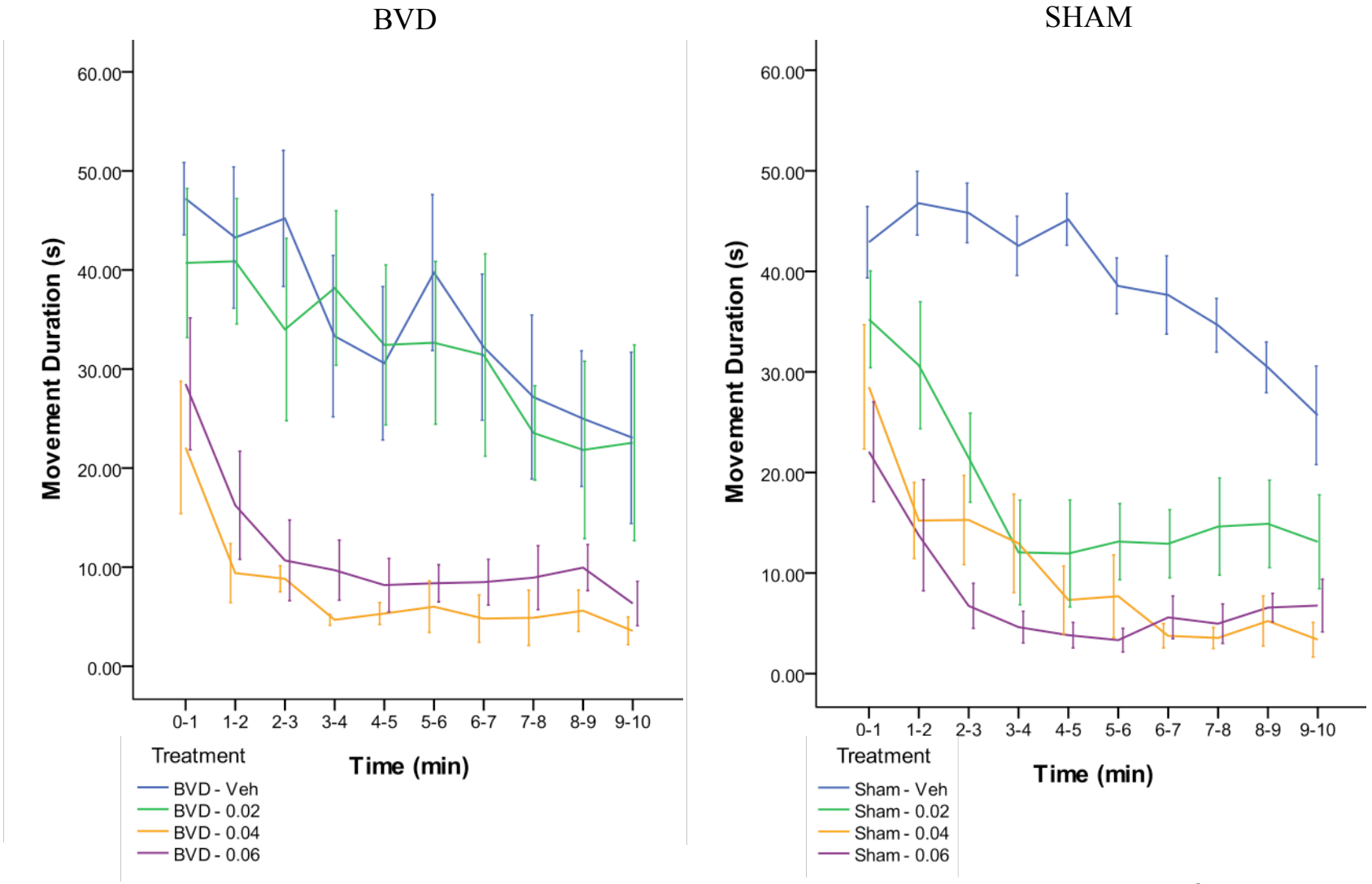


Figure 3.4: Duration of movement per minute in the open field maze showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

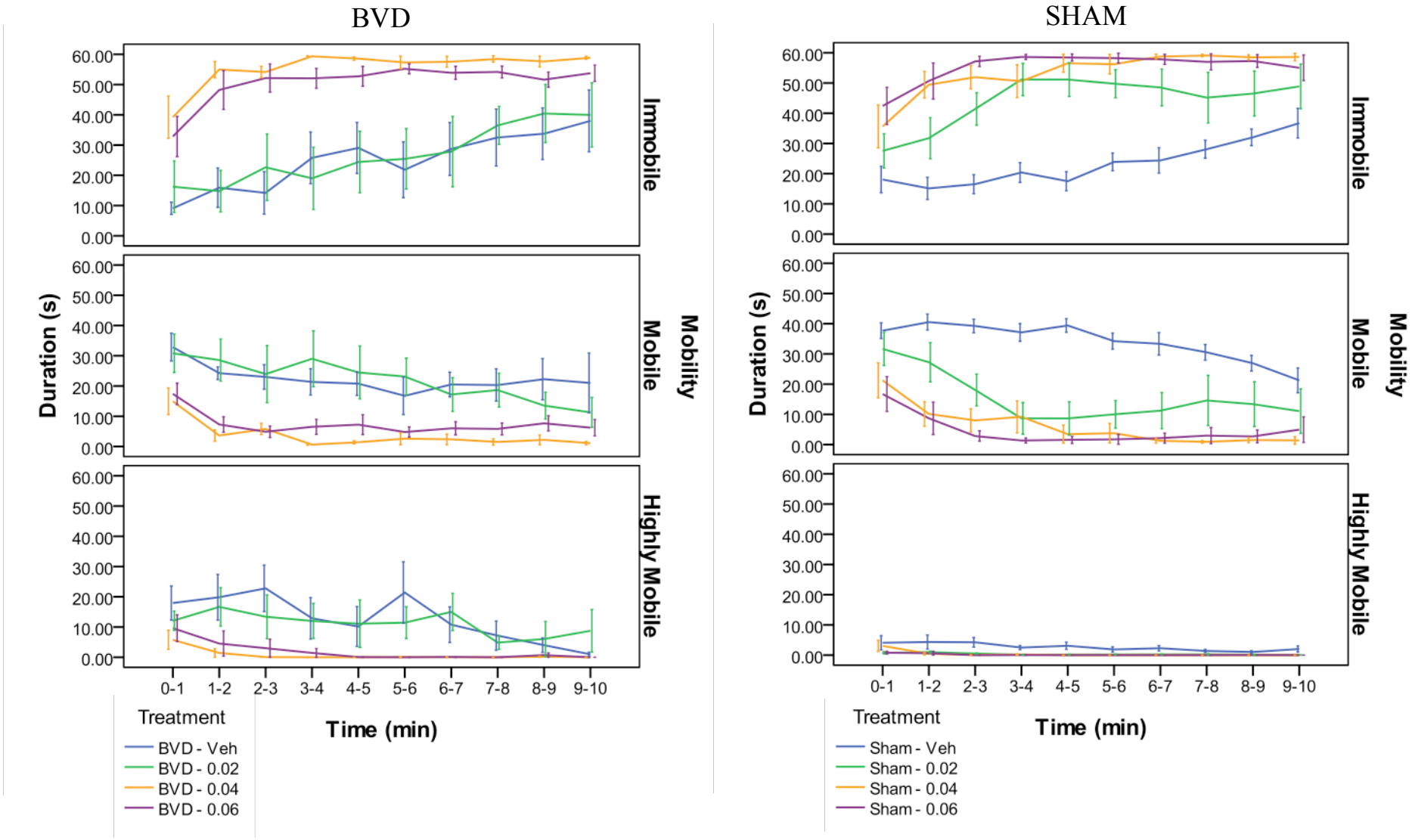


Figure 3.5: Duration of mobility per minute in the open field maze divided into immobile, mobile and highly mobile showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

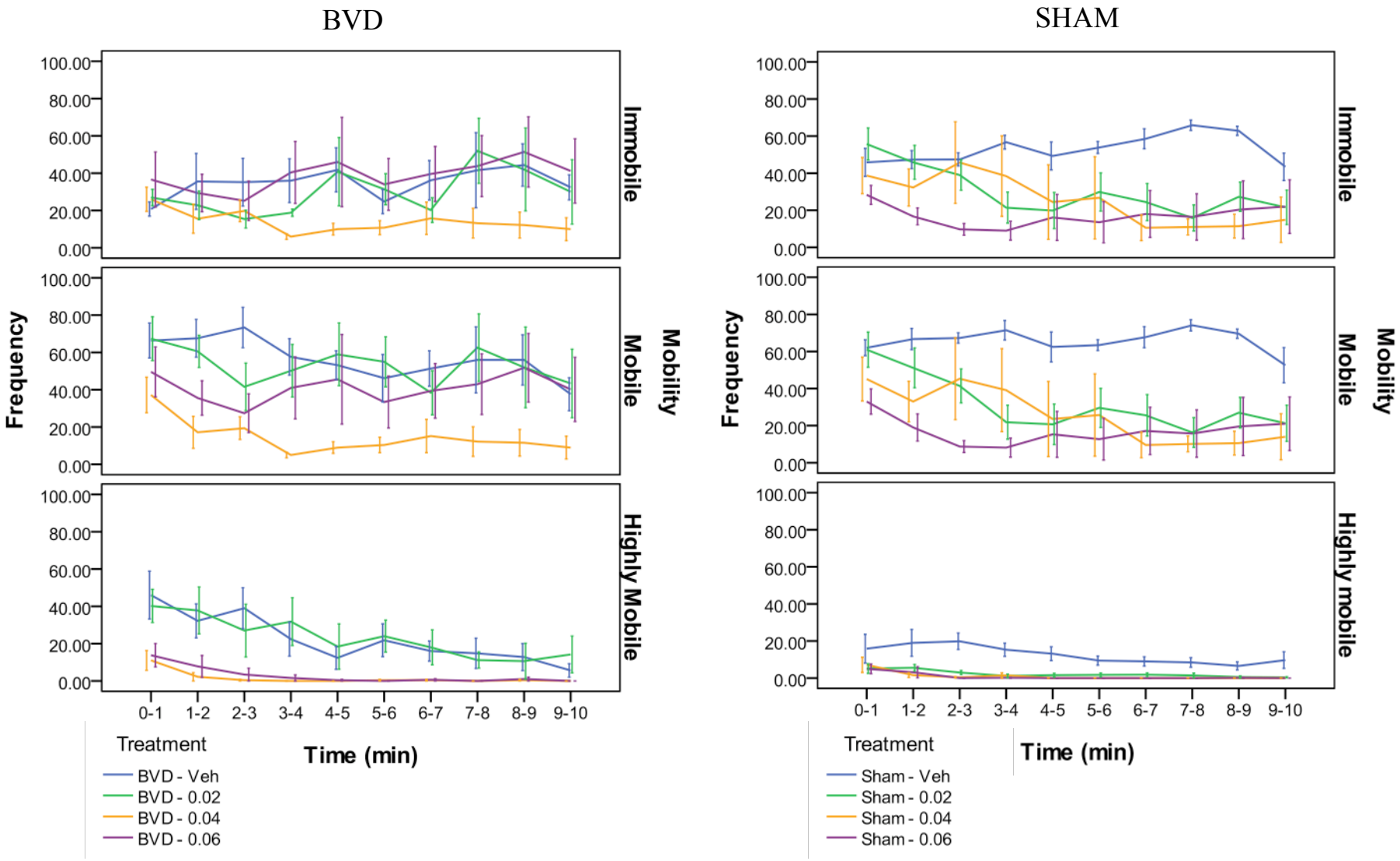


Figure 3.6: Frequency of mobility per minute in the open field maze divided into immobile, mobile and highly mobile showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

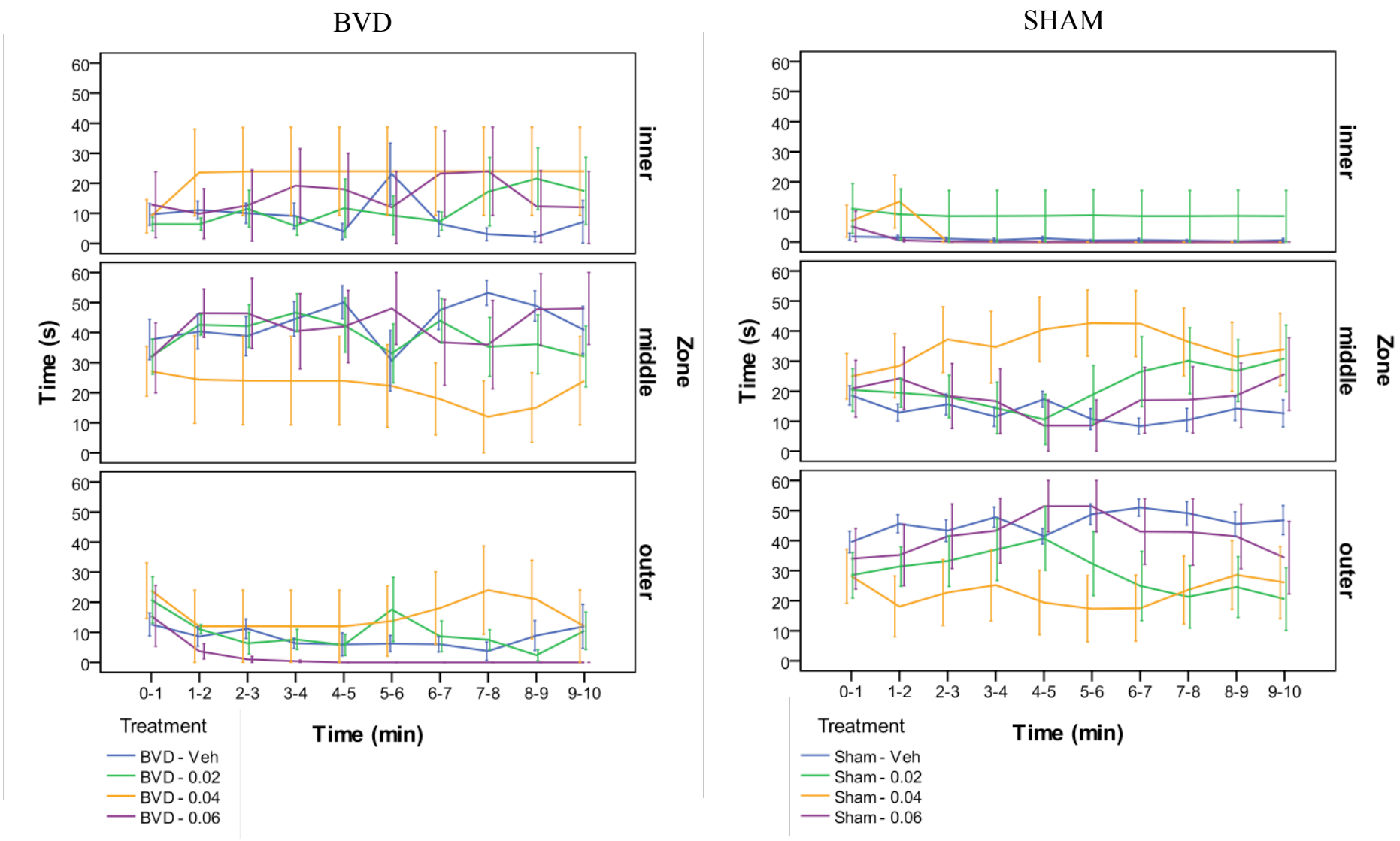


Figure 3.7: Amount of time spent in the inner, middle or outer zone per minute in the open field maze showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

The mobility of the animals was affected in a number of ways by both surgery and drug treatment (see Figures 3.5 and 3.6). Drug treatment caused a significant decrease in the frequency of mobility ($F(3,127.0) = 16.7$, $p < 0.001$; see Figure 3.6) at the 0.04 and 0.06 mg/kg doses compared to the vehicle (pairwise comparison = $p < 0.001$ for both) and 0.02 mg/kg dose (pairwise comparison = $p < 0.001$ for both) but had no effect on mobility duration. There was, however, a significant interaction of surgery × mobility for both the duration ($F(2,1401.8) = 26.5$, $p < 0.001$) and frequency of mobility ($F(2,1106.8) = 12.3$, $p < 0.001$), with BVD rats being highly mobile more frequently and for longer (see Figure 3.5 and 3.6). Sham rats were also seen to be immobile for longer durations. A interaction between drug and both duration ($F(6,1397.7) = 165.0$, $p < 0.001$) and frequency of mobility ($F(6,1113.1) = 8.6$, $p < 0.001$) was also seen, with vehicle-treated animals being more highly mobile and 0.06 mg/kg drug-treated animals being more immobile. For both frequency and duration there was a three way drug × surgery × mobility interaction ($F(6,1113.0) = 13.9$, $p < 0.001$ and $F(6,1396.9) = 21.0$, $p < 0.001$, respectively; see Figure 3.5 and 3.6).

A significant drug × surgery interaction was seen in many of the variables including total distance ($F(3,72.0) = 4.5$, $p = 0.006$), velocity ($F(3,153.7) = 7.5$, $p < 0.001$) and mobility frequency ($F(3,188.0) = 5.8$, $p < 0.001$; see Figure 3.1, 3.3 and 3.6). These variables all consistently showed a decreased drug response to the 0.02 mg/kg drug dose in BVD rats but not in sham rats.

The in zone variable showed a number of significant interactions including zone × surgery ($F(3,1415.0) = 147.7$, $p < 0.001$), as BVD rats spent more time in the inner and middle zones while the sham rats spent most of their time in the outer zone (see Figure 3.7). A significant

zone × drug interaction (F(3,1414.3) = 3.484, $p = 0.002$) was also seen as well as a surgery × drug × zone interaction (F(3,1414.1) = 25.8, $p < 0.001$; see Figure 3.7).

3.2 5-Choice Serial Reaction Time Task

Both sham and BVD rats took approximately 14 days to reach the criterion of 4 consecutive sessions with >85% responses recorded, except for two rats, one sham and one BVD, that took longer. The BVD animals did not appear to have any problems performing the task.

3.2.1 5 second ITI

Drug treatment caused a significant decrease in the total number of responses (F(3,19.9) = 151.4, $p < 0.001$) and therefore a significant increase in the percentage of omissions (F(3,20.1) = 148.3, $p < 0.001$; see Table 3.1). Pairwise comparisons showed significant changes at all doses compared with vehicle (pairwise comparison = $p < 0.001$ for all). However, there was no significant difference in the number of responses or the percentage of omissions as a result of the surgery. Both the percentage of correct responses (F(3,40.0) = 3.7, $p = 0.018$) and the number of premature responses (F(3,16.6) = 5.1 $p = 0.011$) were significantly decreased by drug treatment, but there were no significant differences seen in the pairwise comparisons and no significant differences due to surgery or drug × surgery interaction (see Table 3.1).

Both surgery (F(1,21.5) = 13.4, $p = 0.001$) and drug treatment (F(3,16.3) = 19.6, $p < 0.001$) had a significant effect on the number of perseverative responses, with pairwise comparisons showing a significant difference between all drug doses compared to the vehicle

(pairwise comparison = $p < 0.001$ for all). A significant interaction was also seen between the drug treatment and surgery ($F(3,16.3) = 3.2$, $p = 0.05$; see Table 3.1).

There were no significant changes in latency to make a correct or incorrect response or latency to collect a reward with respect to drug or surgery, nor was there any significant drug × surgery interaction (see Table 3.1).

Table 3.1
Effect of Eticlopride on 5 Second ITI 5-CSRTT

Variable	Surgery	Eticlopride (mg/kg)	Mean ± SEM
Total Responses *	BVD	vehicle	87.0 ± 3.6
		0.02	26.2 ± 16.4
		0.04	16.6 ± 15.4
		0.06	10.8 ± 4.2
	Sham	vehicle	82.0 ± 5.6
		0.02	50.6 ± 14.1
		0.04	3.4 ± 2.0
		0.06	3.7 ± 2.5
Correct Responses (%) *	BVD	vehicle	88.8 ± 3.9
		0.02	46.0 ± 19.2
		0.04	48.7 ± 20.6
		0.06	78.3 ± 9.1
	Sham	vehicle	90.9 ± 2.0
		0.02	81.1 ± 13.7
		0.04	26.0 ± 16.9
		0.06	36.1 ± 17.3
Omissions (%) *	BVD	vehicle	13.0 ± 3.6
		0.02	73.6 ± 16.4
		0.04	83.0 ± 15.3
		0.06	88.6 ± 4.4
	Sham	vehicle	18.0 ± 5.6
		0.02	49.2 ± 14.1
		0.04	96.3 ± 2.1
		0.06	96.0 ± 2.5
Premature Responses *	BVD	vehicle	0.8 ± 0.5
		0.02	3.4 ± 3.4
		0.04	0.6 ± 0.4
		0.06	0.4 ± 0.4
	Sham	vehicle	5.7 ± 1.6
		0.02	3.6 ± 1.4
		0.04	0.3 ± 0.2
		0.06	0.0 ± 0.0
Perseverative Responses †	BVD	vehicle	21.4 ± 3.8
		0.02	3.4 ± 1.6
		0.04	2.0 ± 1.4
		0.06	2.6 ± 1.9
	Sham	vehicle	9.0 ± 1.5
		0.02	3.6 ± 1.0
		0.04	0.0 ± 0.0
		0.06	0.3 ± 0.2
Correct Latency (s) *	BVD	vehicle	1.7 ± 0.1
		0.02	2.2 ± 0.2
		0.04	2.6 ± 0.4
		0.06	3.0 ± 0.4
	Sham	vehicle	1.7 ± 0.2
		0.02	2.2 ± 0.3
		0.04	3.1 ± 0.4
		0.06	3.8 ± 0.8
Incorrect Latency (s)	BVD	vehicle	1.9 ± 0.7
		0.02	2.3 ± 0.5
		0.04	5.0 ± 3.5
		0.06	3.6 ± 1.2
	Sham	vehicle	2.8 ± 0.9
		0.02	3.3 ± 0.5
		0.04	5.1 ± 1.7
		0.06	3.8 ± 1.9
Reward Latency (s)	BVD	vehicle	0.9 ± 0.0
		0.02	4.9 ± 3.9
		0.04	2.4 ± 1.6
		0.06	10.4 ± 7.6
	Sham	vehicle	1.3 ± 0.1
		0.02	1.9 ± 0.4
		0.04	6.7 ± 5.2
		0.06	22.6 ± 19.0

* significant drug effect ($p < 0.01$) of eticlopride doses versus vehicle but no surgery effect or surgery drug interaction.

†significant drug effect ($p < 0.001$) of eticlopride doses versus vehicle. Significant surgery effect ($p < 0.001$) and significant surgery × drug interaction ($p < 0.05$).

3.2.2 Variable ITI

Following drug treatment, the percentage of correct responses showed a significant drug effect ($F(3,62.7) = 23.2$, $p < 0.001$; see Figure 3.8). Associated pairwise comparisons for the drug effect showed a significantly higher percentage of correct responses for the vehicle and 0.02 mg/kg drug dose versus the 0.04 (pairwise comparison = $p < 0.001$ for all) and 0.06 mg/kg doses (pairwise comparison = $p < 0.001$ for both; see Figure 3.8). A significant drug × surgery interaction ($F(3,62.7) = 3.8$, $p = 0.014$) was seen with the 0.02 mg/kg dose, decreasing the percentage of correct responses in the sham rats but not the BVD rats (see Figure 3.8). Drug treatments resulted in a significant decrease in the percentage of omissions ($F(3,14.8) = 198.7$, $p < 0.001$), with pairwise comparisons showing significance for all of the eticlopride doses versus the vehicle (pairwise comparison = $p = 0.02$ for 0.02 mg/kg dose and $p < 0.001$ for 0.04 and 0.06 mg/kg doses; see Figure 3.9).

Both drug treatment and ITI durations were shown to have a significant effect on the number of premature responses ($F(3,23.5) = 11.4$, $p < 0.001$ and $F(3,27.6) = 11.0$, $p < 0.001$, respectively; see Figure 3.10). Significant increases were seen at the longer ITI durations (7.5 and 9 seconds; pairwise comparison = $p < 0.01$ and $p < 0.05$ respectively), while the higher doses of eticlopride produced a significant decrease in the number of premature responses (0.04 and 0.06 mg/kg; pairwise comparison = $p < 0.025$ and $p < 0.002$ respectively). Analysis also showed a significant drug × ITI interaction ($F(3,51.0) = 3.4$, $p = 0.002$; see Figure 3.10) with the drug decreasing the number of premature responses even at the higher ITIs.

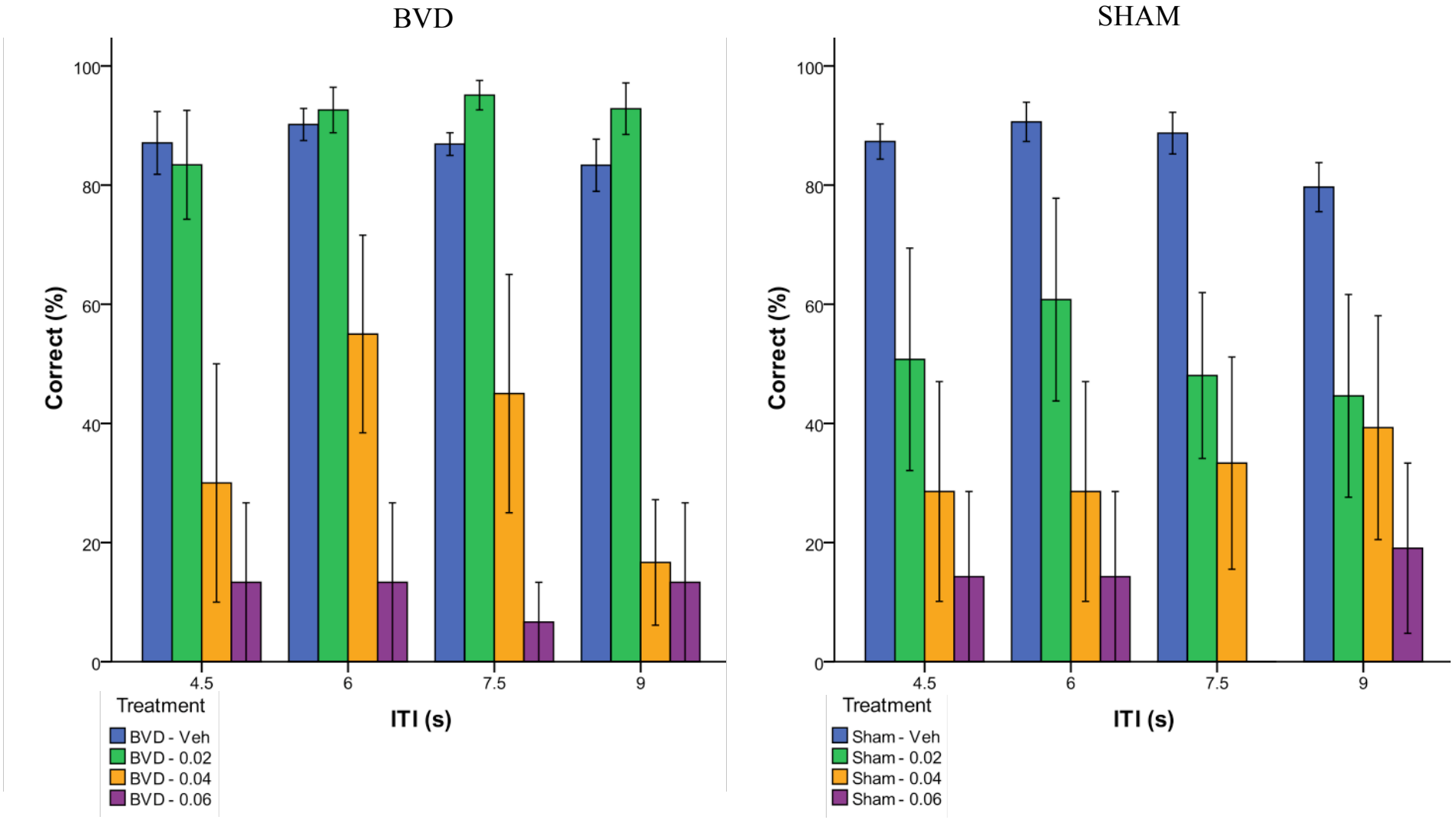


Figure 3.8: Percentage of correct responses recorded in variable ITI 5-CSRTT showing the effect of surgery (BVD and Sham) and eticlopride drug treatment at different ITIs (4.5, 6, 7.5 and 9 seconds). Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.2.

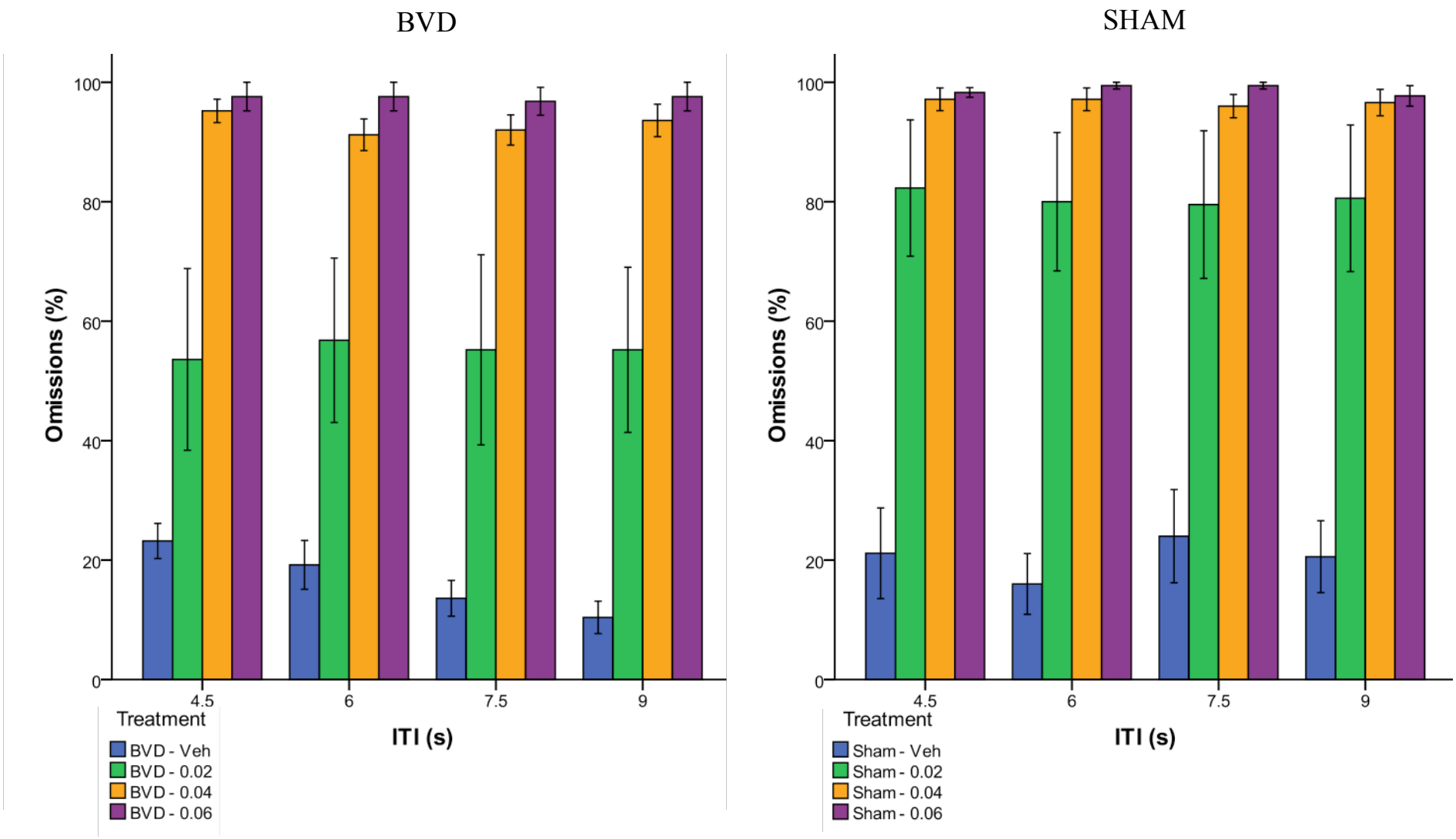


Figure 3.9: Percentage of omissions recorded in variable ITI 5-CSRTT showing the effect of surgery (BVD and Sham) and eticlopride drug treatment at different ITIs (4.5, 6, 7.5 and 9 seconds). Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.2.

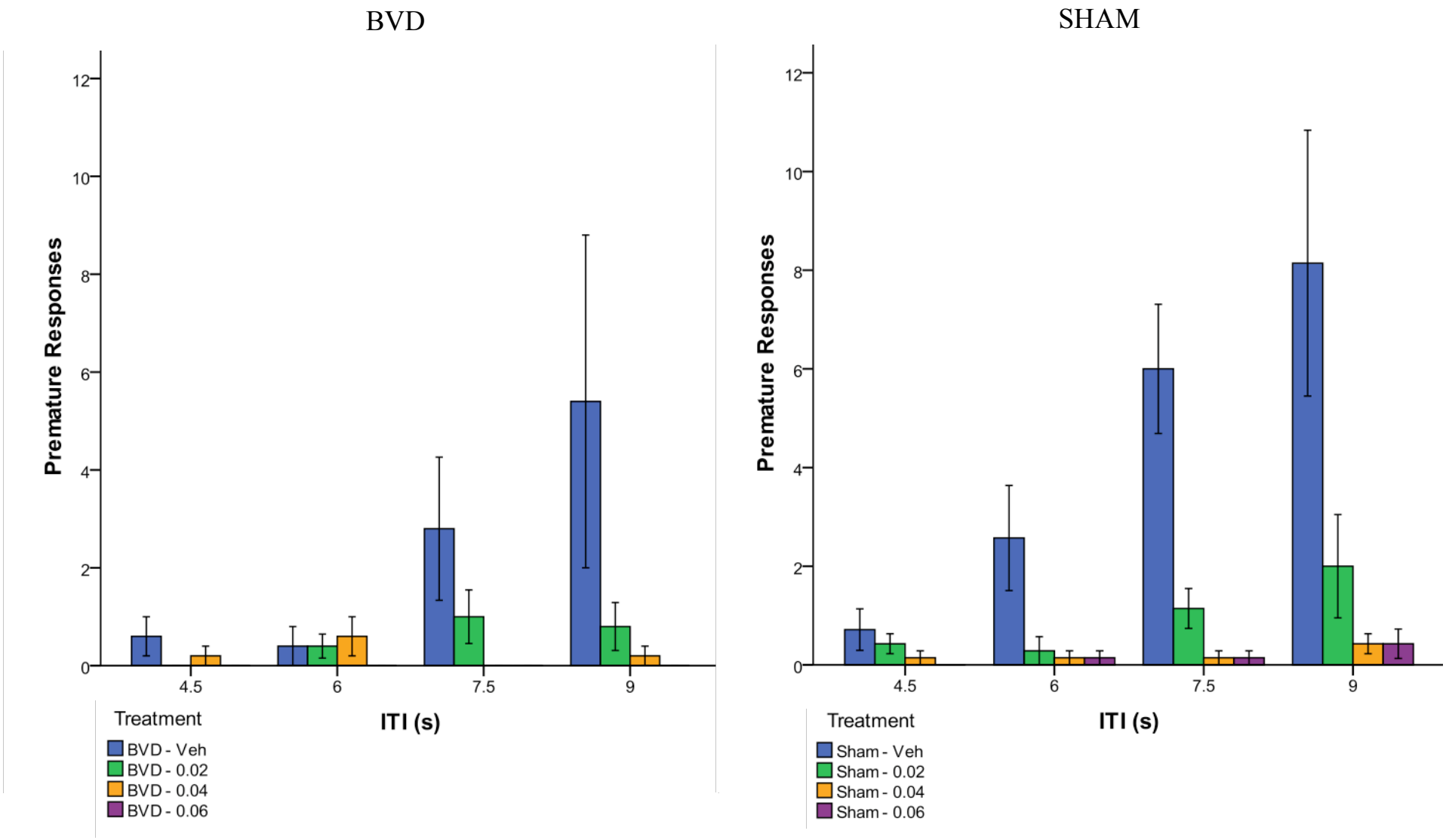


Figure 3.10: Number of premature responses recorded in variable ITI 5-CSRTT showing the effect of surgery (BVD and Sham) and eticlopride drug treatment at different ITIs (4.5, 6, 7.5 and 9 seconds). Drug treatment includes vehicle (veh) and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.2.

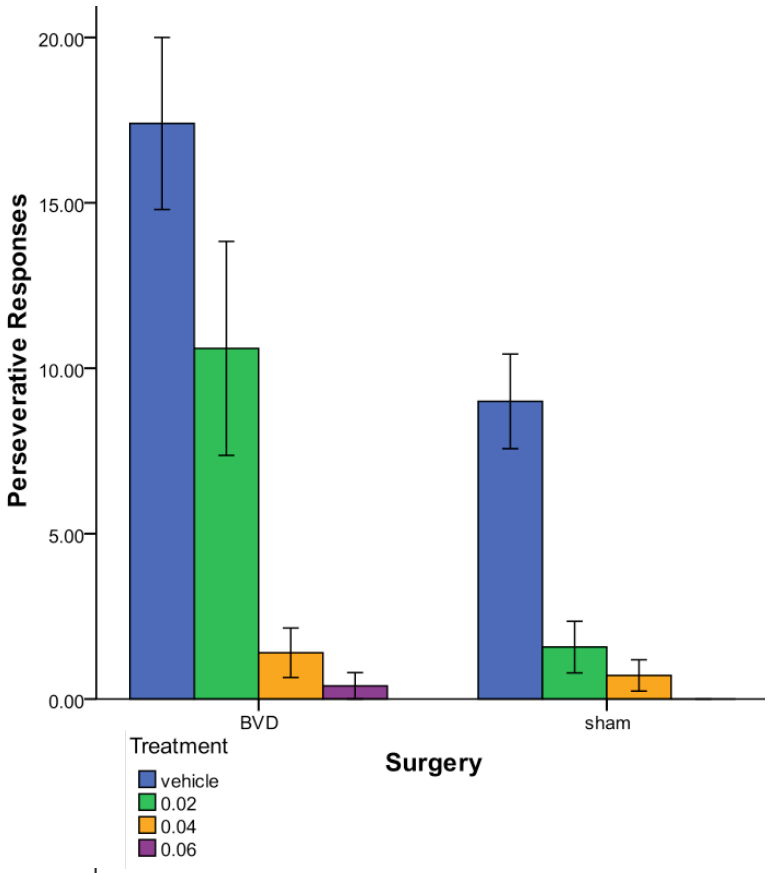


Figure 3.11: Total number of perseverative responses recorded in all periods of variable ITI 5-CSRTT showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

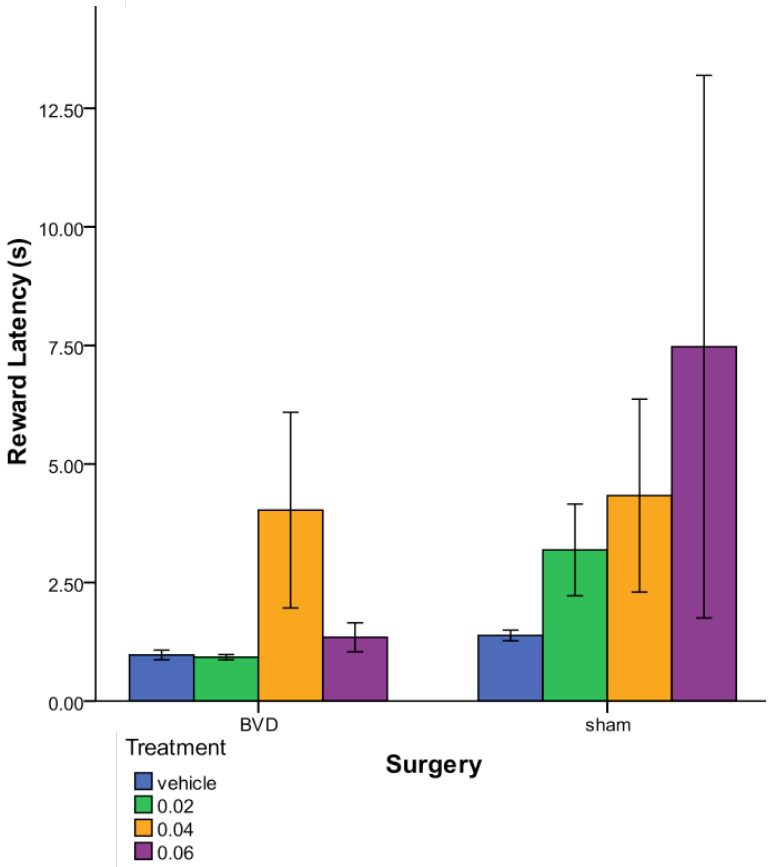


Figure 3.12: Latency to collect reward recorded in all periods of variable ITI 5-CSRTT showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

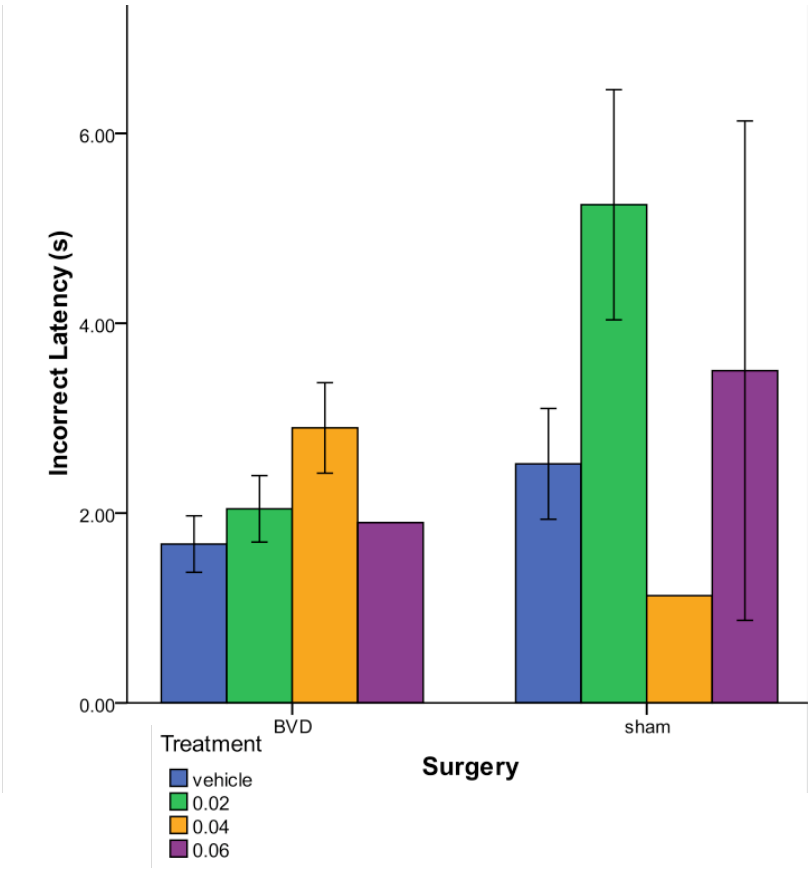


Figure 3.13: Latency to make an incorrect response recorded in all periods of variable ITI 5-CSRTT showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

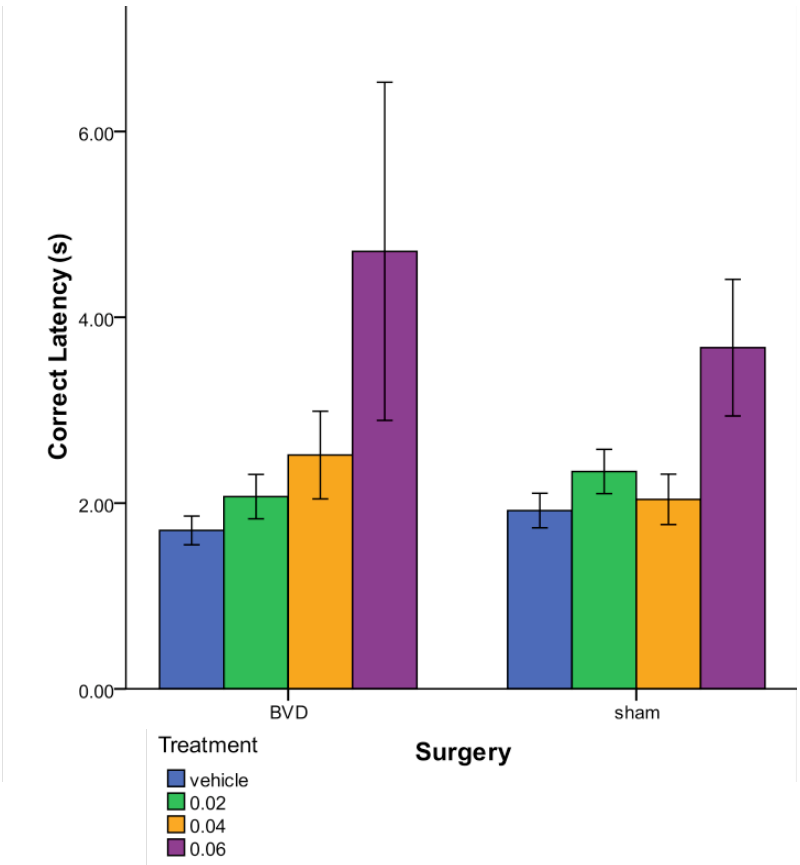


Figure 3.14: Latency to make a correct response recorded in all periods of variable ITI 5-CSRTT showing the effect of surgery (BVD and Sham) and eticlopride drug treatment. Drug treatment includes vehicle and eticlopride doses of 0.02, 0.04 and 0.06 mg/kg. Data points are represented as mean ± SEM. For statistical analysis see Results section 3.1.

As with the 5 second ITI interval, a significant difference was seen, due to both the surgery ($F(1,19.5) = 21.8$, $p < 0.001$) and the drug treatment ($F(3,12.3) = 36.7$, $p < 0.001$), in the number of perserverative responses (see Figure 3.11) in the long variable ITI trials. BVD rats showed significantly more perserverative responses compared with shams. Vehicle treatment resulted in significantly more perserverative responses than all other treatments (pairwise comparison = $p < 0.02$ for all) and the 0.02 mg/kg drug condition showed increased perserverative responses compared with 0.04 and 0.06 mg/kg doses (pairwise comparison = $p < 0.02$ and $p < 0.01$ respectively; see Figure 3.11). A significant drug × surgery interaction was also seen, with BVD rats having significantly more responses at the 0.02 mg/kg dose than sham rats ($F(3,12.3) = 12.3$, $p = 0.008$; see Figure 3.11).

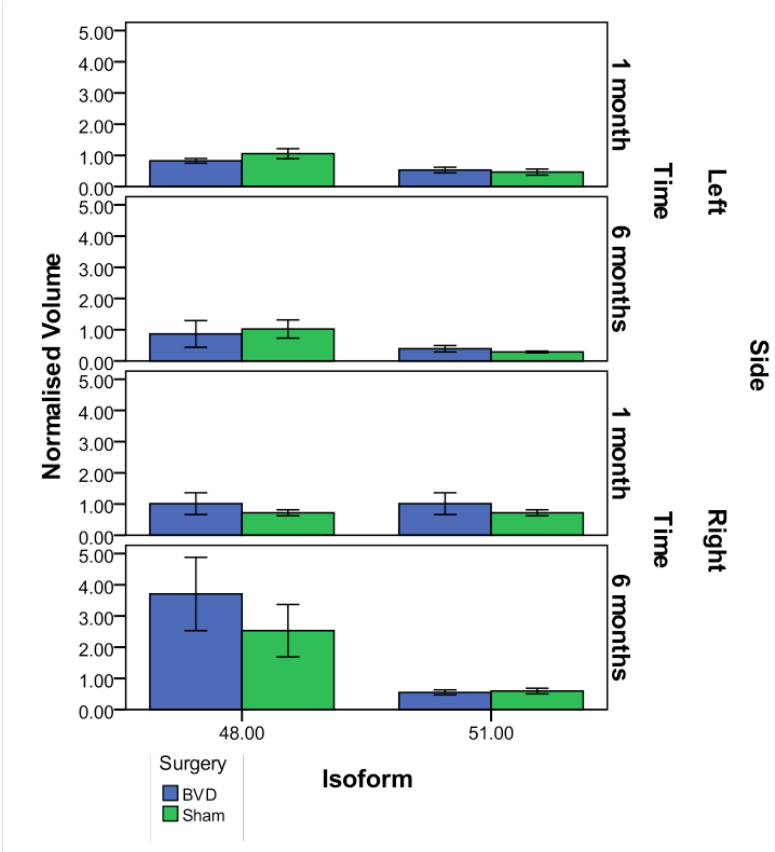
While there was no significant difference seen in relation to reward latency (see Figure 3.12), there was a significant drug × surgery interaction for the incorrect latency ($F(3,8.4) = 4.6$, $p = 0.035$; see Figure 3.13). There was a significant drug effect on the latency to make a correct response ($F(3,9.4) = 6.3$, $p = 0.021$; see Figure 3.14), but no significant differences in the pairwise comparisons.

3.3 Western Blotting

Analysis of the western blotting results showed that surgery had no significant effect on the amount of the D_2 dopamine receptor in the striatum or the frontal cortex (see Figure 3.15 and 3.16). A significant side effect was found ($F(1,41.7) = 47.2$, $p < 0.001$) with more D_2 receptor found in the right side of both the striatum and the frontal cortex and a significant interaction between side and time ($F(1,41.8) = 50.5$, $p < 0.001$) (see Figure 3.16). A

significant 3-way interaction was seen involving side × time × isoforms ($F(1,56.9) = 4.2$, $p = 0.045$) and area × time × isoforms ($F(1,45.3) = 5.4$, $p = 0.024$) as well as a 4-way interaction involving side × area × time × isoforms ($F(1,42.3) = 16.1$, $p < 0.001$). These interactions all show a significant change in the amount of receptor in the 48 kDa isoform on the right side at 6 months post surgery particularly in the striatum. There were no other significant interactions. The combination of the volumes of the two isoforms (results not shown) showed no other significant differences.

(A)



(B)

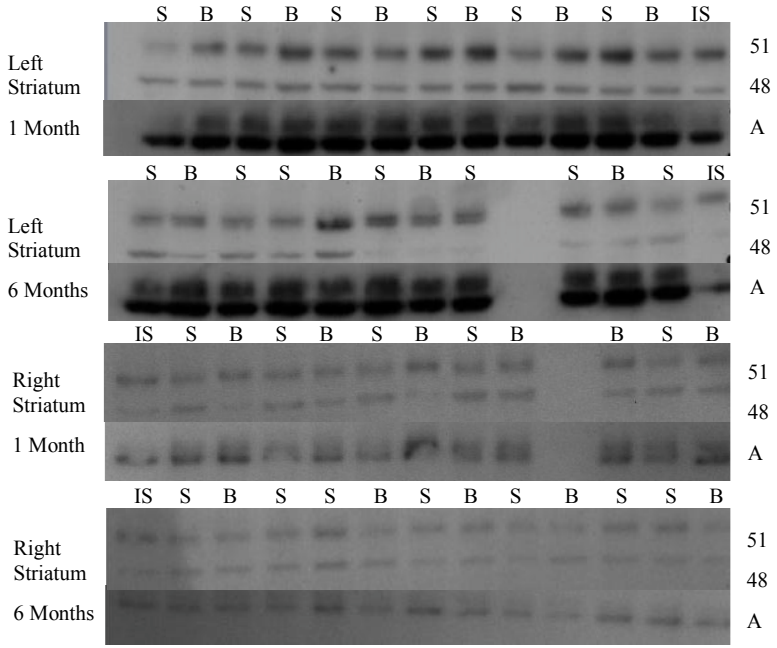
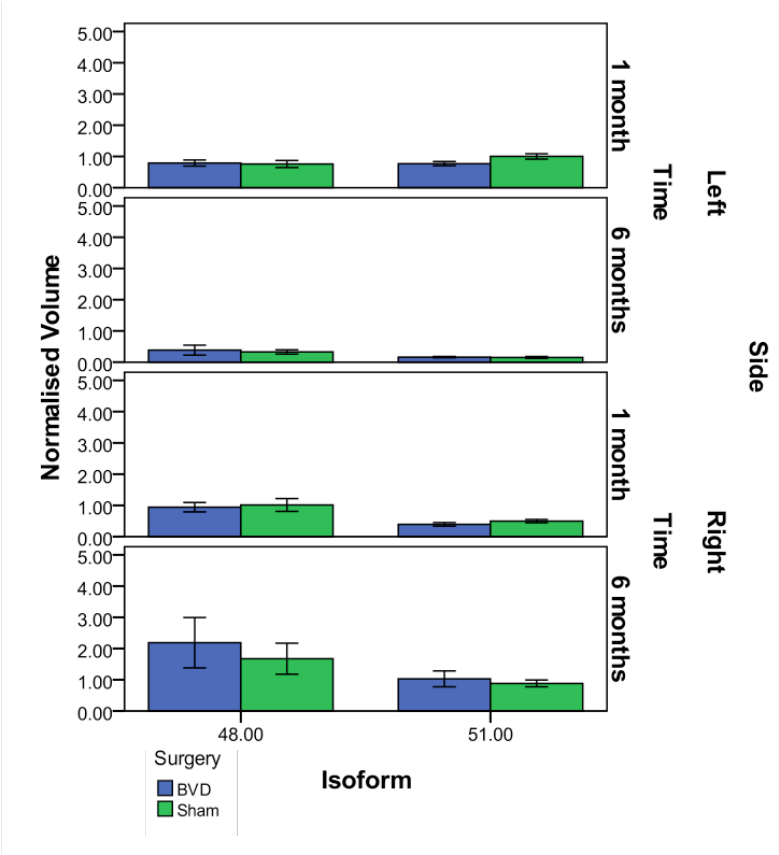


Figure 3.15: Changes seen in the amount of the D_2 dopamine receptor isoforms following BVD or sham surgery at 1 month and 6 months post surgery in the striatum. (A) Graphs show the volume of D_2 dopamine receptor isoforms normalised as a percentage of the actin volume and then divided by the volume of the internal standard. Data points are represented as mean ± SEM. (B) Western blots showing the target proteins D_2 dopamine receptor (51 (long isoform) and 48 (short isoform) kDa) and actin (A, 42 kDa). Well Labels B =BVD, S = Sham and IS = Internal Standard.

(A)



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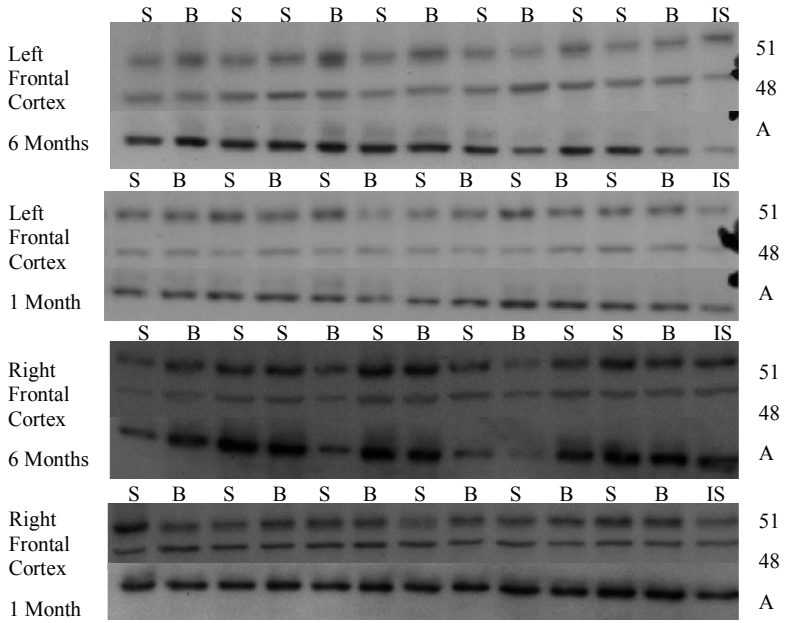

Figure 3.16: Changes seen in the amount of the D_2 dopamine receptor isoforms following BVD or sham surgery at 1 month and 6 months post surgery in the frontal cortex. (A) Graphs show the volume of D_2 dopamine receptor isoforms normalised as a percentage of the actin volume and then divided by the volume of the internal standard. Data points are represented as mean ± SEM. (B) Western blots showing the target proteins D_2 dopamine receptor (51 (long isoform) and 48 (short isoform) kDa) and actin (A, 42 kDa). Well Labels B =BVD, S = Sham and IS = Internal Standard.

Chapter 4: Discussion

The aim of this study was to investigate changes in locomotor behaviours and impulsivity following bilateral vestibular deafferentation (BVD) surgery in rats compared to sham controls, before and following treatment with the D_2 dopamine receptor antagonist, eticlopride. These behavioural changes are well recognised in most vestibular research but have only been studied in a limited capacity.

4.1 Behavioural Changes Following BVD Surgery

In the open field maze, BVD rats were found to display many behavioural traits seen in vestibularly deficient rats, including a significant increase in the distance travelled compared to sham animals. An increase in circling behaviour was also recorded as has been seen in previous studies (Basile *et al.*, 1999; Cryns *et al.*, 2004; Fedrowitz *et al.*, 2003; Fedrowitz *et al.*, 2000; Igarashi *et al.*, 1983; Russell *et al.*, 2003; Vidal *et al.*, 2004; Zheng *et al.*, 2006). However, unlike the genetic animal models, as a group BVD rats in the present study were not found to have a preferred direction for turning. The reason for this is unknown but it could be because of the differences in dopamine release and dopamine receptor expression in animal models. Circling behaviour has been linked to imbalances in dopamine release from the substantia nigra (Schwarting *et al.*, 1996). In one genetic model, *ci2/ci2* rats have been found to have a decrease in the amount of dopamine in the striatum ipsilateral to the preferred direction of turning (Fedrowitz *et al.*, 2000). When analysed separately, individual BVD animals also did not show a preference for direction of turning. The differences in these results may be due to the confounding effects of the genetic deficits those genetic models possess. For example, $Isk^{-/-}$ mutant mice have been shown to have deficits in cardiovascular and renal function (Vidal *et al.*, 2004). It is possible that the changes that cause the vestibular loss in these models may also cause changes in other physiological systems and these effects may then confound any study of the effects of vestibular loss in these animals.

The present study did not find a significant difference between the movement velocity of BVD and sham animals in the open field maze. This result is different from that seen previously by Goddard *et al.* (2008). The main reason for this difference is likely to be due to the way that velocity was calculated in the two studies. The present study calculated velocity as the mean velocity over 1 minute time periods, however Goddard *et. al.* (2008) calculated the velocity as the distance travelled over the time it took to travel and therefore did not include periods of non-movement. Consequently, this produced the mean travelling velocity as opposed to the mean velocity overall given in this study. It is therefore possible that the present BVD rats did have increased velocities compared with shams but that it was not detected. BVD rats did however show significantly longer and more frequent periods of high mobility in this study, suggesting that they produced more body movements like head weaving even when in a stationary position.

BVD rats were shown to be no more impulsive (as shown by the number of premature responses) than sham animals in the 5-CSRTT. However, in the same task BVD rats were more compulsive, as shown by the number of perseverative responses. Increases in perseverative responses have been linked to the prelimbic-intralimbic cortex (Passetti *et al.*, 2002). Following lesions of the prelimbic-intralimbic cortex there was a significant increase in the number of perseverative responses but no change in premature responses; however, when the entire medial prefrontal cortex was lesioned, an increase in premature responses was also seen (Passetti *et al.*, 2002). These results suggest that there may be a specific dysfunction in this region in BVD rats post-surgery.

The causes of the behavioural changes seen in BVD rats are currently still unknown but due to their immediate onset post surgery (Goddard *et al.*, 2008), it is reasonable to assume that the changes are due to altered signalling from the vestibular labyrinth and VNC due to the loss of vestibular information. This change in signalling would then cause changes in D_2 receptor activation and therefore possible receptor changes due to glycosylation and down regulation of surface expression.

4.2 D_2 Dopamine Antagonism in BVD Rats

The present study was the first to test the response of BVD rats to D_2 receptor inhibition. The higher doses of the D_2 receptor antagonist inhibited most behaviours in both the open field maze and the 5-CSRTT in both BVD and sham rats. This appears to be due to a sedative effect of eticlopride. Some D_2 receptor antagonists have been shown to produce catalepsy in healthy animals at high doses (Sanberg *et al.*, 1988), which have also been seen in vestibularly deficient stargazer rats after treatment with haloperidol (Brock *et al.*, 1996). Cataleptic animals (including humans) are unable to correct an unusual posture due to muscle rigidity (Sanberg *et al.*, 1988). Catalepsy was not tested for specifically in this study. However, observations of the animals' behaviour during testing periods suggest the animals were not experiencing catalepsy. When animals were placed into a test environment they would move around before settling into a particular location and once removed from the environment and returned to the carry cage, they would freely move around again. These observations suggest that animals were not showing classic signs of catalepsy such as muscle rigidity and loss of the righting reflex. The assessment of these rats is supported by a study of the cataleptic and sedative effects of eticlopride. Ferrari (1995) found that over a range of doses (0.01 – 0.12 mg/kg), healthy Long Evans rats had dose dependent levels of sedation

compared to saline, but no catalepsy was detected. These results are relevant to the present study as the eticlopride doses used were within this range.

Using the 5-CSRTT as a measure for impulsivity in rodents, van Gaalen *et. al.* (2006a) found that blockage of the D_2 dopamine receptor did not affect the number of premature responses and therefore did not change impulsivity in naïve healthy animals. This result differs to that seen in the present study, which showed a significant decrease in the number of premature responses in both BVD and sham rats when treated with eticlopride. One significant difference between the two studies is that the doses used in the present study were lower (0.02, 0.04 and 0.06 mg/kg) than those used by van Gaalen *et al* (0.06, 0.08 and 0.1 mg/kg). The difference was due to the fact that we found that the 0.06 mg/kg dose used by van Gaalen *et al* resulted in sedation of the animals in the present study and they therefore did not perform the task; therefore, lower doses were used for the study. Van Gaalen *et al* did not see this level of sedation at this dose and it is possible that the difference in injection techniques resulted in a different distribution of the drug. There is also a chance that the drug used was compromised at some time during preparation for one of the studies. However, this would not explain the difference seen in the changes in the number of premature responses. The original van Gaalen *et al* finding was replicated in a second study by the same group in which eticlopride alone was administered to naïve rats and there was no change in premature responding (van Gaalen *et al.*, 2006b). The conflicting nature of the results between the van Gaalen studies and the present study suggests that further investigation into the control of impulsivity by D_2 dopamine receptors is needed.

The main drug effect seen in the present study was a decreased response to the low dose (0.02 mg/kg) of eticlopride in BVD animals compared to shams. The decrease in response

was seen in measured variables in both the open field maze and the 5-CSRTT. This response to dopamine receptor inhibition was previously seen in vestibularly deficient stargazer rats following treatment with haloperidol (Brock *et al.*, 1996). In stargazer rats, a 0.1mg/kg dose of haloperidol produced no catalepsy whereas there was significant catalepsy in their wild-type littermates. When given a higher dose of haloperidol (0.3 mg/kg), catalepsy was significantly increased compared to vehicle in both the stargazer and wild-type rats. In the only other study to look at the effect of dopamine antagonism in rats with vestibular dysfunction, there was no dose dependent change in response (Schirmer *et al.*, 2007b). Brock *et al* (1996) suggested that the cause of the change in drug response was due to a genetic change in the central dopaminergic system in the stargazer rats. This hypothesis now seems less likely as the same result is seen in rats following surgical deafferentation. If the change in drug response was a genetic effect, then it would not be seen in the surgical model of vestibular loss.

A search of the dopamine literature did not find any studies that had been performed in the manner of the present study. Most have looked at the effect of antagonism on a single variable i.e., the dosing of cocaine. This does not allow comparable results as the present study looked at the effect on the different surgical groups. The lack of data makes explaining the current results difficult.

4.3 Neurochemical Measures

Western blotting showed there were no significant differences in the amount of the D_2 dopamine receptor in the striatum or frontal cortex on either side of the brain following BVD surgery when compared to shams. These results differ from a study conducted by Giardino *et al* (1996) in which they found that at 1 month following BVD surgery in rats there was a

significant decrease in the amount of D_2 receptors in the striatum. The variability between the results in the present study and that of Giardino *et al* (1996) as well as other studies using different models of vestibular dysfunction (Giardino *et al.*, 1996; Richter *et al.*, 1999; Schirmer *et al.*, 2007a; Seth *et al.*, 1982), suggests that it is possible that changes in the amount of the D_2 dopamine receptor in the striatum of rats may be compensatory due to other changes in the brain, and that these changes are not consistent among different breeds and models.

While there was no surgery effect on the levels of the D_2 receptor in the areas examined there was an increase in the amount of D_2 receptor between the 1 month and the 6 month time points on the right side of the brain. This increase was seen particularly in the 48 kDa isoform. While it is possible that the increase was simply due to aging it is also possible that it could be explained by the eticlopide treatment as it has been shown that rats that are treated with haloperidol have an increased expression of the short (48 kDa) isoforms of the D_2 receptor in the anterior pituitary (Arnauld *et al.*, 1991). This explanation would explain why the difference is seen in both BVD and sham rats as both were given identical treatment.

The lack of change in the receptor levels is consistent with the hypothesis that due to the sudden onset of behaviours post surgery, it is more likely that the behaviours seen in BVD rats are due to the changes in the vestibulo-striatal pathways causing changes in the activation and efficacy of receptors than changes in receptor levels.

4.4 Overall conclusions

This study demonstrated that BVD rats show most but not all locomotor behaviours seen in previous studies, however the mechanisms behind these behaviours are still unknown. Also, while inhibition of the D_2 dopamine receptor did not eliminate the behaviours seen, suggesting that the behaviours are not the result of a classic hyperkinetic disorder, the difference in drug response between BVD and sham animals suggests some surgical effect on the basal ganglia. As there was no change in the amount of D_2 dopamine receptor in the striatum in rats following BVD surgery, the reason for the change in drug response in the rats could be due to a number of different causes. It is possible that changes did occur but they were region-specific in the striatum and western blotting analysis was not specific enough to detect the changes. A specific neuron population that could undergo changes in D_2 receptor levels are cholinergic interneurons. In the striatum these interneurons fire tonically (Bennett *et al.*, 1999; Pisani *et al.*, 2007) and have been shown to affect the γ-aminobutyric acidergic (GABAergic) medium size spiny neurons (Tepper *et al.*, 2004), the major striatal projection neurons in the striatum, which account for 90-95% of striatal neurons (Kemp *et al.*, 1971). This population of cholinergic neurons is regulated by a number of receptors, including the D_2 dopamine receptor (Pisani *et al.*, 2007). A specific change in these D_2 receptors could cause a change in striatal activity. A more region-specific analysis using a method like immunohistochemistry would allow for these changes to be detected.

The change in response to D_2 receptor antagonism in BVD rats with no change in the amount of D_2 receptors gives some indication about what could be occurring in the rats. There may be a change in the response of the D_2 receptor, resulting in changes in drug response and/or there may be changes occurring at a different part of the motor loop and the decreased response is due to compensatory mechanisms.

The decrease in drug response in BVD rats may be due to changes in the pharmacodynamics of the receptors. Changes affecting the binding of the drug to the receptor, i.e. reduced affinity, would therefore produce the decrease in drug response seen in BVD rats. Another option is that the drug response is due to a change in the efficacy of the receptors once dopamine has bound to them. If dopamine were binding with a higher affinity or producing a larger response then it is possible that the blockade of the receptors by the antagonists was insufficient to decrease the response.

Another possibility is that there is a change in firing of neurons in the substantia nigra and that this could result in increased dopamine release in the striatum and therefore lead to increased competition for receptor binding sites. Increased cell firing rates in the substantia nigra have been seen bilaterally in *ci2/ci2* rats irrespective of the preferred direction of turning (Fedrowitz *et al.*, 2003). However, this has never been studied in BVD rats. This change would be the similar to that resulting from changes in affinity and efficacy as an increase in dopamine concentration would lead to an increase in receptor activation due to competitive binding at the receptor. Electrophysiological studies of the activation of the substantia nigra as well as the striatum, and measuring the levels of striatal dopamine, would allow the measurement of these changes if present.

While it could be expected that changes in activation of the D_2 dopamine receptor would cause compensatory changes in the receptor number, it is possible that changes are occurring in other non-dopaminergic sites to compensate for the changes in dopaminergic activity. Dopamine release into the striatum from the substantia nigra acts upon GABAergic output neurons (Alexander *et al.*, 1990; Alexander *et al.*, 1986; Delong, 2000) as well as playing a

role in the control of interneurons (Pisani *et al.*, 2007; Tepper *et al.*, 2004). It is possible that changes in dopamine activity are causing changes in these neurons and therefore when treated with eticlopride, the blockade of neuronal activity accounts for the change in drug response.

Another option is that the dopaminergic system itself is not changed following BVD surgery and changes in the function of other receptors in the striatal motor loop could be causing changes seen in behaviour. Receptors for neurotransmitters including GABA and acetylcholine play dominant roles in the striatum and it may be that changes in these receptors are causing the behavioural changes displayed in BVD rats. GABA is the main neurotransmitter in the striatum as more than 90% of striatal neurons have been shown to be GABAergic (Bolam *et al.*, 2000; Kemp *et al.*, 1971). While these projection neurons are involved in the relay of information though the striatum, there is also another important group of GABAergic neurons involved in the control of the motor loop. GABAergic interneurons account for only a small number of neurons in the striatum but due to connections to both input and output neurons in the striatum (Bennett *et al.*, 1994; Bolam *et al.*, 2000), they play an important role in striatal function. These neurons receive information from the cortex (Bennett *et al.*, 1994) and are therefore in a position to control the activity of neurons in the direct and indirect pathways and could be responsible for the changes in BVD behaviours. Studies into the changes in the activity of these neurons and of the GABA receptors in the motor loop would allow this to be determined. Regulation of the firing of GABA neurons in the striatum by the release of dopamine from the substantia nigra could be responsible for the difference in response to eticlopride treatment between BVD and sham rats.

Following BVD surgery, rats have been shown to demonstrate a number of behaviours including increases in locomotor activity, circling and compulsions. Antagonism of the D_2

dopamine receptor was found not to inhibit these behaviours. However, there was a dose dependent decrease in the response to D_2 antagonism in BVD rats compared to shams in these behaviours as well as others. These results suggests that while there may not be a direct link between the D_2 dopamine receptor and the behavioural changes, there is an as yet unknown change in the activity of the striatal dopaminergic system following BVD. Further studies into different striatal neuron populations including GABAergic and cholinergic neurons as well as further studies into the changes in the dopaminergic system may aid in identifying the cause of the behavioural changes in BVD rats.

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