Detection of Behavioural and Cognitive Dysfunction in Mucopolysaccharidosis IIIA Affected Dogs.

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Abstract

This study investigated whether behavioural and cognitive dysfunction caused by mucopolysaccharidosis (MPS) IIIA can be detected early in affected dogs' lives, and to describe the behaviours of these dogs. No other scientific papers have been published on this topic and the population of dogs examined in this study are the only MPS IIIA affected dog colony available worldwide for study.

Three main tests were performed on the population of MPS IIIA affected dogs. Physical behavioural assessment tests were performed at six and eight weeks of age and from twenty weeks of age a cognitive function task was taught and then tested to measure the dogs' performance. A previously validated questionnaire, the canine behavioural assessment and research questionnaire (C-BARQ), was completed at three, six and twelve months of age. The researchers in these studies were blinded to the MPS IIIA status of the dogs examined.

The behaviours shown by the MPS IIIA puppies at six and eight weeks of age were not significantly different from the behaviours of the unaffected puppies. This finding supported the research of other MPS IIIA studies and suggests that clinical behavioural changes do not occur at such a young age. The behaviours shown by the MPS IIIA affected puppies appeared to be normal puppy behaviours similar to those described in previous research on puppies.

The C-BARQ measured the behaviours shown by the MPS IIIA affected and unaffected dogs. Most of the MPS IIIA affected dogs' behaviours were not significantly different from the unaffected dogs' behaviours, but MPS IIIA affected dogs did retrieve significantly more than unaffected dogs at three months of age, and were less distractible at twelve months of age. It would be worth investigating these findings further to decide whether it suggests a subtle alteration in brain functioning.

The cognitive function test showed a significant decrease in the success of the MPS IIIA affected dogs in the final maze test. This is the first study on dogs affected with MPS IIIA to find a decline in cognitive function before the occurrence of cerebellar clinical signs and this new knowledge may lead to future developments measuring therapy response and disease progression. The T-shaped maze testing may be valuable in future research on cognitive function in dogs with other diseases such as epilepsy. Thus this thesis provides valuable information on canine MPS IIIA and provides a foundation for future disease investigations.

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Ethics Approval:

This project has been reviewed and approved by the Massey University Animal Ethics committee, Palmerston North, Protocol 05/01.

CHAPTER 1

Introduction

Mucopolysaccharidosis (MPS) IIIA is an inherited lysosomal storage disease which is characterised by severe central nervous degeneration. Children with this condition typically develop progressive neurodegeneration and their life expectancy is greatly diminished. Naturally occurring MPS IIIA occurs in a colony of Huntaway dogs in New Zealand, and these dogs typically develop obvious neurological signs of the disease at approximately one and a half years of age. This thesis is part of an overall programme to produce a working animal model of MPS IIIA so that new treatments for the disease can be tested on the dogs. This may lead to effective treatment of the disease in children.

The main hope for developing therapies for MPS IIIA is based on animal models. Animals have a different clinical presentation of the disease to that seen in humans (Bhaumik et al., 1999; Jolly et al., 2000). More investigation interpreting and understanding the animal models is needed. MPS IIIA mouse and dog models provide significant possibilities for the development of therapies. In recent years the development of these models has advanced significantly (Bhattacharyya et al., 2001; Hemsley et al., 2008; Jolly et al., 2000; Jolly et al., 2001; Jolly et al., 2007; Meyer et al., 2007a; Meyer et al., 2007b; Yogalingam et al., 2002).

The dogs used in this study are in the only MPS IIIA affected dog colony in the world and thus information gathered in this study may be extremely valuable (Jolly et al., 2000). If MPS IIIA affected dogs can be identified when the animal is young this may lead to the development of more effective therapies. This possibility has led to this

thesis in which the behavioural signs and cognitive function in MPS IIIA affected dogs are monitored.

The main objective of this thesis was to detect early behavioural and cognitive dysfunction indicative of MPS IIIA in young dogs so as to enable the more effective use of the MPS IIIA dog model.

The thesis consists of five chapters. The literature review in chapter one looks at MPS and the measurement of behaviour and cognitive function. The lysosomal storage diseases including MPS are described, and clinical signs and diagnosis discussed. Details on MPS IIIA diagnosis, treatment and therapy are explored. The second part of chapter one reviews the literature on the measurement of behaviour and cognitive function in dogs.

The low numbers of studies on behaviour and cognitive function in MPS IIIA dogs led to the tests performed in chapters two, three and four. Behavioural tests were performed at six weeks, eight weeks, three months, six months, and twelve months. A cognitive function test was also carried out to compare MPS IIIA affected and unaffected dogs and to detect any decline in cognitive function in the affected animals.

Chapter two reports on behavioural tests carried out on six and eight week old MPS IIIA affected and unaffected puppies. Chapter three describes the use of a previously tested behavioural questionnaire on these same dogs at three, six and twelve months. Chapter four reports on cognitive function in these dogs through the use of a maze test. Chapter five is a general discussion of the results of all three studies.

Literature Review

1.1 Introduction

Children affected with MPS IIIA are born clinically normal with no signs of the severe and inevitably fatal problems they will develop. They are deficient in the enzyme N-sulphoglucosamine sulphohydrolase (sulphamidase) which degrades heparan sulphate glycosaminoglycans. It is the accumulation of glycosaminoglycans which usually causes clinically evident signs after the first year of life but the increase begins very early after conception. In the second trimester of the foetus' life intracellular inclusions are already evident (Ceuterick et al., 1980).

There is no treatment to stop this inevitable accumulation of glycosaminoglycans and progressive neurodegenerative signs develop. The development of new treatments for MPS IIIA is centered around the use of animal models and a thorough knowledge of the clinical progression of the disease in mice and dogs is essential (Hemsley et al., 2008).

1.2 Lysosomal Storage Diseases (LSDs) and Mucopolysaccharidoses

The lysosome is a membrane surrounded acidic organelle which is essential in the degradation and recycling of both exogenous and endogenous molecules. This organelle is of extreme importance in cell physiology. LSDs occur when there is a deficiency of specific lysosomal proteins, and the effects on the organism are often catastrophic.

More than 40 LSDs have been described (Scriver et al., 2001) including MPS. Gaucher disease was the first LSD described in 1882 but it was not recognised as such at that time (Grabowski, 2008). Pompe disease was identified in 1963 as the first recognised LSD. A seven month old girl was presented to Pompe after she had died of

hypertrophic cardiomyopathy and he identified that she had accumulated glycogen in her tissues (Merk et al., 2009).

MPS are a group of lysosomal storage diseases which typically cause severe disease in young children. They occur when there is a deficiency of proteins which degrade glycosaminoglycans. Clinical disease occurs due to glycosaminoglycan accumulation in lysosomes leading to widespread dysfunction, and to the excretion of some partially degraded glycosaminoglycans in urine. These conditions lead to the death of affected children.

MPS cause severe and rapidly progressive disease in affected individuals. They occur naturally in man, dogs, cats, rats and goats and in mice through genetic engineering (Neufeld & Muenzer, 2001). There are eleven different enzyme deficiencies in this group of disorders. These deficiencies lead to seven different MPS (Table 1.1).

Table 1.1:   Mucopolysaccharidosis types and corresponding enzyme deficiencies

MPS Type	Subtype	Enzyme Deficiency
MPS I		α-L-Iduronidase
MPS II		Iduronate sulphatase
MPS III	A	Heparan *N*-sulphatase
	B	α-*N*-Acetylglucosaminidase
	C	Acetyl-CoA:α-glucosaminide acetyltransferase
	D	*N*-Acetylglucosamine 6-sulphatase
MPS IV	A	*N*-Acetylgalactosamine 6-sulphatase
	B	β-Galactosidase
MPS VI		*N*-Acetylgalactosamine 4-sulphatase
MPS VII		β-Glucuronidase
MPS IX		Hyaluronidase

Adapted from Neufeld and Muenzer (2001)

Except for MPS II these disorders are inherited in an autosomal recessive fashion. Metabolism which occurs within cells is facilitated by degradative lysosomal enzymes and when these enzymes are deficient glycosaminoglycans accumulate. MPS I to VII involve the lack of one or more of ten specific enzymes. There is also a new condition called MPS IX which involves the lack of hyalronidase. There has only been one human case of MPS IX although there is a mouse model of this disease (Martin et al., 2008).

1.3   Clinical signs and diagnosis

MPS affected individuals are born without clinical signs and routine neonatal screening does not detect these conditions. Nowadays most testing occurs later in the patient's life once they are showing clinical signs.

Glycosaminoglycan accumulation causes progressive organ dysfunction. Symptoms for these disorders are similar in many cases. Multiple organs are involved with organ enlargement, coarse facies and dysostosis multiplex being common (Neufeld & Muenzer, 2001). Individuals with MPS are clinically normal when born. Their first year of life is normal and signs appear at varying times later in life.

Neuro-cognitive deterioration is the main clinical sign seen in these diseases. Disease progression in MPS affected mice is very similar to that in humans (Gliddon & Hopwood, 2004). These studies have suggested that some similarities between the animal models and the human models exist.

 To date there are no tests that determine the severity of disease or responsiveness of the disease to treatment (Randall et al., 1980).

When therapies are being developed it is necessary to have an accurate measure of the stage of the disease and ability of the individual with MPS. Thus a sensitive measure is needed if improvements due to therapy are to be measured. Various tests have been used. For MPS I a physical performance measure has been developed (Haley et al., 2006).

MPS occurs in a number of species apart from humans and the symptoms are different in each species. MPS I occurs in mice and causes memory deficits and cognitive impairment. Affected mice show a reduction in exploratory behaviour and long term memory deficits (Reolon et al., 2006).

1.3.1   MPS III

MPS III (Sanfilippo syndrome) occurs infrequently in people at a rate of 1 birth in 63700 in Germany (Meyer et al., 2007a). There are four subtypes in this group namely subtypes A to D. These subtypes are differentiated by the deficiency of one of four enzymes involved in the breakdown of heparan sulphate.

Some of these subtypes are more prevalent than others. In Australia MPS IIIA is the most common (Meikle et al., 1999) whereas in Greece MPS III B accounts for 81% of all MPS disease seen (Beesley et al., 2004).

The accumulation of glycosaminoglycans leads to lysosome swelling and this leads to deformation of the cell outline. With time and further accumulation this leads to organomegaly. Many of the clinical signs seen are due to this increase in the size of the organs except for the changes seen in the CNS, bone and cartilage.

MPS III is usually detected by characteristic behavioural and neurological signs. These occur most typically when the person is still an infant. The accumulation of heparan sulphate leads to the similar clinical signs in these four subtypes. Severe neurologic degeneration is characteristic with mild somatic disease (Neufeld & Muenzer, 2001). The clinical signs include aggression, hyperactivity, developmental delays, hepatosplenomegaly, sleep disorders, hirsutism and coarse hair (Neufeld & Muenzer, 2001).

In humans sleep disturbances are very common in MPS III. In a survey carried out on 141 people 91.5% of the children with MPS III had sleep disturbances (Fraser et al., 2005). The problems include difficulties in getting to sleep, frequent waking and wandering around at night. The sleep disturbances occurred at the highest frequency between three and five years of age (Fraser et al., 2005).

1.3.2 MPS IIIA

MPS IIIA is an inherited MPS with reduced activity of sulphamidase being the primary problem (Savas et al., 2004). Sulphamidase is required for the degradation of heparan sulphate glycosaminoglycans. Glycosaminoglycan accumulation occurs due to the specific enzyme deficiency and potential therapies have investigated providing the missing enzyme (Gliddon & Hopwood, 2004; Savas et al., 2004).

The prevalence of this disorder in New Zealand is unclear. Nelson (1997) reported an incidence of 0.36 in 100,000 live births in Northern Ireland and Poorthuis et al. (1999) reported the prevalence being 1.16 in every 100,000 live births in the Netherlands. Meikle (1999) reported an incidence of 1 in 114,000 live births in Tasmania.

There is a colony of dogs in New Zealand which is a model for MPS IIIA. These dogs have naturally occurring MPS IIIA and they have been selectively bred to have the disease (Jolly et al., 2000). The dogs are Huntaways and the mutation involved is an insertion of adenosine (Jolly et al., 2007; Yogalingam et al., 2002). The insertion causes a frame shift and the chain terminates at position 228 (Yogalingam et al., 2002).

The mutation was found to occur at a frequency of 3.8% in a sample of Huntaways (Yogalingam et al., 2002). In these dogs the disease occurs with a severe phenotype. The dogs are clinically normal for the first one and a half years of their lives and then they start to show neurodegeneration as evidenced by ataxia. Clinical signs also include high stepping, exaggerated patella, cranial tibial and gastrocnemius reflexes and loss of learned behaviour occurs (Jolly et al., 2000).

MPS IIIA has also occurred in wire-haired Dachshunds (Fischer et al., 1998; Jolly et al., 2001). Two of the dogs were male and female siblings who first showed symptoms at three years of age. The male showed head tilt and nystagmus and the female ataxia. They progressed to severe ataxia of the hind legs and proprioceptive deficits. Coarse tremor of the head was seen but no behavioural changes were evident. The third case was a four year old male who had hind limb ataxia which progressed to include the forelimbs two years later.

MPS IIIA also occurs in mice. Dogs with MPS IIIA have a very different disease progression from that of affected mice (Gliddon & Hopwood, 2004; Jolly et al., 2000). At three weeks of age affected mice show hyperactivity. At sexual maturity affected mice show aggressiveness and their fur starts to become coarse. At the same time they become hunched and their faces become broader. Activity levels decrease and affected mice usually die by one year of age. On autopsy hepatosplenomegaly and distended bladders are seen (Gliddon & Hopwood, 2004). The disease progression and presentation in mice is similar to that in humans affected with MPS IIIA. The neuropathology of murine MPS IIIA has been well documented with it causing lysosomal vacuoles in the glia, neurons and perivascular cells (Savas et al., 2004). It also causes the formation of ectopic dendrites and axonal spheroids.

Central nervous system pathology is the main problem in MPS IIIA. The neurodegeneration leads to cognitive decline and shortens the affected person's lifespan enormously. Alterations in the affected person's brain white matter are correlated with mental retardation (Gabrielli et al., 2004). MPS IIIA is usually detected by six years of age in humans. It has been hard to detect MPS IIIA any earlier than this due to the non-specificity of the clinical presentation seen however early developmental milestones were not met in 74% of patients. In two thirds of MPS IIIA patient's speech was delayed and in one third motor development was delayed (Meyer et al., 2007a). In affected children cognitive function starts declining at three year of age, and at approximately four years of age motor function declines (Meyer et al., 2007a).

The natural progression of the disease has been followed from conception to try to diagnose it as early as possible (Gabrielli et al., 2005; Meyer et al., 2007a). It was found that, 80.3% of the pregnancies were normal, 6.2% had cervical insufficiency, 5.6% had infections, 4.2% vaginal bleeding, 29.2% had had previous miscarriages and 8.5% were born prematurely (Meyer et al., 2007a).

In 38% of patients the first signs of the disease were either sleep disturbances or behavioural abnormalities including aggression, lack of awareness of danger and hyperactivity. These symptoms in humans are similar to the findings in mice (Meyer et al., 2007a).

In humans the first symptoms were seen, on average, at seven months of age, however although the first signs of disease were noticed early (67.6% of patients within the first year) the diagnosis is made on average at four and a half years of age (Meyer et al., 2007a).

This disease progresses to a moderate degree of cortical atrophy. The main clinical signs seen are; progressive dementia, insomnia, sleep problems, short attention span, hyperactivity, temper tantrums, destructive behaviour and aggression, and mental retardation (Neufeld & Muenzer, 2001). Most affected people die in their late teens to early twenties. The median age at the time of death was 15.2 years of age.

MPS IIIA in humans is usually suspected with the detection of the clinical signs mentioned above. Imaging studies may be carried out, including radiographs to detect dysostosis multiplex, magnetic resonance imaging to examine brain structure and ultrasonography to look for organomegaly (Neufeld & Muenzer, 2001). Urinary heparan sulphate excretion is increased and then the diagnosis is confirmed through enzyme level assays from tissue samples and through gene sequencing (Valstar et al., 2008). It is also possible for a diagnosis to be made prenatally by measuring the activity of particular enzymes in chorionic villi cells or amniocytes (Ceuterick et al., 1980).

1.4 Progress in treatment of MPS

1.4.1 Use of animal models

As MPS occurs in humans and other species this has allowed the development of animal models. The two major limitations on the research of MPS in humans are that the disease frequency is low and the onset of disease is slow. This has led to the development of animal models of MPS.

The animal models of MPS are numerous and help in the understanding of disease and in the development of new therapies (Table 1.2). Recent research has focused on therapy for these diseases and a lot of that research depends heavily on the use of animal

models. There is a dog model for MPS IIIB as well as for MPS IIIA (Ellinwood et al., 2003).

MPS has been extensively researched and the use of animal models has provided much of the available knowledge on potential therapies. The disease occurs in a number of species and mice models are especially common (Table 1.2). In mice the signs of disease include aggression, cognitive dysfunction, startle response, and gait alterations. It is extremely useful to have murine models since mice are inexpensive and easy to maintain and produce. Their short life span and the early occurrence of disease lends towards their use in developing therapies.

There are a number of naturally occurring MPS in animals. These naturally occurring mutations are often bred from in order to establish breeding populations for use in research. The first recognised occurrence of MPS in an animal was in a Siamese cat called 'Suzie'. This cat's disease (MPS VI) was recognised at the University of Pennsylvania in 1976 (Cowell et al., 1976; Haskins et al., 2002).

The next two MPS found in animals were also found at the University of Pennsylvania. They were MPS I in a cat and MPS VII in a German Shepherd / Labrador cross (Haskins et al., 2002). Numerous dog models of MPS have been established (Table 1.2). A model of MPS VII was established in German Shepherd dogs (Dombrowski et al., 2004). In these dogs the disease occurs early when the dogs are one month old. The dogs have been used to investigate therapies.

There are also other MPS animal models which have allowed rapid advances in developing potential treatments (Table 1.2). A MPS I genetic mouse model has been developed, and MPS VI is seen as a fairly frequent inherited disease in cats (Crawley et al., 2003; Reolon et al., 2006). There is a naturally occurring mouse-model of MPS III (Bhaumik et al., 1999). In the murine model there are changes in the open field test from three weeks of age (Hemsley & Hopwood, 2005).

Table 1.2:   **Animal Models of Mucopolysaccharidosis**

MPS type	Subtype	Species	Reference
MPS I		Canine	(Menon et al., 1992)
		Feline	(Reolon et al., 2006)
		Murine*	(Clarke et al., 1997)
MPS II		Canine	(Wilkerson et al., 1998)
		Murine	(Muenzer & Fu, 1999)
MPS III	A	Canine	(Jolly et al., 2000)
		Murine	(Gliddon & Hopwood, 2004)
	B	Canine	(Ellinwood et al., 2003)
		Murine	(Li et al., 1999)
		Emu	(Aronovich et al., 2001)
	D	Caprine	(Thompson et al., 1992)
MPS IV		Murine	(Haskins et al., 2002)
MPS VI		Canine	(Neer et al., 1995)
		Feline	(Cowell et al., 1976)
		Murine	(Evers et al., 1996)
		Murine(rat)	(Yoshida et al., 1993)
MPS VII		Canine	(Haskins et al., 1984)
		Feline	(Crawley et al., 2003)
		Murine	(Birkenmeier et al., 1989)
MPS IX		Murine	(Martin et al., 2008)

*Murine refers to mouse unless rat specified.

Various tests are used to measure the alterations in the animals' condition as their disease progresses. In the murine model for MPS VII, the behavioural abnormalities of affected mice have been well described (Chang et al., 1993). These diseased mice spent five to ten percent of the normal amount of time spent on grooming. They also spent about sixty percent of the normal amount of time face grooming when stimulated by a

light mist. In a Morris water maze the mice were poor at remembering a platform's location in the maze, and deficient at developing means of locating the platform.

There is a mouse model and a dog model of MPS IIIA (Fischer et al., 1998; Jolly et al., 2000; Jolly et al., 2001). In the murine MPS IIIA model that has been developed the disease is caused by a problem in the sulfamidase gene with a base substitution at codon 31 (Gliddon & Hopwood, 2004). This causes aspartic acid to be changed to asparagine (Bhattacharyya et al., 2001). These mice also store secondary gangliosides as do the Huntaways in the New Zealand dog model (Jolly et al., 2000).

Various therapies have been trialed on the murine model. Recombinant human sulfamidase has been injected into the CSF of the mice and this led to a reduction in one of the heparan sulphate derived mono-sulphated disaccharides. These treated mice also had improved behaviour (Hemsley et al., 2007)

In the MPS IIIA affected mice cognitive function has been measured by the use of the Morris Water Maze. The affected mice show decreased cognitive abilities by 20 weeks of age (Gliddon & Hopwood, 2004). Scientists used the Morris water maze to gauge the response to therapy.

1.4.2   Current therapies

Although progress in the development of therapies has only been recent it has been very rapid. Enzyme replacement therapy (ERT) is developing especially fast (Galan-Gomez et al., 2008; Hemsley et al., 2008; Merk et al., 2009; Rohrbach & Clarke, 2007; Savas et al., 2004; Wagner, 2007). The explosion in research into therapies means that animal MPS models are essential. Multiple studies have investigated potential markers or tests or symptoms for MPS IIIA (Haley et al., 2006; Randall et al., 1980).

The somatic symptoms of Hurler syndrome (MPS II) in a 9 year old boy were significantly reduced through the use of bone marrow transplantation (BMT) (Hughjones et al., 1982). In a later study the same boy and eight others who were treated were examined and there were reductions in visceral problems such as airway obstruction, and hearing either remained normal or improved but the dysostosis

multiplex worsened (Downie et al., 1995). The difficulty with patient follow-up is deciding how the patients would have progressed even without treatment, but in these cases the improvements appeared to have significantly improved the patients' quality of life. BMT is however a high risk procedure and the results overall from therapy are variable.

In animals MPS VI BMT has shown positive results. In cats the treatment leads to correction of a somatic sign, facial dysmorphology. The treated cats' corneas also cleared. In rats that were treated by BMT clinical improvement was seen in only one of eight treated rats. MPS VII affected mice were treated and good results were seen. In humans large numbers of patients have been treated by BMT. In people the ocular and skeletal abnormalities have not been improved by this therapy but somatic abnormalities have been reduced. The neurological problems showed a very variable response to the BMT.

ERT with idursulfase for Hunter syndrome (MPS II) is now available (Galan-Gomez et al., 2008). ERT is also available for MPS I and clinical trials have been carried out for MPS II and MPS VI (Muenzer et al., 2009). The main problem however is that for the neurodegenerative MPS there is limited opportunity for therapy since medications cannot get across the blood brain barrier. Enzyme replacement therapy for MPS VI shows that the therapy is more effective the earlier in the cat's life that therapy is performed (Auclair et al., 2003).

This encourages researchers to study other therapies such as using substrate reduction therapies including genstein (Piotrowska et al., 2006). Other researchers are trying to improve therapy by overcoming the blood brain barrier problem by using high dose enzyme replacement therapy (LeBowitz, 2005). Another factor to be taken into account with MPS therapy is the need for a reduced immune response to the proteins being given therapeutically. In MPS I dogs this has been successfully addressed by tolerisation (Kakkis et al., 2004).

The main problem with gene therapy for MPS is that it is difficult to place the gene into the central nervous system and the gene is not expressed for a long enough time for effective treatment. Recent advances in therapy however have led to more success in

this field. Therapy often fails due to an immune response but more recently neonatal gene therapy in a MPS I dog model has been effective (Traas et al., 2007). There have also been exciting therapy developments using gene transfer in viral vectors under the control of liver specific promotors which lead to the production of arylsulfatase (the enzyme missing in MPS VI) thus leading to a treatment for MPS VI. This research was carried out in MPS VI affected cats and rats (Tessitore et al., 2008).

Therapy has been approved in the USA and European Union for the treatment of MPS II using the product Elaprase as an enzyme replacement therapy for MPS II (Garcia et al., 2007). In the mouse model of MPS VIII a corrective gene has been inserted into the fetal mouse brain before the disease has shown pathology (Karolewski & Wolfe, 2006). This treatment corrected the central nervous system disease and led to a significant improvement in the survival time of the mice (Karolewski & Wolfe, 2006). Retroviral vectors appear to be a good way to attempt therapy for MPS (Anson & Fletcher, 2007). The early detection of MPS will enable the earlier use of therapies in affected individuals (Meikle et al., 2006).

Animal models provide opportunities to investigate these diseases and in the last few years progress has been made in their treatment. MPS are different in animals than in humans and there is a need to investigate the clinical signs of MPS in young animals in order to detect MPS at an early age so as to aid in research into these diseases.

Currently there is no treatment for MPS IIIA that halts or reverses the disease. Unfortunately lysosomal enzymes cannot get into the brain unless they are given at very large doses. Future research into potential therapies is likely to focus on how to overcome this problem and the dog model for MPS IIIA will be crucial in this endeavour.

1.5   Detection of behavioural and cognitive function alterations

There has been little investigation of the behavioural and cognitive function of MPS IIIA dogs. MPS IIIA has been detected in two breeds of dogs, Huntaways and Dachshunds. The research has been published in four main papers (Fischer et al., 1998;

Jolly et al., 2000; Jolly et al., 2001; Jolly et al., 2007). An 18 month old male Huntaway dog with a one month history of progressive ataxia and defecation in his kennel was euthanased and MPS IIIA confirmed (Jolly et al., 2000). A pathologic study of two MPS IIIA affected Huntaway dogs was also performed (Jolly et al., 2007). The clinical signs are not mentioned but typical cerebellar signs are assumed. The pathology described in the cerebellum including loss of Purkinje cells would be consistent with ataxia.

One male and one female Dachshund with MPS IIIA were examined clinically (Fischer et al., 1998). Both dogs had ataxia, decreased flexor reflexes, exaggerated myotactic reflexes with clonus, and some loss of vestibuloocular reflexes. No behavioural or cognitive function alterations were noted. The female was euthanased and a post mortem confirmed MPS IIIA (Fischer et al., 1998). Another Dachshund was examined by post mortem and MPS IIIA confirmed (Jolly et al., 2001). This dog showed ataxia but no behavioural or cognitive function alterations were noticed. Behavioural or cognitive function changes have not been identified in these studies, but few dogs were examined, and apart from clinical history no specific behaviour measures were made.

1.5.1 Behaviour monitoring

When identifying the behavioural signs of disease, validity and reliability are critical (Paroz et al., 2008). The ability to detect minimal differences between affected and unaffected dogs is important and tests used must be able to identify and measure the behaviours of the individual being tested (Gosling & Vasire, 2002). Thus, clear descriptions of behaviour are key to the accurate detection of behavioural alterations. A lot of the phrases used to describe behaviours are subjective which has made repeatability low in many previous studies of dogs (Goddard & Beilharz, 1982; Knol et al., 1988; Murphy, 1998; Pfaffenberger et al., 1976; Weiss & Greenberg, 1997).

Studies of pet dogs using behaviour tests (Hennessy et al., 2001; Netto & Planta, 1997; Taylor & Mills, 2006; Vanderborg et al., 1991) are very variable and the accuracy of the tests used is dependant on the appropriateness of the test for measuring a specific behaviour. In one study a measurement used was probably inappropriate in that 97% of

the subjects were found to be aggressive (Netto & Planta, 1997), which suggests that the test was overly sensitive or was forcing the dogs to show an inappropriately aggressive reaction compared to their usual response. Many studies measure behaviours and then examine whether there is a relationship between them. Often no attempt is made to understand what those measurements mean in the dog's life (Svartberg, 2002).

Several new behavioural tests incorporate considerations of feasibility and repeatability (Ley, 2008; Serpell & Hsu, 2001; Svartberg, 2005). The canine assessment and research questionnaire (C-BARQ) has been validated (Serpell & Hsu, 2001). The questionnaire was developed from questions developed by Serpell and Hsu with extra questions from Svartburg (Hsu & Serpell, 2003; Svartberg, 2005) It is a quantitative owner-answered questionnaire which measures the behaviour of dogs (Hsu & Serpell, 2003; Serpell & Hsu, 2001). Recently it has been used as the gold standard for test comparisons (Diederich & Giffroy, 2006; Svartberg, 2005). These questionnaires are often performed at one year of age but have been completed at many different ages (Svartberg, 2005). The validity of the questionnaire was measured for its use in dogs between one and seven years of age (Hsu & Serpell, 2003).

1.5.2   Measuring behaviour at different ages

According to John Scott (Pfaffenberger et al., 1976) there are nine major groups of behaviours; ingestive, shelter-seeking, investigative, sexual, epimeletic, et-epimeletic, agonistic, allelomimetic and eliminative. As these develop different tests can be used to assess behavioural patterns. Sensitive periods are described as a stage of development in which an individual can be more strongly influenced by a given event than at any other stages (Martin & Bateson, 2005).

A two to three day old puppy isolated from its mother will crawl slowly forward, moving its head from side to side and whining (Pfaffenberger et al., 1976). This is et-epimeletic behaviour designed to attract its mother's attention. The puppy is unable to see until its eyes open at about thirteen days of age and it cannot hear until its ear canals open at eighteen to twenty days. Thus a puppy's behaviours are adapted to its abilities at a given time.

The transition period is a time of great behavioural development. At about three weeks the puppy begins to respond to people and other dogs at a distance and agonistic behaviours start to appear. Vocalisation occurs when the puppy is alone even if it is not cold or hungry.

The socialisation period comes next. Weaning gradually occurs during this time. The puppy's yelping in a strange place starts to decrease at about six to seven weeks of age (Pfaffenberger et al., 1976). In contrast to this as the puppy is more able to look after itself investigatory behaviour increases. The critical period occurs between three and eight weeks (Pfaffenberger et al., 1976). This is the time that pups can learn to socialise with people and dogs and situations more easily than at any other time in their lives. It is with this in mind that a lot of behavioural testing is done at this time. By eight weeks of age the puppy has developed a wide repertoire of behaviours that appear to show the puppy's behavioural qualities.

1.5.3   Standardisation of behavioural measurements

Murphy (1998) attempted to standardise the assessment and identification of behaviours to reduce problems encountered with the lack of repeatability in behaviour testing of dogs. At twelve months of age the dogs came into a training facility and were videotaped on an assessment walk. Several trainers assessed the tapes for behaviours and noted where they occur.

Sections of videos where two or more trainers independently commented that a specific behaviour was occurring were analysed. Analysis looked for specific classifiable behavioural characteristics. For example pulling the base of the ears back was a behaviour that occurred more than 80% of the time when the trainers said the dog was exhibiting fear.

People have also tried to address the problem of repeatability by using a number of people to assess one animal's behaviour (Goddard & Beilharz, 1983; Murphy, 1998; Slabbert & Rasa, 1997). The between trainer correlation is then measured. If the correlation is high then test reliability is increased.

Another way to increase the validity of qualitative analysis is to use knowledge of normal behaviour. The Bar Harbour study (Pfaffenberger et al., 1976) was one of the first studies done on domestic dog behaviour that catalogued the progression of behavioural changes in the first year of life. Erik Wilson and Per-Erik Sundgren (1998a) wrote a series of papers on this topic. Their studies were carried out on 2107 dogs at the Swedish Dog Training Centre. They did puppy tests at 8 weeks of age and tests at 450-600 days of age. There were 10 characteristics assessed in the adult tests but the categories may have had a lot of unintentional overlap. The categories of courage and nerve stability are an example of this. The author defines nerve stability as the appropriateness of a dog's reaction to a situation such as being able to overcome a frightening situation whereas courage is defined as the ability to overcome fear. From these definitions it can be seen that in frightening situations these categories are similar. The positive side of these overlaps in categories is that if these similar categories are correlated a higher degree of confidence may be achieved.

The naming of categories is always problematic with behavioural studies as they are subjective classifications. Prey Drive is a category Erik Wilsson and Per-Erik Sundgren (1998a) defined as "the willingness to engage in competitive games". This is subjectively gauged by the game "tug of war" but its relationship to "drive" is not discussed. This is likely to be play behaviour and the term play may be more appropriately descriptive. The other problem with this study was that only one person assessed the adult dogs and the tests were not repeated. This allows for bias and thus reliability is in doubt. If the assessor subconsciously has preconceived ideas about German Shepherds and Labrador Retrievers then this may have led to inconsistencies in behaviour interpretation. The subjective nature of assessments also allows for bias. This study did use more than one assessor in the puppy tests which may have increased the test's reliability. This could have been calculated by determining the inter-observer reliability.

In the Wilsson and Sundgren (1998a) study the puppy test used was composed of twelve units. There was a medium high to high heritability for the tests but lack of correlation between puppy and adult results. This may be due to there being no real correlation between the behaviours or due to the measurement of different behaviours.

There are several important differences in how puppies were tested in comparison to adult dogs. In the puppy test the puppy is first left alone for five minutes. In a pack situation it would be stressful for an un-weaned puppy to be left alone without any litter-mates in an unfamiliar non-den area. After five minutes the test leader, comes into the room. What is "normal" behaviour for a puppy in this situation?

In the adult test the first situation is an unfamiliar test leader in an area familiar to the dog. The person tries to make contact with the dog and then a familiar trainer runs the dog through a series of leash exercises. In an adult dog these environments are more familiar and the dog's reactions will be different to those of a puppy under stress. The adult test increases in situation stress whereas the puppy tests begin stressful and move onto non-stressful situations quickly. However, the puppy test is only thirty minutes long whereas the adult test is an hour long and gives the dog longer to show a more natural response to each situation.

In the puppy test one experience may influence many responses if the puppy does not have time to adjust to the new conditions. In a young puppy it may be necessary to carry out the test in small steps with longer intervals between them. Another problem is that in a puppy, yelps and shrieks are a care-soliciting behaviour pattern (Fox, 1978; Pfaffenberger et al., 1976) and decrease after six to seven weeks of age. Therefore this may be just a behaviour relevant to a short period of life.

The author points out that the behaviour patterns of puppies are different to the behaviour patterns of adult dogs and this is why they made the two tests so dissimilar. However this does make it more difficult to find correlations between behaviours. In other studies the tests for detecting fear were more similar and there was a correlation between these tests (Goddard & Beilharz, 1983; Goddard & Beilharz, 1986) . The Goddard and Beilharz (1983, 1986) studies were carried out on dogs of a wide range of ages and thus may provide information on when the optimal time for a puppy test would be. The adult tests were performed both at 12-18 months of age when the dogs came in for training and also when they were fully trained. When the dogs came in for training the testing was carried out over five days. There were a large number of tests that could elicit fear and a number of walks included in the tests also (Goddard & Beilharz, 1986).

Puppy tests were carried out weekly from four to ten weeks, and the procedures altered at each time. These tests had fear-inducing situations such as throwing a surfboard on the footpath in front of the dog. This meant that correlations between more similar emotional states could be made. Tail position, approach response to a human and activity scores were all positively correlated with each other at each young age.

On specific learned tasks there was no consistent correlation between the immature and the adult scores (Goddard & Beilharz, 1986). Immature responses to a situation were different for puppies than for adult dogs. Puppies showed low activity when scared whereas adult dogs had a wider range of responses. In this study playful retrieving was negatively correlated with adult fearfulness. This may mean that retrieving is a play task that puppies can learn which decreases fear or that it is a behaviour which indicates an already defined characteristic which decreases fearfulness or that it is a spurious result. The predictive value of tests increased with age and with more intense stimuli (Goddard & Beilharz, 1986).

A popular test that has been in use for a long time is a test proposed by Campbell in 1975. This test involves five simple situations and is fast to perform (Beaudet et al., 1994). It does not correlate with social status at later ages. This may be because the test situations are not natural situations and thus interpretation of the results are difficult. The restraint dominance test involves placing the puppy on its back on the handler's lap and scoring the reaction, however this is not a situation which happens to pups in normal play or fighting and interpretation is difficult. If the puppy's movement was also measured during the test then the total score for movement and social standing had some predictive value for social tendencies.

There are many new tests being produced in the behavioural field. One of these is the socially acceptable behaviour (SAB) test (Planta & De Meester, 2007). This test was designed to measure aggression. As mentioned previously it detected aggression in 97% of the dogs tested suggesting that the test was probably too sensitive or was forcing the dogs to show aggression. However when just biting or attack behaviour were analysed, the test showed a statistically significant relationship to the biting history of the dogs and thus is useful for assessing aggressive tendencies. In a follow up study both the qualitative and quantitative elements of fear in that test were analysed and

compared with the standardised C-BARQ (De Meester et al., 2008). The dogs' postural scores related to stranger contact was significantly correlated with the C-BARQ score related to "fear for strangers". This suggests that the SAB test could also be used to classify whether a dog shows shy or fearful behaviours.

1.5.4 Environmental and maternal influences on puppy behaviours.

The concept of a sensitive period for socialisation has significant ramifications for the possibility of influencing the expression and development of puppy behaviours (Pfaffenberger et al., 1976). This suggests that in order to effectively test and analyse behaviours environmental and maternal influences must be addressed.

Early handling has been shown in a number of studies to be beneficial in young animals (Birkenmeier et al., 1989; Denenberg & Morton, 1962; White & Castle, 1964). At five weeks of age 'handled' puppies are more exploratory than their non-handled siblings and they perform better in problem-solving tests (Fox & Stelzner, 1966). In other work it was shown that puppies that are either over-indulged or over-disciplined tend to be more disobedient (Freedman et al., 1961). Thus the possibilities for influencing puppy behaviour are significant.

Social interactions between mother and offspring can also have significant effects on puppy behaviour (Pfaffenberger et al., 1976; Wilsson, 1984). Wilsson (1984) showed that there were positive correlations between maternal aggressiveness and the puppy's ability to fetch. Puppies from mothers who used a lot of inhibited bites, nibbling and licking were less likely to make contact with a passive person. This suggests that a puppy's attraction to humans may be influenced by its mother.

1.6   Measurement of cognitive function

Cognition relates to the capacity of an individual to function on an intellectual level (McCune et al., 2008). It is often considered to comprise intelligence with additional

facets such as simple and complex learning, memory, language acquisition and attention (McCune et al., 2008).

Cognitive function has been examined in aging dogs with a reduction in memory, attention and learning being seen in older individuals. A number of the features of geriatric dog brains are similar to the brains of elderly people (Head, 2001). A number of researchers have developed a canine model of cognitive aging (Ikeda-Douglas et al., 2005; Ikeda-Douglas et al., 2004; Studzinski et al., 2006). They found that visiospatial and landmark discriminative learning both decrease in old age.

The specific cognitive functions which have been tested in canines are discriminative and reversal learning, procedural learning, visual recognition memory, allocentric spatial learning, spatial learning and memory (Adams et al., 2000). A modified version of the Wisconsin Testing apparatus was used for these tests. The format of a cognitive function test must take the age of the dog into account.

Latency during cognitive function tests can be used as a measure of cognitive function (Nippak & Milgram, 2005). In humans latency relates to central processing speed and older people have longer latencies than young people but in dogs this has not been found to be true (Nippak & Milgram, 2005). Apart from cognitive function, two additional factors are involved in how fast a dog completes a task; individual dogs have different latencies when performing a task and the difficulty of the task. The harder the task the longer the latency (Nippak & Milgram, 2005).

An association exists between the results of cognitive function tests and particular disease states. For example there is an association between a size-discrimination task and beta-amyloid accumulation in dogs (Head et al., 1998). Aged dogs in a canine model of age-dependant cognitive decline performed worse than young dogs in a visiospatial task (Tapp et al., 2003b). Some studies involved very long trials and many days of testing. These trials often involve older dogs which influences the test results when compared to a young dog (Tapp et al., 2003a). Research in young dogs using a T-shaped maze measured dogs' cognitive function by recording the dogs' ability to choose where to go in the maze to obtain a food reward (Kelley & Lepine, 2005; Kelley et al., 2004) . Studies using other species have looked at cognitive function in MPS affected

animals. Open field tests and an novel object recognition memory task were performed in idua mice (Reolon et al., 2006). They found an impairment of memory for an aversive task but no difference for a non-aversive task. The affected mice were less exploratory. MPS VII affected mice showed altered spatial memory in the Morris water maze and in a learning protocol (Chang et al., 1993).

Most cognitive function tasks in dogs involve the use of training protocols with positive reinforcement. The reinforcers used are usually food (Kelley & Lepine, 2005; Roudebush et al., 2005). Testing often involves the use of agility and obedience pre-training and then testing as a measure of cognitive function. In one study no impairment of cognitive function in dogs was detected on physical assessment but it was not defined how this was assessed (Fretwell et al., 2006). Other standard tests have been adjusted to incorporate a cognitive component. For example the standard measure of palatability is a two pan test and now this has been modified to be a three choice object discrimination task for dogs (Araujo & Milgram, 2004).

Recent studies in laboratory rats examined emotional bias (Burman et al., 2008) The theory was that the emotional state of an individual influences cognitive processes and will bias the individual towards either a positive or negative response to an ambiguous stimulus. This finding was borne out by the results which showed that if rats housed in a negative manner were presented with an ambiguous stimuli they anticipated a negative outcome (Burman et al., 2008). This showed that cognitive function tests may be used to measure the emotional state of an animal and thus as another means of examining the brain functioning of the individual.

1.7   Summary

Neurodegeneration as evidenced by ataxia is first seen between one and a half to four years of age in MPS IIIA affected dogs (Jolly et al., 2000; Jolly et al., 2001). Clinical signs include high stepping, exaggerated patella, cranial tibial and gastrocnemius reflexes and loss of learned behaviour (Jolly et al., 2000). In contrast in humans and mice with MPS IIIA the first clinical signs seen are sleep disturbances, hyperactivity

and aggressiveness (Gliddon & Hopwood, 2004; Meyer et al., 2007a). Cognitive decline occurs as the disease progresses.

There are numerous behavioural and cognitive function tests which could be useful for detecting MPS IIIA symptoms early in affected dogs' lives. The main hope for developing therapies for MPS IIIA is based on animal models. If affected dogs can be identified earlier this would aid in the testing of new treatments which could ultimately lead to the treatment of MPS IIIA in children.

1.8   Objectives of Thesis

The objectives of the research described in this thesis are:

1. To determine whether there are cognitive alterations in MPS IIIA affected dogs at a young age.

2. To determine whether there are behavioural alterations in MPS IIIA affected dogs at a young age.

3. To describe the behaviours of MPS IIIA affected dogs during their first year of life.

CHAPTER 2

Behavioural Tests at Six and Eight Weeks of Age
Comparing MPS IIIA Affected and Unaffected Dogs

2.1 Introduction

MPS IIIA causes accumulation of glycosaminoglycans which lead to severe neurodegeneration and death in young children (Neufeld & Muenzer, 2001). Accrual of glycosaminoglycans begins early as evidenced by intracellular inclusions being detectable in the second trimester of pregnancy (Ceuterick et al., 1980). The appearance of clinical signs is much delayed however with the first symptoms often being noticed at seven months of age, and diagnosis is frequently not made until four and a half years of age (Meyer et al., 2007a). Animal models of this disease also have delayed symptom onset but this varies according to species.

MPS IIIA occurs in both mice and dogs. There are mouse, wirehaired Dachshund and Huntaway dog models of MPS IIIA. The Huntaway dogs are apparently clinically normal for the first one and a half years of their lives and then neurodegeneration becomes evident. Ataxia, high stepping, exaggerated patella, cranial tibial and gastrocnemius reflexes, and loss of learned behaviour are seen (Jolly et al., 2000). In contrast by three weeks, mice show hyperactivity, then aggressiveness (Gliddon & Hopwood, 2004).

In the Huntaway model, due to MPS IIIA symptoms becoming evident at one and a half years of age, any detection of response to puppy treatment is much delayed. As there are

changes in the brain at a young age due to MPS IIIA some evidence of brain dysfunction may also be measurable early. This study focused on the effects of MPS IIIA on behaviour and probably reflects pathology of the frontal lobe whereas the symptoms described in this colony of dogs until now has focused on cerebellar signs (Jolly et al., 2007).

To detect any effects of MPS IIIA on behaviour, baseline behaviours, behavioural alterations and cognitive function need to be accurately measured. This study focuses on the baseline behaviours and alterations in behaviours. It investigates whether the behavioural signs caused by MPS IIIA can be detected early in affected dog's lives. As a part of this investigation it will also document behaviours of MPS IIIA affected dogs at six and eight weeks of age.

2.2 Materials and methods

2.2.1 Aim

The aim of this study was to determine if MPS IIIA causes changes in behaviour at six and eight weeks of age. The method used for these tests was derived from Wilsson and Sundgrens' extensive work studying puppies (Wilsson & Sundgren, 1997; Wilsson & Sundgren, 1998a; Wilsson & Sundgren, 1998b).

2.2.2 Subjects

The subjects were 33 puppies from a breeding population of MPS IIIA affected and unaffected Huntaway dogs. All puppies produced in this colony were recruited into this study during 2004 and 2005. These puppies were also a part of a multi-faceted study into MPS IIIA. All puppies had a physical examination carried out within 48 hours of birth and a microchip was inserted (BackHome electronic identification program, Virbac, Auckland, New Zealand). A blood spot test was carried out at the same time.

The blood spot test was a deoxyribonucleic acid / polymerase chain reaction blood test to determine whether each puppy had MPS IIIA or not (Yogalingam et al., 2002). This

research dog population has both MPS IIIA homozygous dogs, MPS IIIA heterozygote dogs and normal dogs. The 33 puppies were composed of 18 male and 8 female unaffected puppies, and 4 male and 3 female homozygous MPS IIIA affected puppies. The testing and identification of the puppies was carried out by a veterinarian who was not a part of this current study and thus the researchers in this study were blinded to the puppies' MPS IIIA status.

2.2.3   Procedure

Management of each puppy was planned to standardise each puppy's learning and socialisation experiences. Puppies were handled daily by kennel staff. A trainer handled the puppies at least four times a week, and each session lasted for 10 minutes per puppy. This handling began at six weeks of age. It involved; (1) gentle handling including lifting the puppy off the ground and patting it and (2) teaching sit, retrieve and down using food, praise and games as reinforcers.

The same location was used for all of the tests. This location was novel to all of the six week old puppies. Testing was always carried out in daylight hours and the pen used was under cover so that environmental conditions did not alter significantly between the tests. The floor of the test pen was concrete and it was marked into squares 0.6m by 0.6m and a circle 2m diameter (Figure 2.1). There was also a plank bisecting the room.

All the training and the six and eight week tests were carried out at the facility by one trainer. The testing followed a similar protocol to that described by Wilsson and Sundgren (1998a) and is described in Table 2.1. The data for the six and eight week tests was transcribed at the time of testing. The tests were also videotaped and an independent assessor re-scored five percent of the six and eight week tests to determine inter-observer reliability.

Figure 2.1   Floor Plan of the site for the six and eight week tests
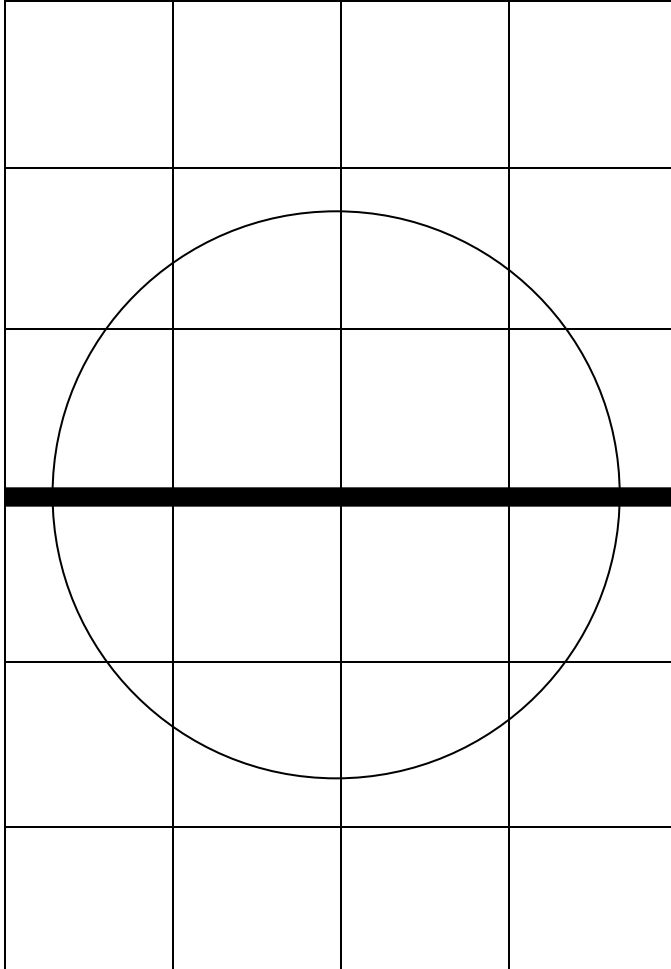
Key: Each square is 0.6m x 0.6m.

The circle is 2m diameter

▬▬▬   - 10cm high plank of wood

Table 2.1   Puppy testing methodology

A. Isolation Test

One puppy is randomly picked out and placed in the pen (approx 2.5m by 3 m). Floor marked in squares- 0.6 x 0.6m. In the middle of the arena a circle 2m in diameter is marked. A low (10 cm high) plank of wood bisects the room across the centre of the room.

The puppy is left alone in the pen and the door to the pen is closed.

1. Yelp: time until puppy whines or yelps is noted. If does not vocalise within **5** minutes a latency of **300** seconds is noted.

2. Shriek: Time until puppy gives three distinctive shrieks is noted. If doesn't do three shrieks within **5** minutes the score time of **300** seconds is noted.

3. Contact One: Tester (T) enters pen without paying attention to the puppy. Puppy's reaction scored from 1-5.

  1- puppy not making contact with T
  2- puppy initially withdraws and then makes contact
  3- puppy spontaneously makes contact after hesitation
  4- puppy spontaneously making contact without hesitation
  5- puppy overly willing to make contact- i.e. jumping up, vocalising

B. Object Retrieval Test.

Once score is determined then T shows puppy a ball. When T has the puppy's attention the ball is rolled over the floor. There are two parts to the retrieve test,   fetch and retrieve

4. Fetch: Time it takes for puppy to take the ball in its mouth, from time of becoming attentive to the ball, is noted. If does not take the ball in its mouth within **3** minutes then the score of **180 seconds** is written down.

5. Retrieve: The puppy's willingness to retrieve the ball is scored from 1 to 5

  1- puppy shows no interest in the ball
  2- puppy running after the ball but not taking it in its mouth
  3- puppy carrying the ball but not bringing it back to the T
  4- puppy retrieving once or twice
  5- puppy who retrieves the ball spontaneously three or more times

C. Object investigation test

6. Large ball: Larger ball (13cm diameter) is placed in the centre of the pen. The puppy's immediate reaction to the ball is scored

    1- puppy withdraws from the ball

    2- puppy running after the ball without investigating it further before or after it has been rolled

    3- puppy investigating the ball only after it has been rolled

    4- puppy starting to play with the ball after it is rolled over the floor

    5- puppy starts to play spontaneously with the ball.

7. Tug of war: T draws the puppy's attention to a cotton rag. If the puppy grasps the toy the T doesn't let go.

    1- puppy doesn't pay attention to the rag

    2- puppy investigates the rag but does not take it in mouth

    3- puppy bites and chews but lets go of rag as soon as T pulls

    4- puppy highly engaged but not doing the death shake

    5- highly engaged and doing the death shake

This test will give measures of the puppy's temperament and confidence.

D. Arena Test

Spread four objects out on the floor:- a piece of wood 3 x 7 x 20 cm, a tennis ball, a large ball and the cotton rag used in part one of the test.

For 5 minutes tester sits quietly in the middle of the circle on the plank going across the room.

Measure:

8. Contact two: time in seconds spent inside the circle during the 5 minutes is noted down.

9. Activity- number of squares that the puppy moves over is noted.

10. Objects visited- number of objects visited during the test.

Adapted from Wilsson and Sundgren (1998a)

These behavioural tests were performed on all puppies at six and eight weeks of age.

2.3   Data collection and statistical analysis:

All statistical analyses were carried out using Minitab statistical software (release date 31st January 2007, version 15).   The level of $\alpha = 0.05$ was used to indicate statistical significance. The data has been included in a DVD-ROM (Appendix One).

The contact two, yelp, shriek and fetch tests produce continuous quantitative data. The activity and objects visited tests produce discrete quantitative data. The contact one, retrieve, large ball and tug of war tests produce ordinal qualitative data. The most appropriate ways to display the data are boxplots and histograms for the quantitative data and histograms for the qualitative data.

Histograms were used to visually test for the normality of the data.   Statistical analysis mainly used non-parametric analysis because this allowed a relaxation of the assumption of normality. Normality is often not observed in behavioural or cognitive data. The data was summarized using diagrams, numerical measures and reference ranges.

Simple statistics were performed. The results of the testing were tabulated to allow analysis. Descriptive statistics examined the distribution and tendencies of the data gathered. The level of inter-observer reliability was calculated. Determination of normality was measured both by examining the general trends of the data in graphs and by the use of the Anderson-Darling test.

The sample size for this study was relatively small. Only seven MPS IIIA affected puppies were available for comparing with the unaffected carriers (heterozygotes) of MPS IIIA and normal puppies. This disease only occurs in homozygotes and thus heterozygote puppies and normal puppies were compared with the affected puppies together.

The small sample size influenced the statistical analyses that were performed. Chi squared calculations and Mood's median tests were avoided since they were for a normally distributed population which this was not and chi squared calculations are not

robust with small sample sizes. The data was graphically displayed to look for general trends (Figure 2.1).

Mann-Whitney *U* tests were performed on the six and eight week data to examine the data for differences between the medians of the data for the MPS affected and unaffected puppies. Spearman's rank correlation coefficients were calculated for the six and eight week test results separately to see if particular tests are related to each other.

2.4   Results

2.4.1   Tests for normality

The results of the Anderson-Darling tests for normality for the six and eight week tests are shown in Tables 2.2 and 2.3. These show that at both six and eight weeks of age the null hypothesis that the distribution of the data was normal was rejected. Thus the distribution is not likely to be normally distributed. For the affected puppies a number of the tests had p-values which did not allow the rejection of the null hypothesis and thus these questions may be normally distributed. However due to the relatively low numbers of animals in the colony normality was not conclusively determined thus non-parametric statistics were performed. Also the literature in this field uses non-parametric analysis and so this study followed the current literature (Serpell & Hsu, 2001).

Table 2.2   Anderson-Darling test for normality for six week tests in MPS IIIA affected and unaffected puppies.

Test	AD value	P-value
Unaffected puppies:		
A. Isolation Test		
1. Yelps	1.760	<0.005
2. Shriek	2.746	<0.005
3. Contact One	2.602	<0.005
B. Object retrieval test		
4. Fetch	6.880	<0.005
5. Retrieve	3.617	<0.005
C. Object recognition test		
6. Large Ball	2.247	<0.005
7. Tug of war	1.350	<0.005
D. Arena test		
8. Contact Two	0.594	0.111
9. Activity	0.221	0.813
10. Objects visited	0.964	0.013
MPS IIIA affected puppies		
A. Isolation test		
1. Yelps	0.163	0.903
2. Shriek	1.235	<0.005
3. Contact One	0.271	0.551
B. Object retrieval test		
4. Fetch	2.000	<0.005
5. Retrieve	1.139	<0.005
C. Object recognition test		
6. Large Ball	0.675	0.043
7. Tug of war	0.998	0.005
D. Arena test		
8. Contact Two	0.316	0.440
9. Activity	0.363	0.328
10. Objects visited	0.349	0.359

Table 2.3   Anderson- Darling Test for normality for eight week tests in MPS IIIA affected and unaffected puppies.

Test	AD value	P-value
Unaffected puppies:		
A. Isolation tests		
1. Yelps	3.845	<0.005
2. Shriek	1.524	<0.005
3. Contact One	2.803	<0.005
B. Object retrieval tests		
4. Fetch	2.982	<0.005
5. Retrieve	1.021	0.008
C. Object recognition tests		
6. Large Ball	2.656	<0.005
7. Tug of war	1.130	<0.005
D. Arena test		
8. Contact Two	0.286	0.586
9. Activity	0.773	0.037
10. Objects visited	2.107	<0.005
MPS IIIA affected puppies		
A. Isolation tests		
1. Yelps	0.300	0.457
2. Shriek	0.461	0.174
3. Contact One	0.374	0.307
B. Object retrieval tests		
4. Fetch	1.063	<0.005
5. Retrieve	0.759	0.025
C. Object recognition tests		
6. Large Ball	0.783	0.021
7. Tug of war	0.271	0.551
D. Arena test		
8. Contact Two	1.003	0.005
9. Activity	0.434	0.208
10. Objects visited	0.967	0.006

2.4.2   Descriptive statistics

The general trends in the six and eight week data gathered were determined by calculating the descriptive and summary statistics (Table 2.4). The six week tests were carried out on 33 puppies. Out of the total puppies 26 were unaffected and 7 were MPS affected puppies.

2.4.2.1 Summary statistics for the six week tests

Figures 2.2 and 2.3 show the distribution of the six week test data. As can be seen in Table 2.4 and Figure 2.2 the vast majority of the puppies yelped quickly once they were left on their own. The puppies without MPS IIIA yelped on average within 75.7 seconds whereas the MPS IIIA affected puppies yelped on average within 82.9 seconds. Shrieking in comparison occurred much later in the observation period being on average more than three minutes into the observation period. On average fetching and retrieving were both poorly performed by most of the puppies. It can be seen clearly that there was not a lot of variability on the fetch test but the other tests do have differences between individual responses.

From these plots it appears that the data for the contact two, activity, tug, large ball and objects revisited questions are evenly distributed. The data for the retrieve, contact one, yelp, shriek and fetch tests appear to have some variation skew. No large differences are seen between the MPS IIIA affected and unaffected puppies in these tests.

Table 2.4   Summary statistics for six week tests for MPS IIIA affected and unaffected puppies

Test	Mean	Standard Error Mean	Standard Deviation
Unaffected puppies:			
Time until Yelps (seconds)	75.7	15.6	79.7
Time until Shrieks (seconds)	204.5	22.7	115.7
Contact test	3.538	0.194	0.989
Fetch (seconds)	146.7	13.7	69.6
Retrieve	1.769	0.237	1.210
Large Ball	3.962	0.316	1.612
Tug	2.231	0.178	0.908
Contact Two (seconds)	195.8	15.0	76.4
Activity (number of squares)	25.96	3.04	15.48
Objects visited	2.038	0.245	1.248
MPS IIIA affected puppies:			
Time until Yelps (seconds)	82.9	20.5	54.4
Time until Shrieks (seconds)	243.6	38.3	101.4
Contact test	3.000	0.488	1.291
Fetch (seconds)	155.0	25.0	66.1
Retrieve	1.429	0.202	0.535
Large Ball	3.857	0.508	1.345
Tug	2.000	0.218	0.577
Contact Two (seconds)	171.4	21.4	56.6
Activity (number of squares)	19.00	6.33	16.75
Objects visited	1.429	0.369	0.976

Figure 2.2 Boxplots comparing MPS IIIA affected and unaffected puppies for the six week quantitative tests: time until yelps, contact two, objects visited, fetch, time until shrieks and activity.
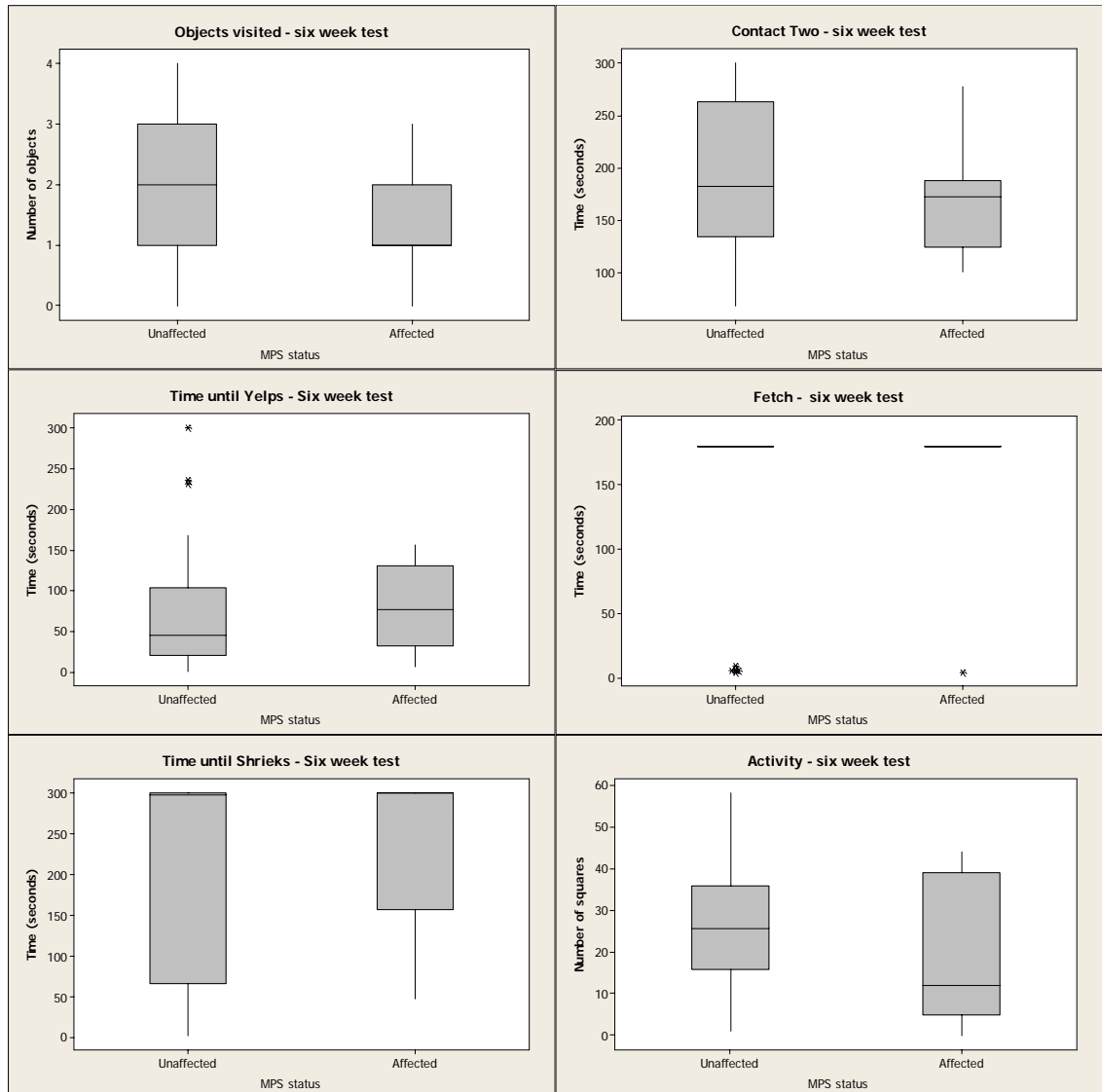
Figure 2.3 **Histograms comparing MPS IIIA affected and unaffected puppies for the six week ordinal qualitative tests: large ball, contact one, retrieve and tug of war.**
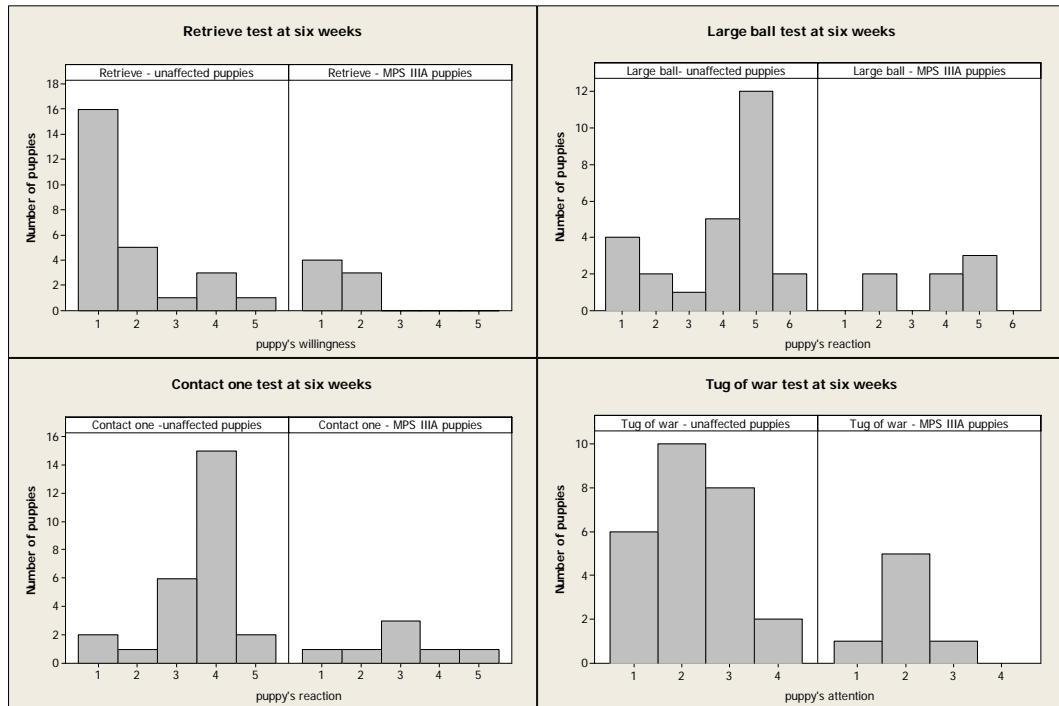
2.4.2.2   *Summary statistics for the eight week tests*

The data in Table 2.5 shows that the results for the affected and non affected puppies at first gaze are fairly similar. There was very little difference between the affected and unaffected puppies for the retrieve, contact one and large ball tests. The unaffected puppies when isolated in a room take approximately 33 seconds before they yelp or whine whereas the affected puppies take approximately 28.2 seconds.

There were some small differences in several of the test results. The unaffected puppies took on average 97.1 seconds before emitting 3 distinct shrieks whereas it took the affected puppies on average 124.2 seconds. The affected puppies were slightly slower to fetch a ball. In the arena test they spent less time in a 2 m circle in the room and visited less objects. Figures 2.4 and 2.5 show the distribution of the eight week data.

Table 2.5   Summary statistics for eight week tests for MPS IIIA affected and unaffected puppies

Variable	Mean	Standard Error Mean	Standard Deviation
Unaffected puppies:			
Time until Yelps (seconds)	33.0	11.3	49.3
Time until Shrieks (seconds)	97.1	23.6	102.7
Contact test	3.579	0.221	0.961
Fetch (seconds)	81.5	19.8	86.4
Retrieve	2.579	0.336	1.465
Large Ball	4.158	0.308	1.344
Tug	2.789	0.211	0.918
Contact Two (seconds)	157.4	17.8	77.7
Activity (number of squares)	52.05	8.35	36.38
Objects visited	3.105	0.295	1.286
MPS affected puppies:			
Time until Yelps (seconds)	28.21	8.11	19.87
Time until Shrieks (seconds)	124.2	45.5	120.5
Contact test	3.286	0.522	1.380
Fetch (seconds)	107.7	34.1	90.3
Retrieve	2.857	0.459	1.215
Large Ball	4.429	0.297	0.787
Tug	3.000	0.488	1.291
Contact Two (seconds)	122.4	30.8	81.4
Activity (number of squares)	68.4	20.1	53.2
Objects visited	2.857	0.508	1.345

Figure 2.4 **Boxplots comparing the MPS IIIA affected and unaffected puppies for the eight week quantitative tests: fetch, time until yelps, activity, time until shrieks, contact two and objects visited.**
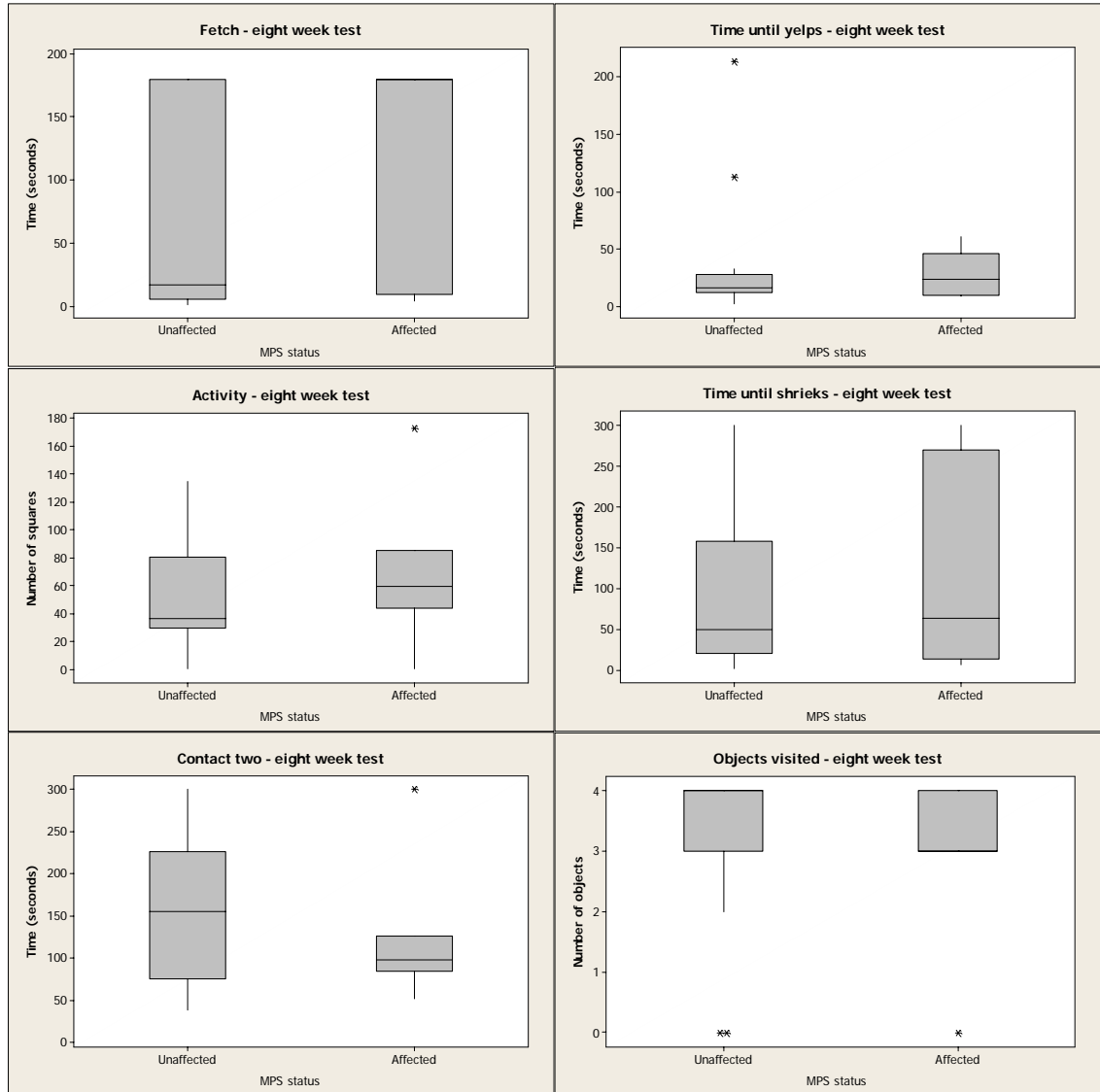
Figure 2.5   Histograms comparing the MPS IIIA affected and unaffected puppies for the eight week ordinal qualitative tests: tug of war, large ball, retrieve and contact one.
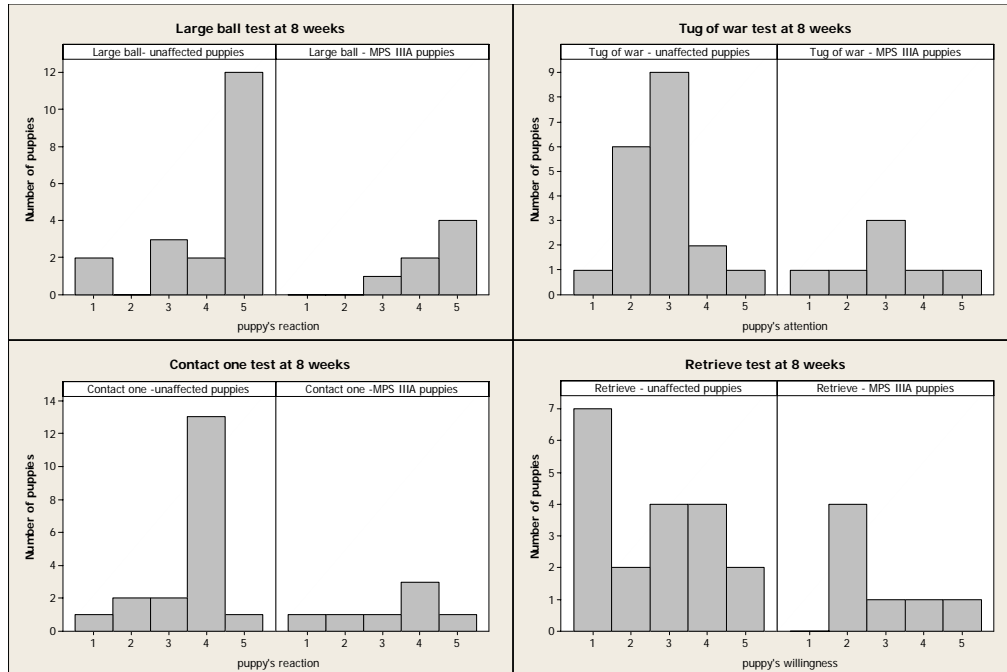
2.4.3   Comparing the six and eight week tests

When comparing the six and eight week tests it was seen that in the eight week test (Figure 2.6) puppies yelped a lot sooner than they had in the six week test. The affected puppies yelped slightly sooner than the unaffected puppies and it still took longer for the affected puppies to shriek three times (Figure 2.4). The puppies spent less time in the circle at the eight week test than they did in the initial six week test. They were also faster to fetch and to shriek at the eight week test (Figure 2.6).

Examination of the histograms (Figures 2.3 and 2.5) appeared to show differences between affected and unaffected puppies however this was mainly due to the large difference in the total numbers of affected and unaffected puppies tested. Taking this into account the general trends for the results of the MPS IIIA affected and unaffected puppies were similar.   This supports the summary statistics in Tables 2.4 and 2.5. Mann-Whitney U tests were also performed to confirm this suspicion (Table 2.6 and 2.7). The results in Table 2.6 and Table 2.7 did not show any statistically significant differences between the behaviours seen in the unaffected and affected puppies.

Table 2.6 Mann-Whitney *U* six week test results comparing the MPS IIIA affected and unaffected puppies

Test	Number of Unaff	aff	Median Unaff	Aff	W	P Value	P Value *	Confidence Interval
Yelps	26	7	46	77	423.0	0.4153	0.4152	-74.7, 36.5
Shriek	26	7	298.5	300	423.0	0.4153	0.3735	-153.6, .01
Contact Test	26	7	4	3	470.0	0.2259	0.1928	0.001, 2
Fetch	26	7	180	180	439.5	0.9298	0.8958	-0, 0
Retrieve	26	7	1	1	445.5	0.8949	0.8798	-1, 1
Large Ball	26	7	5	4	450.0	0.7412	0.7268	-1, 1
Tug	26	7	2	2	455.0	0.582	0.5566	-1, 1
Contact Two	26	7	183	173	456.0	0.5522	0.552	-35, 96.7
Activity	26	7	25.5	12	467.0	0.2806	0.2797	-8, 22
Objects visited	26	7	2	1	468.0	0.2615	0.2469	-0, 2

significance level 0.05
* adjusted for ties
Key: Aff -   MPS IIIA affected puppies
                    unaff-   unaffected puppies

Table 2.7 Mann-Whitney *U* eight week test results comparing the MPS IIIA affected and unaffected puppies

Test	Number of Unaff	aff	Median Unaff	Aff	W	P Value	P Value *	Confidence Interval
Yelps	19	6	17	24	240	0.6792	0.6791	-24, 10.7
Shriek	19	7	50	64.1	248.5	0.6646	0.6641	-141, 44.9
Contact Test	19	7	4	4	263.5	0.7071	0.6673	-1, 1.0
Fetch	19	7	17.5	180	244.5	0.5062	0.4835	-163.3, 3
Retrieve	19	7	3	2	247	0.6029	0.5942	-1, 1
Large Ball	19	7	5	5	255.5	0.9769	0.9736	-1, 1
Tug	19	7	3	3	248.5	0.6646	0.6442	-1.0, 1.0
Contact Two	19	7	155	98	272.5	0.3702		-38.2, 122.6
Activity	19	7	37	60	241.5	0.4019	0.4017	-48.0, 29.0
Objects visited	19	7	4	3	268	0.5249	0.4928	-1, 1

Significance level 0.05
* adjusted for ties
Key: Aff -   MPS IIIA affected puppies
                    unaff-   unaffected puppies

Figure 2.6 Boxplots comparing the results of the six and eight week test items
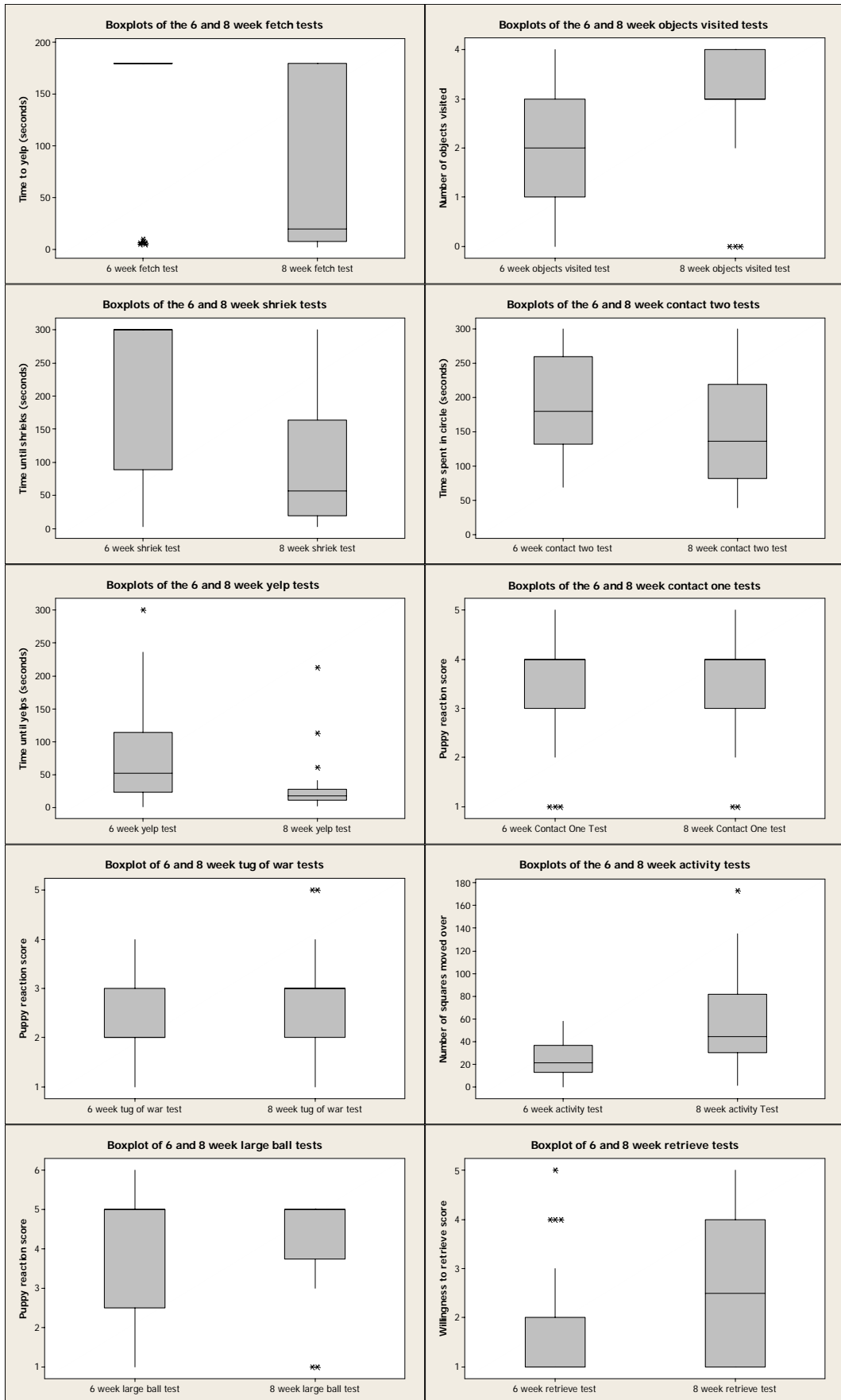
2.4.4   Interobserver reliability

To provide a measure of the objectivity and validity of the six and eight week tests interobserver reliability was calculated.   An independent observer re-examined the videotape of five percent of the six and eight week tests and scored the tests from that tape.   The level of agreement between the two observers, otherwise known as the interobserver reliability was 83%.

2.4.5   Spearman's rank correlation coefficient

Spearman's rank correlation coefficients were also calculated to determine the associations present between the different test components (Tables 2.8, 2.9, 2.10 and 2.11).   These results showed that there were associations between several of the test behaviours seen.   The associations were seen in the unaffected puppies and they mainly showed relationships between the active behaviours namely objects visited, contact two, activity and tug.   The other strong associations were between retrieve and fetch and between shriek and yelp.

There was a negative correlation between fetch and retrieve, thus as the time to fetch increased, so the retrieve score decreased.   This is because the fetch score is the time taken to pick up a ball and the retrieve is a measure of willingness to retrieve, thus these scores are closely related.   The lowest retrieve score would indicate no interest in retrieving and the highest fetch score would indicate that the puppy did not fetch. Likewise contact two (remaining in the circle) was negatively correlated with the active measures such as activity.

In terms of comparing between the affected and unaffected puppies the only significant association seen for the affected puppies was at eight weeks a very significant ($p < 0.01$) negative association was seen between the fetch and the retrieve.

Table 2.8 Spearman's rank correlation coefficient –Six week unaffected puppies

	Time until Yelps	Shriek	Contact test
Shriek	0.433 *		
Contact test	-0.401 *	-0.384	
Fetch	0.159	-0.088	-0.048
Retrieve	0.087	0.244	0.017
Large Ball	-0.323	-0.094	0.085
Tug	-0.335	-0.071	0.324
Contact Two	0.012	0.060	-0.115
Activity	-0.123	-0.267	0.310
Objects visited	-0.008	0.089	0.143

	Fetch	Retrieve	Large Ball
Retrieve	-0.776 ***		
Large Ball	-0.157	0.224	
Tug	-0.236	0.152	0.444 *
Contact Two	-0.348	0.166	-0.150
Activity	0.358	-0.285	0.072
Objects visited	0.158	-0.035	0.150

	Tug	Contact Two	Activity
Contact Two	-0.351		
Activity	0.212	-0.644 ***	
Objects visited	0.448 *	-0.680 ***	0.542 ***

Number of pairs: 26

Table 2.9 Spearman's rank correlation coefficient - Six week MPS IIIA affected puppies

	Time until Yelps	Shriek	Contact test
Shriek	0.668		
Contact test	0.296	0.000	
Fetch	-0.408	-0.255	-0.424
Retrieve	0.144	0.000	0.749
Large Ball	0.416	0.542	-0.726
Tug	-0.134	0.000	-0.555
Contact Two	-0.286	-0.579	-0.371
Activity	0.468	-0.112	0.150
Objects visited	-0.019	-0.420	0.524

	Fetch	Retrieve	Large Ball
Retrieve	-0.471		
Large Ball	0.108	-0.535	
Tug	0.764	-0.540	0.354
Contact Two	0.000	0.000	0.057
Activity	-0.618	-0.073	0.105
Objects visited	0.214	-0.076	-0.604

	Tug	Contact Two	Activity
Contact Two	0.267		
Activity	-0.472	0.198	
Objects visited	0.000	-0.243	0.208

Number of pairs: 7
*   p <0.05
**   p <0.02
***   p <0.01

Table 2.10 Spearman's rank correlation coefficient – Eight week unaffected puppies

	Yelps	Shriek	Contact test
Shriek	0.271		
Contact test	-0.598 ***	-0.216	
Fetch	-0.065	-0.272	-0.181
Retrieve	-0.190	0.061	0.189
Large Ball	-0.288	-0.346	0.409
Tug	-0.175	-0.005	0.647 ***
Contact Two	0.324	0.172	-0.396
Activity	-0.218	-0.184	0.406
Objects visited	-0.111	-0.228	0.248

	Fetch	Retrieve	Large Ball
Retrieve	-0.444		
Large Ball	-0.091	0.502 *	
Tug	-0.218	-0.009	0.195
Contact Two	0.027	-0.147	-0.553 *
Activity	-0.023	0.352	0.518 *
Objects visited	-0.033	0.335	0.475 *

	Tug	Contact Two	Activity
Contact Two	-0.369		
Activity	0.151	-0.595 **	
Objects visited	0.229	-0.614 **	0.630 ***

Number of pairs: 19

Table 2.11 Spearman's rank correlation coefficient – Eight week MPS IIIA affected puppies

	Yelps	Shriek	Contact test
Shriek	0.600		
Contact test	0.213	0.152	
Fetch	0.152	-0.698	0.032
Retrieve	-0.152	0.698	-0.032
Large Ball	0.778	0.676	0.467
Tug	0.203	0.812	0.092
Contact Two	-0.600	-0.200	-0.516
Activity	-0.086	-0.143	0.395
Objects visited	0.676	0.507	0.539

	Fetch	Retrieve	Large Ball
Retrieve	-1.000 ***		
Large Ball	-0.108	0.108	
Tug	-0.801	0.801	0.600
Contact Two	-0.334	0.334	-0.845
Activity	0.152	-0.152	0.338
Objects visited	0.000	0.000	0.700

	Tug	Contact Two	Activity
Contact Two	-0.203		
Activity	0.058	-0.657	
Objects visited	0.171	-0.676	0.507

Number of pairs: 6
*   p <0.05
**   p <0.02
***   p <0.01

2.5   Discussion

The main finding of this study was that no statistically significant behavioural differences were detected between the unaffected puppies and the MPS IIIA affected puppies at six and eight weeks of age. Behavioural alterations in MPS IIIA puppies before the appearance of cerebellar signs such as ataxia have not been detected in previous studies (Fischer et al., 1998; Jolly et al., 2000; Jolly et al., 2001). These previous studies did not document detailed descriptions of the behaviours shown by young MPS IIIA affected puppies whereas this study did include exploratory data on the range of behaviours shown by the puppies.

2.5.1   Correlations

The correlations between the characteristics measured in the tests were tested to check for convergent validity. Did the tests measure what they should? This was identified by determining whether items which should have correlated did. The repeatability and reliability of these tests was analysed by comparing them to previously performed analyses (Wilsson & Sundgren, 1998a). Wilsson and Sundgren (1998a) had similar correlation matrixes for the puppies that they studied which suggest that these tests do measure repeatable characteristics.

Examining the Spearman's rank correlation coefficients for the six and eight week test characteristics for the affected and unaffected puppies showed mainly only significant associations for the unaffected puppies. This is likely to be due to the small numbers of affected puppies available for analysis. The scores gathered for the unaffected puppies had similar associations to those seen in Wilsson and Sundgren (1998a) which suggests that there was some repeatability from that work.

The only significant association for the MPS IIIA affected puppies was a strong negative correlation between the fetch and the retrieve scores for the puppies at eight weeks of age, thus as the time to fetch increased, so the retrieve score decreased. The two tests are related because the fetch score is the time taken to pick up a ball and the retrieve is a measure of willingness to retrieve. The lowest retrieve score would

indicate no interest in retrieving and the highest fetch score would indicate that the puppy did not fetch.

2.5.2 Limitations of these tests

The interobserver reliability was calculated to provide a measure of the objectivity and validity of the six and eight week testing. Parallel independent coding of a random sample (five percent) of the videotapes was performed. The agreement between the observers was 83% and thus this suggests that it is unlikely that there was significant bias in the measurement of the puppies' behaviours. It is only in recent years that interobserver reliability has been calculated routinely for behavioural studies but it certainly increases confidence in the accuracy of the results gathered (Frank et al., 2007). In the original Wilsson and Sundgren (1998a) study there was an attempt at interobserver reliability by having two people present on each test occasion but it was unclear whether both people are scoring the tests and if so whether the scoring was independent.

Inconsistencies in the performance of the testing may have caused some inaccuracies in the measurements of these puppies' behaviours. The tester knew the puppies very well and was also the main trainer for these puppies. This led to some inconsistencies in the tests in that some puppies received more attention than others during the testing. This reduced the reliability and accuracy of these tests. In comparing these studies' results with those of Wilsson and Sundgren (1998a) the general trends are very similar so it is unknown how much effect these inconsistencies had but they will have influenced the results. In future testing it would be better to have an independent tester who has not previously interacted with the puppies.

2.5.3 Comparison between the six week and eight week findings

Although there are no statistically discernable differences between the unaffected puppies and the MPS IIIA affected puppies there is still useful information in describing the basic behaviours shown by these puppies at six and eight weeks of age for documentation of the puppies' behaviours for future analysis and comparison.

In a previous study younger puppies were found to vocalise more than older dogs (Frank et al., 2007). In this study since it was two groups of young puppies being compared it is difficult to know whether significant differences could be expected. The eight week puppies actually yelped and shrieked faster than they did at six weeks. This may be due to them becoming more socially aware and thus vocalising faster when people leave.

2.5.4   Areas for improvement in future testing

To enable more statistical analysis and potentially to find any differences between the affected and unaffected puppies it would be good to carry out these tests on a larger sample size. If this was done there are several ways in which the testing could be improved. More consistent and impartial testing of the puppies would help with the reliability and repeatability. In these tests the researcher knew the puppies well and some of the puppies had more interaction than others during the course of the testing.

If this testing was to be performed it would also be good to perform the retesting with an observer watching the testing directly since it was difficult to adequately determine where the puppies moved on the video since the view was not inclusive of all of the area.

2.5.5   Six and eight week test behaviours

The Mann-Whitney U test p-values comparing the MPS IIIA affected and unaffected dogs in the individual six and eight week tests ranged from 0.1928 to 0.9769 and thus no significant differences in the median behaviours of the affected or unaffected puppies were seen. This finding is supported by the previous studies on MPS IIIA which have not detected behavioural changes this early in MPS IIIA (Fischer et al., 1998; Jolly et al., 2000; Jolly et al., 2001).

The six week old puppies yelped quickly, took a longer time to shriek, made good contact with person entering pen and were not particularly interested in retrieving. They were interested in playing with a big ball and investigated a tug toy.

In the eight week test the puppies yelped a lot sooner on average than they had in the six week test. The affected puppies yelped slightly sooner than the unaffected puppies. It still took longer for the affected puppies to shriek three times.

Both the six and the eight week tests covered a wide range of the behaviours seen in puppies at this age. These tests are very dependant on the manner in which they are performed and it was important that the testing was validated and tested by an outside agent.

This study showed that the behaviours shown by the MPS IIIA puppies at six and eight weeks of age were not significantly different from those of the unaffected puppies. The behaviours shown appeared to be normal puppy behaviours similar to those shown in other studies on puppies (Goddard & Beilharz, 1983; Wilsson & Sundgren, 1998a).

This study supports the findings of the other studies on MPS IIIA in finding no significant behavioural alterations in the behaviours of young MPS IIIA affected dogs (Fischer et al., 1998; Jolly et al., 2000; Jolly et al., 2001). These findings suggest that clinical behavioural changes do not occur at such a young age and that future research should try to find when behavioural changes may first be seen.

CHAPTER 3

Canine Behavioural Assessment and Research Questionnaire to Measure Behaviour in MPS IIIA Affected and Unaffected dogs

3.1 Introduction

The canine behavioural assessment and research questionnaire (C-BARQ) was developed by Serpell and Hsu with additional questions from Svartburg (Hsu & Serpell, 2003; Svartberg, 2005) It is a validated survey for measuring the behaviour and temperament traits of pet dogs (Hsu & Serpell, 2003; Serpell & Hsu, 2001). This is a quantitative and reliable measure based mainly on the assumption that a dog's owner or carer knows most about the dog's behaviour. The C-BARQ was used in this study for two reasons. Firstly it was used to characterise the behaviours of MPS IIIA affected dogs and secondly it was used to determine whether there were behavioural differences between MPS IIIA affected dogs and unaffected dogs.

The measurement of dog behaviour by survey has been hampered by the lack of an accepted system for the classification and description of behaviour. In addition there has been a lack of stringent rigor in the reliability, repeatability and accuracy of behavioural research (Mills, 2003). Many behavioural studies were based on the ambition to develop a test for a particular type of dog or a particular behaviour problem (Goddard & Beilharz, 1982; Goddard & Beilharz, 1983; Goddard & Beilharz, 1984a; Goddard & Beilharz, 1984b; Goddard & Beilharz, 1985; Goddard & Beilharz, 1986;

Knol et al., 1988; Pfaffenberger et al., 1976). In the 1980s and early 1990s there was a relative dearth of good tests. The development of the C-BARQ was a breakthrough (Serpell & Hsu, 2001). This provided the clarity and impetus for a renewed flurry of research in this field. The data being gathered using this new tool has increased quality research since other researchers use the C-BARQ as a gold standard for comparing their data or as a data gathering tool (Diederich & Giffroy, 2006; Svartberg, 2005). This questionnaire is a well validated and reliable test (Hsu & Serpell, 2003; Serpell & Hsu, 2001).

3.2   Materials and methods

C-BARQs were completed at three months, six months and twelve months of age. They were completed by the trainer of the affected and unaffected dogs. The trainer, however, was blinded to the MPS IIIA status of the dogs. The questionnaires comprised a series of questions which examined the dogs' behaviours across a range of situations (Appendix Two). There were questions relating to several categories of behaviours including; training and obedience, fear, aggression, separation-related behaviour, attention-seeking behaviour, attachment excitability and sociability. The questionnaire items were answered on a five point rating scale.

3.3   Statistical analysis

Statistics were performed using Minitab statistical software (release date 31st January 2007, version 15). Descriptive statistics examined the distribution and tendencies of the data gathered. The null hypothesis and alternative hypothesis were used as a basis for the analysis performed. The null hypothesis is that there is no difference in behaviours or cognitive function between MPS IIIA affected dogs and unaffected dogs whereas the alternative hypothesis is that the there is a difference in the behaviours of the MPS IIIA affected dogs and the unaffected dogs but the direction of that change is unknown.

The C-BARQs were tabulated in a Microsoft Excel document (Appendix One) and behaviour factor scores from the questions were calculated using formulae obtained

from James Serpell (Table 3.2). Due to a number of irrelevant questions in the questionnaire several of the factors were adjusted and some were removed.

The internal consistency of the C-BARQ factors were originally measured by Serpell and Hsu using the Cronbach α (Serpell & Hsu, 2001). The α values were excellent for all of the factors used and so this internal consistency was assumed for this study except for some factors with altered formulae.

The dogs' trainer completed the questionnaire which comprises of questions about specific situations and scenarios that the dogs encountered in the previous several weeks.

The C-BARQ results were also analysed by developing boxplots of the factors at the three time points measured. Then Mann-Whitney U tests were performed to detect any differences in the medians between the affected and unaffected dogs.

3.4   Results

Of the original thirty-three colony dogs which were tested at six weeks, seventy-three percent completed the C-BARQ at three months, fifty-five percent at six months, and thirty-six percent at twelve months of age (Table 3.1).

Table 3.1 Number of questionnaires completed for the MPS IIIA affected and unaffected dogs at three, six and twelve months

	Data Type	Total Number Tested	Affected Dogs	Unaffected Dogs
C-BARQ				
3 months	Qualitative ordinal	24	7	17
6 months	Qualitative ordinal	18	5	13
12 months	Qualitative ordinal	12	6	6

3.4.1 Descriptive statistics

In the original development of the C-BARQ, factor analysis was carried out to determine the factors which could be derived from the data (Serpell & Hsu, 2001). Factors were identified which accounted for a substantial amount of the variation in the results. These results gave the empirical item groupings to describe behaviours or traits. The item groupings are owner-directed aggression, stranger-directed aggression, stranger-directed fear, dog-directed fear or aggression, non-social fear, separation-related behaviour, attachment or attention-seeking behaviour, trainability, chasing, excitability and pain sensitivity.

When analysing the data the factors of Serpell and Hsu (Serpell & Hsu, 2001) were used but since the subjects in this study are colony dogs their daily activities are different from the activities of the original study dogs. There were a number of the questions in the C-BARQ questionnaire which proved irrelevant for these dogs. This meant that there were missing values in many of the questions and this led to a reduction in the number of factors which could be analysed.

As there were numerous factors with missing data the original factor calculations (Table 3.2) were altered by the removal of several questions and adjustment of the resulting formulae. Some of the factors were completely removed due to irrelevant questions for that age of the dog or due to the dogs being in an enclosed colony situation. The reductions are shown in Table 3.3.

To investigate the data more fully summary statistics were calculated for each of the ages that the questionnaires were used at (Table 3.4).

Table 3.2 C-BARQ scoring method and formulae calculations for the C-BARQ factors

A: C-BARQ scoring method:

Score all frequency scale items as: Never = 0, Seldom = 1, Sometimes = 2, Usually = 3 and Always = 4, except for questions 5, 6 and 7 in Section 1. For these, reverse the scores to: Never = 4, Seldom = 3, Sometimes = 2, Usually = 1 and Always = 0.

B: Original formulae calculations:

Stranger-directed aggression (**SDA**) score	= (questions 10 + 11 + 12 + 15 + 16 + 18 + 20 + 21 + 22 + 28)/10.
Owner-directed aggression (**ODA**) score	= (questions 9 + 13 + 14 + 17 + 19 + 25 + 30 + 31)/8.
Dog-directed aggression/fear (**DDA/F**) score	= (questions 23 + 24 + 26 + 29 + 45 + 46 + 53 + 54)/8
Familiar dog aggression (**FDA**) score	= (questions 32 + 33 + 34 + 35)/4
Trainability (**Train**) score	= (questions 1 + 2 + 3 + 4 + 5 + 6 + 7 + 8)/8
Chasing (**Chas**) score	= (questions 27 + 75 + 76 + 77)/4
Stranger-directed fear (**SDF**) score	= (questions 36 + 37 + 39 + 40)/4
Nonsocial fear (**NSF**) score	= (questions 38 + 41 + 42 + 44 + 47 + 48)/6
Separation-related problems (**SRP**) score	= (questions 55 + 56 + 57 + 58 + 59 + 60 + 61 + 62)/8
Body sensitivity (**BS**) score	= (questions 43 + 49 + 50 + 52)/4
Excitability (**Excit**) score	= (questions 63 + 64 + 65 + 66 + 67 + 68)/6
Attachment/attention-seeking (**Att**) score	= (questions 69 + 70 + 71 + 72 + 73 + 74)/6

From James Serpell (personal communication, July 31, 2003)

Table 3.3 **Reductions and alterations to the original C-BARQ factor formulae due to irrelevant questions causing missing data**

Age	C-BARQ factors	C-BARQ questions removed
Three months		
	Unaltered Factors	
	Separation-related problems	
	Attachment/attention-seeking	
	Trainability	
	Altered Factors	
	Familiar dog aggression	34
	Stranger-directed fear	36, 37
	Excitability	65, 66, 67
Six months		
	Unaltered Factors	
	Dog-directed aggression/fear	
	Familiar dog aggression	
	Trainability	
	Separation-related problems	
	Attachment/attention-seeking	
	Altered Factors	
	Owner-directed aggression	14
	Stranger-directed fear	36, 37
	Nonsocial fear	44
	Body sensitivity	50
	Excitability	65, 67
Twelve months		
	Unaltered factors	
	Owner-directed aggression	
	Trainability	
	Separation-related problems	
	Attachment/attention-seeking	
	Altered factors	
	Body sensitivity	49
	Excitability	65

Table 3.4   Summary statistics for the C-BARQ factors

A: Three months MPS IIIA affected dogs

Variable	Mean	St Dev	Q1	Median	Q3
FDA	0.667	0.793	0.000	0.667	0.667
Train	2.464	0.472	2.125	2.375	2.625
SDF	0.357	0.378	0.000	0.500	0.500
SRP	0.839	0.437	0.375	0.875	1.250
Excit	2.381	1.044	1.667	2.000	3.667
Att	1.952	0.665	1.333	2.167	2.500

Number of MPS IIIA affected dogs:7

Three months unaffected dogs

Variable	Mean	St Dev	Q1	Median	Q3
FDA	0.706	0.686	0.167	0.667	1.000
Train	2.110	0.474	1.625	2.125	2.563
SDF	0.294	0.614	0.000	0.000	0.250
SRP	0.7353	0.4119	0.3750	0.6250	1.1250
Excit	1.980	0.672	1.500	2.000	2.500
Att	1.363	0.610	0.833	1.333	1.833

Number of unaffected dogs:17

B. Six months MPS IIIA affected dogs

Variable	Mean	St Dev	Q1	Median	Q3
ODA	0.1714	0.1195	0.0714	0.1429	0.2857
DDA/F	0.775	0.445	0.313	1.000	1.125
FDA	1.500	1.311	0.375	1.000	2.875
Train	2.675	0.597	2.125	2.625	3.250
SDF	0.200	0.274	0.000	0.000	0.500
NSF	0.840	0.385	0.500	1.000	1.100
SRP	0.900	0.335	0.625	0.750	1.250
BS	1.0000	0.000	1.0000	1.0000	1.0000
Excit	2.000	0.848	1.250	1.750	2.875
Att	1.867	1.089	0.833	2.000	2.833

Number of affected dogs: 5

Six months unaffected dogs

Variable	Mean	St Dev	Q1	Median	Q3
ODA	0.1319	0.0915	0.0714	0.1429	0.1429
DDA/F	0.7500	0.3104	0.5625	0.8750	1.0000
FDA	1.173	0.874	0.375	1.000	2.125
Train	2.413	0.511	1.938	2.500	2.938
SDF	0.462	0.828	0.000	0.000	0.500
NSF	0.969	0.559	0.600	0.800	1.200
SRP	0.856	0.411	0.563	0.750	1.125
BS	0.667	0.492	0.333	0.500	1.000
Excit	1.981	0.505	1.750	1.750	2.125
Att	1.205	0.578	0.667	1.167	1.750

Number of unaffected dogs: 13

C. Twelve months MPS IIIA affected dogs

Variable	Mean	St Dev	Q1	Median	Q3
SRP	0.271	0.406	0.000	0.063	0.625
BS	0.667	0.471	0.333	0.333	1.167
Excit	1.450	0.512	1.000	1.500	1.875
Att	1.361	1.040	0.250	1.500	2.375

Number of affected dogs: 6

Twelve months unaffected dogs

Variable	Mean	St Dev	Q1	Median	Q3
SRP	0.250	0.440	0.000	0.063	0.469
BS	0.667	0.333	0.333	0.667	1.000
Excit	1.650	0.379	1.375	1.500	2.000
Att	1.139	1.123	0.250	0.750	2.333

Number of unaffected dogs: 6

Key:

SDA	- Stranger-directed aggression score
ODA	- Owner-directed aggression score
DDA/F	- Dog-directed aggression/fear score
FDA	- Familiar dog aggression score
Train	- Trainability score
Chas	- Chasing score
SDF	- Stranger-directed fear score
NSF	- Nonsocial fear score
SRP	- Separation-related problems score
BS	- Body sensitivity score
Excit	- Excitability score
Att	- Attachment/attention-seeking score

Boxplots of the C-BARQ factors comparing the affected and unaffected dogs were compiled for three, six and twelve months of age (Figures 3.1, 3.2, 3.3). The boxplots are useful in that they show the median and spread of the scores across the affected and unaffected dogs. The general behaviours of the dogs can be gauged from these graphs.

At three, six and twelve months of age separation-related problems vary slightly but remained low. Trainability was moderate at both three and six months of age. Stranger-directed aggression was very low at both three and six months of age whereas familiar dog aggression was moderate at six months of age.

On examination of these graphs it is clear that there is no significant difference between the affected and unaffected dogs in terms of any of the factors at either three, six or twelve months of age.

Figure 3.1 Boxplots of C-BARQ factors at three months of age comparing the unaffected and MPS IIIA affected dogs
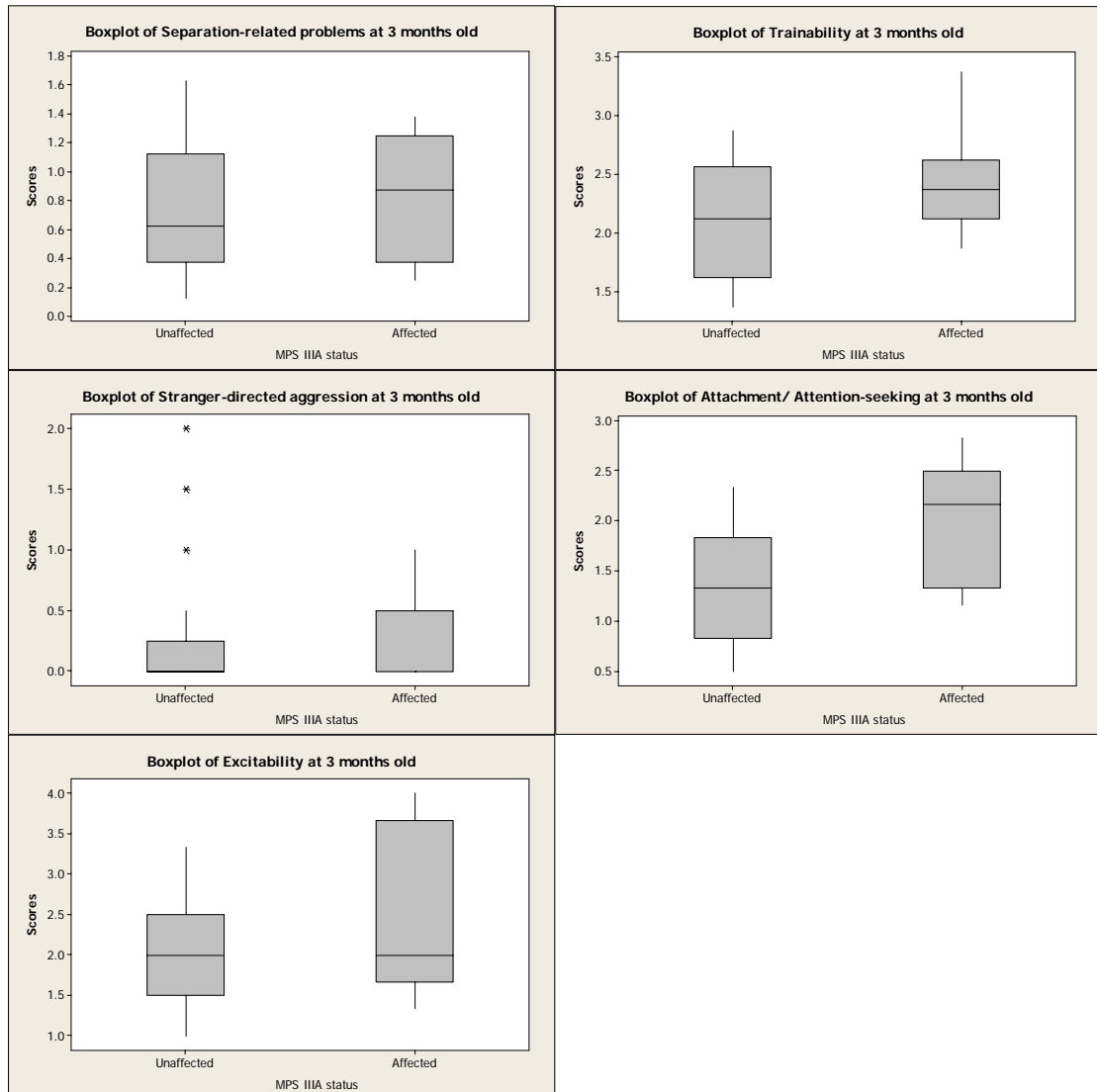
Figure 3.2 Boxplots of C-BARQ factors at six months of age comparing the unaffected and MPS IIIA affected dogs
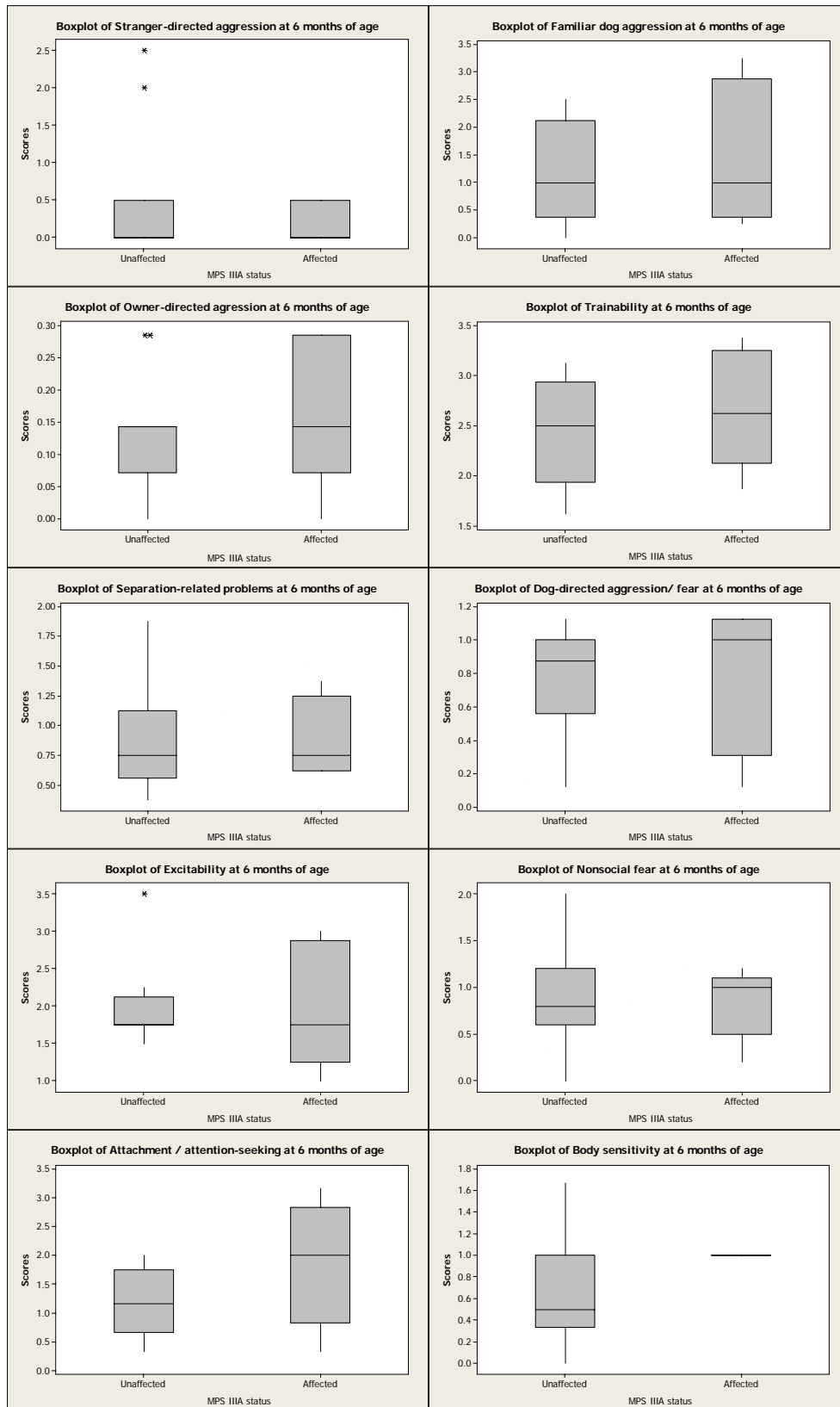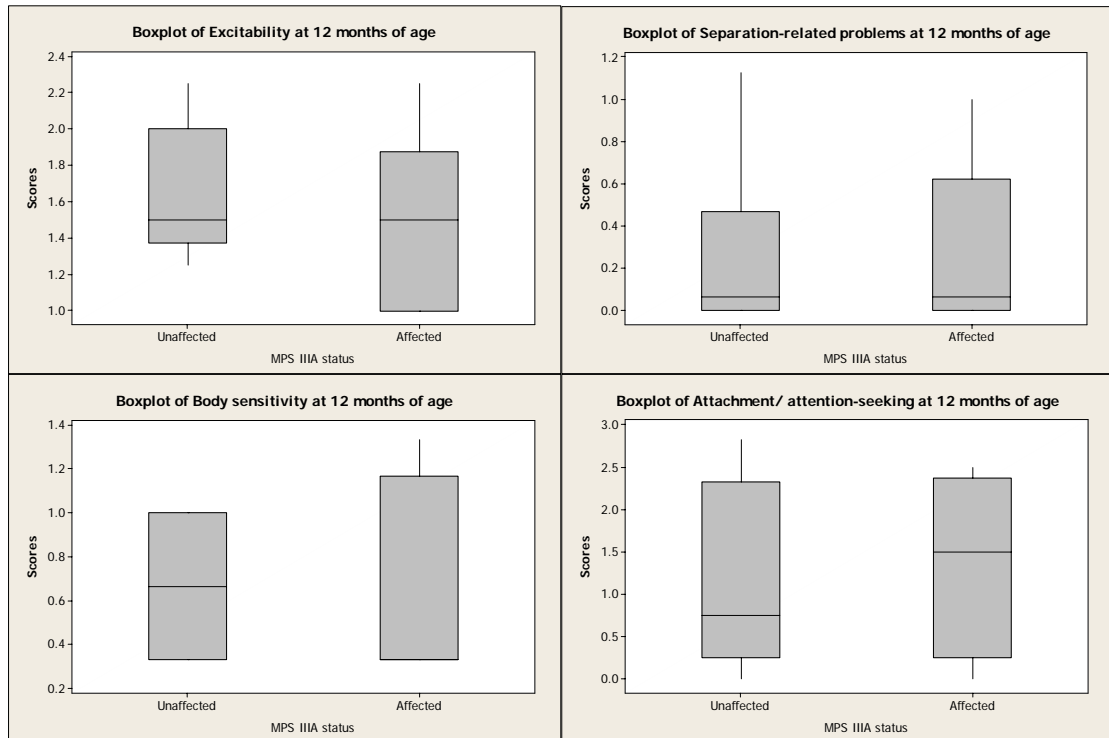
Figure 3.3 Boxplots of C-BARQ factors at twelve months of age comparing the unaffected and MPS IIIA affected dogs

3.4.2   Mann-Whitney *U* tests

To investigate whether there was a significant difference between the medians of the affected and unaffected dogs in terms of the factors derived from the C-BARQ Mann-Whitney U tests were performed (Table 3.5). These tests did not detect any significant differences between the medians.

Questions one, two, three, four, five, seven, eight and ninety-one (Appendix Two) were examined individually because they were about behaviours which MPS IIIA may influence (Table 3.6). For example question ninety-one asks whether the dog is hyperactive, restless or has trouble settling down (Appendix Two).

For question 7 of the 12 month CBARQ there was a significant difference between the affected and unaffected dogs on Mann Whitney U analysis (p <0.05).   This question asked how distractible the dog was to sights, sounds and smells. The MPS IIIA affected dogs were less distractible than the unaffected dogs.

Question 8 of the 3 month CBARQ showed a significant difference between the affected and unaffected dogs. That question asked how much the dog retrieved and the results showed that the affected dogs retrieved more than the unaffected dogs. For the same question for the 6 month CBARQ the differences between the affected and unaffected dogs bordered on being significant (p=0.0720). Thus there was a reasonable likelihood that the affected dogs retrieved or attempted to retrieve more than the unaffected dogs. The trends of the question 8 data were shown in Figure 3.4.

In the 3 month questionnaire, question 5 also approached significance (p = 0.0538). If there was a difference to the population it would be that the unaffected dogs were less likely than the affected dogs to respond to correction or punishment or to be thick-skinned.

Table 3.5   Mann-Whitney *U* comparing the MPS IIIA affected and unaffected dogs for the C-BARQ factors at three, six and twelve months of age.

3 Months	Number unaffected 17, Number affected 7				
	Median				
	Unaffected	**affected**	**W**	**P Value**	**P Value ***
FDA	0.667	0.667	219	0.7032	0.6977
Trainability	2.125	2.375	191.5	0.1929	0.1908
SDF	0	0.5	197.5	0.3571	0.2709
SRP	0.625	0.875	203	0.5676	0.5652
Excit	2	2	201.5	0.5049	0.4997
Att	1.333	2.167	183.5	0.0703	0.0674

6 Months	Number unaffected 13, number affected 5				
	Median				
	Unaffected	**affected**	**W**	**P Value**	**P Value ***
ODA	0.1429	0.1429	116.5	0.5217	0.4763
DDA	0.875	1	118	0.6221	0.617
FDA	1	1	118	0.6221	0.6199
Trainability	2.5	2.625	114	0.375	0.3733
SDF	0	0	125	0.9215	0.9095
NSF	0.8	1	123.5	1	1
SRP	0.75	0.75	118	0.6221	0.6168
Excit	1.75	1.75	127	0.7674	0.7566
Att	1.167	2	109.5	0.1833	0.1815

12 Months	Number unaffected 6, number affected 6				
	Median				
	Unaffected	**affected**	**W**	**P Value**	**P Value ***
SRP	0.0625	0.0625	39	1	1
BS	0.6667	0.3333	28	1	1
Excit	1.5	1.5	31.5	0.4647	0.4477
Att	0.75	1.5	37	0.8102	0.8095

*adjusted for ties

Table 3.6 Mann-Whitney *U* – C-BARQ Results for questions one, two, three, four, five, seven, eight and ninety-one comparing the MPS IIIA affected and unaffected dogs.

Significance Level: 0.05

A. 3 Month Questions

	Number of dogs		Median			P	P Value
	Unaffected	affected	Unaffected	affected	W	Value	*
Question 1	17	7	2	3	201.0	0.4848	0.4372
Question 2	17	7	2	2	216.0	0.8489	0.8416
Question 3	16	7	1	0	203.0	0.4830	0.4548
Question 4	17	7	3	3	188.5	0.1356	0.0848
Question 5	17	7	2	3	184.0	0.0754	0.0538
Question 7	17	7	2	2	199.0	0.4090	0.3743
Question 8	17	7	2	3	181.5	0.0527	**0.0328**
Question 91	17	7	1	1	211.0	0.9494	0.9423

B. 6 Month Questions

	Number of dogs		Median			P	P Value
	Unaffected	affected	Unaffected	affected	W	Value	*
Question 1	13	5	2	3	108.5	0.1529	0.1180
Question 2	13	5	3	3	110.0	0.2000	0.1037
Question 3	13	5	3	3	115.0	0.4304	0.3440
Question 4	13	5	3	3	117.0	0.5542	0.5133
Question 5	13	5	2	2	133.0	0.3750	0.3061
Question 7	13	5	2	2	122.5	0.9607	0.9588
Question 8	13	5	2	4	105.5	0.0845	0.0720
Question 91	13	5	1	1	122.5	0.9607	0.9549

*adjusted for ties

C. 12 month Questions

	Number of dogs		Median			P	P Value
	Unaffected	affected	Unaffected	affected	W	Value	*
Question 1	6	6	3	3	36.0	0.6889	0.5948
Question 2	6	6	3	3	39.0	1.0000	1.0000
Question 3	6	6	1.5	1	39.0	1.0000	1.0000
Question 4	6	6	2.5	3	31.5	0.2623	0.2120
Question 5	6	6	3	3	37.0	0.8102	0.7842
Question 7	6	6	2	3	26.0	0.0453	**0.0225**
Question 8	6	6	1	2	32.0	0.2980	0.2731
Question 91	6	5	2	1	41	0.4113	0.3912

*adjusted for ties

Figure 3.4 Box-plot of question eight on retrieving in the C-BARQ comparing the MPS IIIA affected and unaffected dogs at three, six and twelve months of age
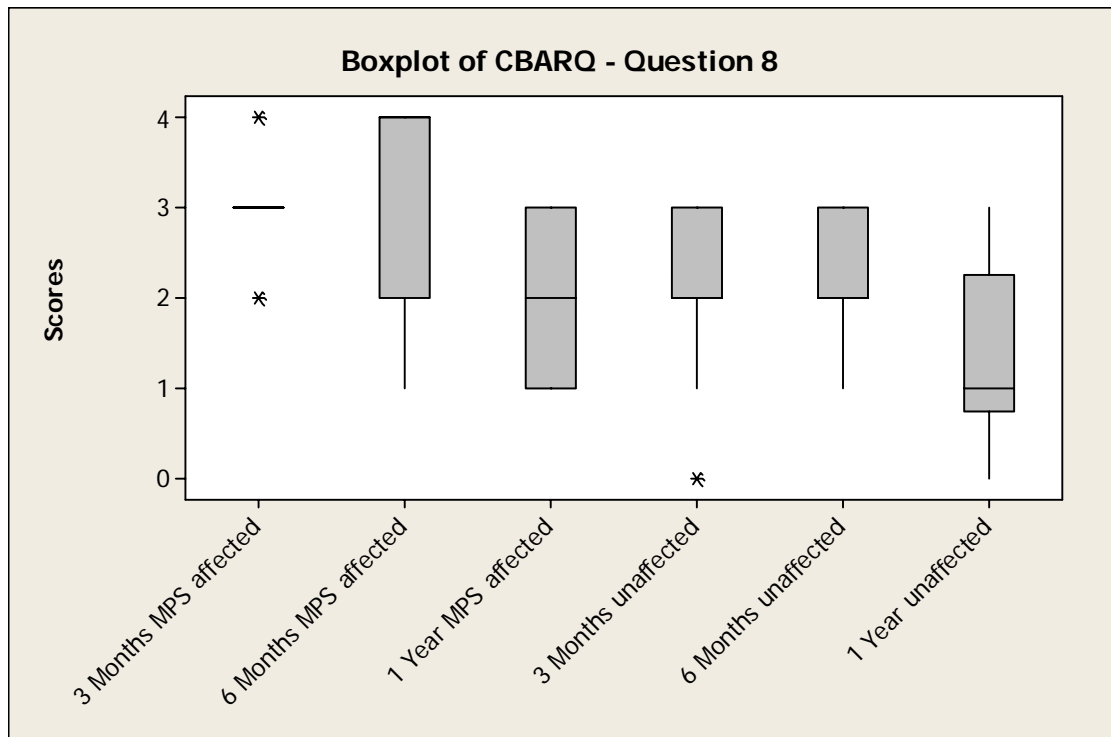
3.5   Discussion

This study is the first to focus on the behaviour of MPS IIIA affected dogs up to twelve months of age and as such provides valuable information on the behaviours seen in these dogs. This study used a 101 item questionnaire with validated factors and nearly all behaviours proved to be statistically similar in both affected dogs and in the unaffected dogs. This finding supports previous studies on MPS IIIA which also did not detect behavioural changes in young MPS IIIA affected dogs including Huntaways and Dachshunds (Jolly et al., 2000; Jolly et al., 2007). It also supports the null hypothesis that there is no difference between the behaviours of unaffected dogs and MPS IIIA affected dogs early in their lives.

The other objective of this study was to document the behaviours shown by MPS IIIA affected dogs and the instrument used for this was of key importance. The C-BARQ has several advantages over the use of a one-instant practical behavioural test once dogs are older than about 8 weeks old. Firstly the C-BARQ is comprehensively validated and repeatable (Hsu & Serpell, 2003; Serpell & Hsu, 2001) and secondly it has the advantage of measuring the dog's behaviour over the few months prior to taking the questionnaire. Thus it gives a reliable picture of the dogs' behaviours.

Completing this questionnaire at three, six and twelve months of age was frequent enough to capture much of the dogs' first year of life in terms of their behaviour. The categories of behaviours that the C-BARQ examines are also appropriate because they include behaviours which one would expect MPS IIIA to alter. In humans this includes progressive dementia, insomnia, sleep problems, short attention span, hyperactivity, temper tantrums, destructive behaviour and aggression and mental retardation and the C-BARQ includes questions related to many of these potential problems (Neufeld & Muenzer, 2001). In mice the signs include hyperactivity and aggression and the C-BARQ includes questions which would detect many of these alterations (Gliddon & Hopwood, 2004).

As numerous behavioural signs are seen in other species with MPS IIIA it seems logical that affected dogs would also have behavioural alterations. From the previous studies

on MPS IIIA in dogs it is unlikely that the behavioural signs of this disease are going to be obvious since previous clinical cases have not had behavioural signs that the owners noticed (Jolly et al., 2000; Jolly et al., 2001). This suggests that using a tool such as the C-BARQ which measures a range of behaviours over a long time period, and gathers together people's recollections of the specifics of the dog's behaviours, would be a useful means of focusing on subtle behavioural changes.

The association between the questions in the questionnaire factor analysis was carried out in the original study which developed the C-BARQ (Serpell & Hsu, 2001). The Serpell and Hsu factors were used but some questions were not relevant and several factors were not calculated or had unused questions removed. Having a number of irrelevant questions reduced the significance of the findings. Even with the validity of some factors reduced the C-BARQ was the most reliable tool for questionnaire-based behaviour measurement for this study.

The summary mean was the overall mean of each factor. The means and other summary statistics were calculated to examine the trends of the dogs' behaviours. When the factors were examined differences were found over the first year. Separation related behaviour problems such as barking, howling or restlessness when left alone, were similar in the MPS IIIA affected dogs at three and six months of age (0.839 at three months, 0.9 at six months) but decreased by twelve months (0.271). However this was not a large decrease in that these factors are an average of the individual's scores for that factor and zero is a behaviour never occurring and one is the behaviour seldom occurring. This suggests that the difference between a 0.839 and a 0.271 is minimal in terms of clinical significance. In pet puppies separation-related behaviours tend to be seen in puppies less than three months of age and within six days of them being adopted which suggests that it is weaning and moving the puppies to a new social grouping which causes separation-related behaviours to be seen (Frank et al., 2007). Puppies in this study do not leave their colony and that is likely to be why they do not show high levels of separation-related behaviours.

The factors compiled were also graphed as comparative boxplots of the affected and unaffected dogs at each time point. These graphs clearly showed no major difference between the behaviours shown by the two groups of dogs. The summary medians were

compared using a two sample test. Mann-Whitney *U* tests have been used in previous studies on the C-BARQ and were appropriate for this data (Serpell & Hsu, 2001). Mann-Whitney *U* tests were performed to determine whether dogs with MPS obtained significantly higher or lower scores on the corresponding factors than the non-affected dogs. In terms of the C-BARQ factors calculated no statistically significant differences were found.

Several questions in the C-BARQ were also individually analysed using Mann-Whitney *U* tests since these questions were where differences between affected and unaffected dogs would be expected, namely in distractibility, obedience and performing a task. There was a significant difference between the affected and unaffected dogs relating to how much the dogs retrieved and to how distractible they were, however the difference was not in the direction expected. The results approached significance for the six month old dogs and were significant for the three month old puppies for how well they retrieved. The results were also significant for the twelve month old dogs as to how distractible they were. The findings are not what would have been expected for a decline in brain function. Namely the MPS IIIA affected dogs retrieved more and were less distractible than the unaffected dogs. It may be that these results are spurious, or there may be a subtle difference in how these dogs' brains are functioning at this early stage in the disease. One possibility is that they have a reduced range of behaviours that they can show in a given situation due to their brain pathology. If this was shown in future studies an entirely different approach for future research would be needed.

An important factor to consider about the C-BARQ is that it is a person based questionnaire. Thus behaviours of the dog are reported using the memory and knowledge of the handler about that dog. One of the limitations of using this questionnaire is that it is a colony of dogs being investigated not one person with a pet dog, and it may be more difficult for the handler to differentiate between each dog. The other limitations of this study are the alterations in the C-BARQ factors and the low number of dogs available for study.

In future studies it would be good to increase the reliability of the questionnaire by ensuring that the colony dogs are exposed to more of the situations addressed in the original study questions such as travelling in a car and meeting more strangers. This

would give a broader range of behaviours to compare the dogs on. Continuing the study on to two years at least would also have increased the reliability of the results.

In summary this study mainly supported other MPS IIIA dog studies in not demonstrating differences between the behaviours of the affected and unaffected dogs (Fischer et al., 1998; Jolly et al., 2000; Jolly et al., 2001). The finding that the affected dogs retrieved more and were less distractible would be worth investigating to decide whether it suggests a subtle alteration in brain functioning.

CHAPTER 4

Cognitive function measurement – maze testing comparing between MPS IIIA affected and unaffected dogs

4.1   Introduction

Cognitive function measurement in dogs has been researched intensively only in the last ten years as the dog became a model of human cognitive aging (Head et al., 2000; Ikeda-Douglas et al., 2005; Ikeda-Douglas et al., 2004; Nippak et al., 2006; Nippak et al., 2007; Siwak et al., 2001; Siwak et al., 2003; Studzinski et al., 2006). Prior to this there was little scientific research into dog cognition and most information was anecdotal. This study will determine if there are signs of cognitive dysfunction in young MPS IIIA affected dogs. This information will be used to improve our understanding of the MPS IIIA dog model and to improve the use of this model for developing therapies.

There is great individual variation in the latency to undertake tasks in dogs and this latency is also influenced by the difficulty of the task. The harder the task the longer the latency (Nippak & Milgram, 2005). Many cognitive tests are used on older dogs (Tapp et al., 2003a). In this study dogs identify an object so as to obtain a food reward. Maze tests measure cognitive function in young dogs by recording their ability to choose where to go in a T-shaped maze to obtain a food reward (Kelley & Lepine, 2005; Kelley et al., 2004).

This maze test is a form of object discrimination learning which examines the acquiring and retention of semantic memory in dogs (Adams et al., 2000). The shortest distance for an object to be in focus is 25 cm (Callahan et al., 2000) and in this test the dogs were able to see the discriminatory object from that distance or greater.

This study's aim will determine if there is a discernable difference between the cognitive function of the MPS IIIA affected or unaffected dogs.

4.2   Materials and methods

4.2.1   Subjects

The subjects were 21 dogs from a breeding population of MPS IIIA affected and unaffected Huntaway dogs. These dogs were held in a research facility in New Zealand. Puppies produced by this colony in 2004 and 2005 were recruited into this study. These dogs had behavioural tests carried out at six and eight weeks of age (chapter two). These dogs were also a part of a multi-faceted study into MPS IIIA.

These dogs were not preselected for training since dog numbers were low and thus all available dogs were used in the study. Physical examinations were carried out on the dogs within 48 hours of their birth and at the same time a blood spot was taken from each dog for genetic diagnosis of their MPS IIIA status. No dogs were removed from the study on the basis of this testing. Each dog was identified using a microchip (BackHome electronic identification program, Virbac, Auckland, New Zealand).

A deoxyribonucleic acid/polymerase chain reaction blood test was carried out on these dogs to determine whether they had MPS IIIA or not (Yogalingam et al., 2002). The testing and identification of the dogs was carried out by a veterinarian who did not collect or analyse any of the behavioural data of this current study and thus the researchers in this study were blinded to the dogs' MPS IIIA status. The 21 dogs were composed of 14 unaffected dogs (8 male and 6 female) and 7 MPS IIIA affected dogs (5 male and 2 female).

4.2.2   Experimental design

A maze was constructed from plywood (3m x 1.8m x 0.6m) (Figure 4.1). The entrance to the maze led to a T-shaped junction and then to the left and right arm of the maze. At the end of each arm of the maze was a wooden box (0.3m x 0.3m x 0.2m). During training and testing each box held a container of food (Schmackos, Mars Petcare Australia, NSW). At the correct location for each test the box was facing so that food was accessible, whereas in the incorrect location the food was inaccessible. Both boxes were positioned so that the dogs could not see the food as they approached. There was a wooden door at the end of each arm of the maze so that the dog could be allowed out of the maze on completion of the task. Training and testing only occurred on relatively fine days as the maze was outside and did not occur every day due to time constraints.

Figure 4.1 Diagram of the T-shaped maze used to test the cognitive function of MPS IIIA affected and unaffected dogs

Adapted from (Kelley & Lepine, 2005)

This study involved two parts, training and testing. From approximately 20 weeks of age all dogs were trained to associate an item (randomly allocated either a road cone or a brick) with moving through either the left or right arm of the maze to obtain a food reward. For example a road cone might indicate that the food was at the end of the right arm of the maze. The dogs were tested in alternate directions and the order of training the dogs on each day was also randomised.

The symbol was placed on the ground ahead of the dog waiting area. The dog was placed on a sit and stay command at the dog waiting area and then released. The time from when the dog entered the maze to when the dog reached either the correct or incorrect box at the end of the maze was measured. Ten trial runs were performed per day of training. It was also noted whether the dog went to the correct or incorrect location for each trial.

Training was repeated for the same symbol and location association until the dog made the correct choice in 90% of the trials for two successive training days. Then training was carried out in the same manner using the alternate symbol, going down the opposite arm of the maze, until again the dog was correct in 90% of the trials for two successive training days.

The second step was testing to quantify how well the dogs had learnt the task. Three days of testing were carried out. On the first day of testing ten trials were performed using the first item the dogs were trained on. Ten trials were carried out on the second day using the second item. On the last day of testing the items presented were randomly selected for ten trials.

For the tests the dog was presented with the item and placed on a sit and stay. It was then released to enter the maze. The time taken to reach the end of the maze and whether the correct location was reached was recorded. The dog received a food reward if it reached the correct location for the item presented. Training or testing days were not necessarily successive.

4.2.3 Data collection and statistical analysis

The data has been included in a DVD-ROM (Appendix One). The data was tested for normality using the Anderson-Darling test. If not normally distributed then the data was analysed for differences between the sample medians using the Mann-Whitney U test. The Mann-Whitney U test was more appropriate than the Chi-squared since it is a non-parametric technique and more reliable for small populations. Boxplots were also developed to analyse the data.

Due to the weather and time limitations on when testing could be carried out the ages of the dogs varied at the time of completion of testing. Thus to determine whether this affected the results Spearman's rank correlation coefficient was carried out on the dogs' ages and the success of the final random testing.

4.3   Results

4.3.1   Training results

Each dog was required to complete at least 90% of the trials correctly on two successive days of training before proceeding to the next stage. Thus the number of training days per dog varied widely. Many of the dogs completed the training quickly but some dogs took several more days of training to learn the tasks. When the number of training days per dog was graphed it was seen to not be normally distributed (Figure 4.2).

Since this was non-normally distributed data a Mann-Whitney U test was performed to determine whether there was a difference between the population medians of the unaffected and affected dogs for the number of training days. There was no significant difference (Table 4.1).

Figure 4.2 **Boxplot comparing the number of days of training carried out by MPS IIIA affected and unaffected dogs**
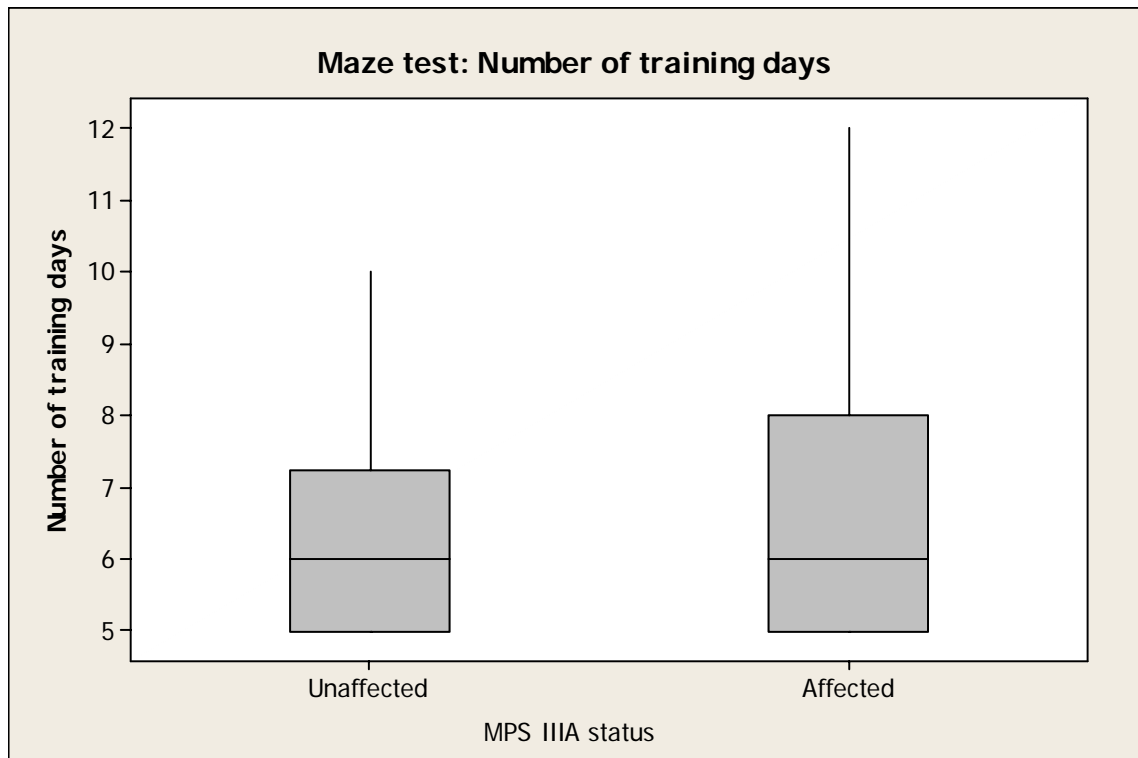

Table 4.1 **Mann-Whitney *U* test comparing the median number of training days for the MPS IIIA affected and unaffected dogs**

	Number	Median
MPS IIIA affected dogs	7	6.000
Unaffected dogs	14	6.000

Confidence Interval for the difference between the two population medians is -1.001 to 1.999

W = 82.0   P = 0.7371

P = 0.7265 (adjusted for ties)

The mean of the correct number of training exercises per day of training was also examined. Figure 4.3 demonstrates the general distribution of the information.

On examination of the graph the MPS IIIA affected dogs appeared to have slightly less correct training exercises per day of training. The data was not normally distributed and to investigate the data more effectively a Mann-Whitney *U* test was performed (Table 4.2). The test showed no significant difference (p = 0.3905 adjusted for ties) between the median correct training scores for the affected and unaffected dogs.

Due to the training taking differing time periods in each dog this meant that the dogs were different ages at the completion of training. To ensure that this did not bias the training results a Spearman's rank correlation coefficient was calculated (Table 4.3). As can be seen from the result the age of the dogs was not statistically correlated to the success of the dogs.

One MPS IIIA affected dog was trained but was not able to achieve the target of two days of 90% correct trials and thus that dog did not complete the training or move onto testing.

Figure 4.3   Boxplot comparing the mean number of correct training exercises for MPS IIIA affected and unaffected dogs per training day
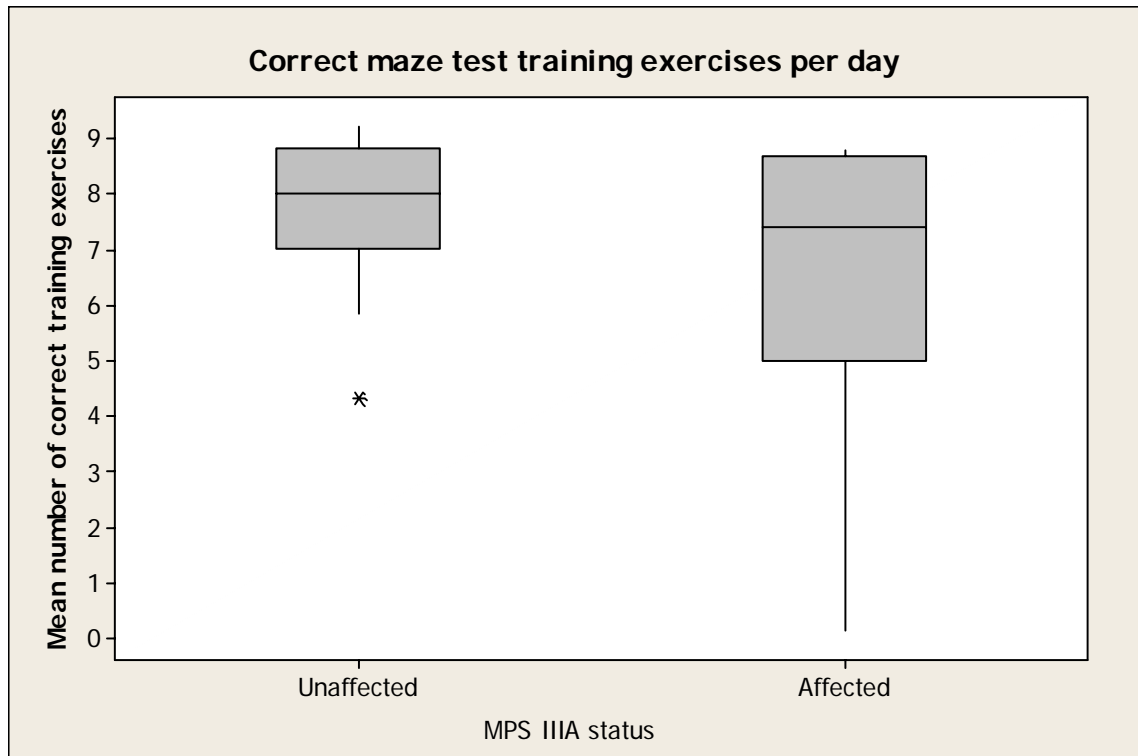

Table 4.2   Mann-Whitney *U* test comparing the median number of correct training exercises for MPS IIIA affected and unaffected dogs per day

	Number of dogs	Median
Unaffected dogs	14	8.000
MPS IIIA affected dogs	7	7.400

Confidence Interval for the difference between the two population medians is -0.799 to 2.833

W = 166

P = 0.3909

P = 0.3905 (adjusted for ties)

Table 4.3 **Spearman's rank correlation coefficient for the number of successful final test results correlated to the age of the dogs**

N 20

Rs -0.2182

t -0.95

Df 18

Two tailed P value   =   0.354694

4.3.2   Time taken for testing

Boxplots were also compiled to examine the general trends on the latency to perform a task. Graphs were made of the: (1) time taken to perform the correct maze tests (Figure 4.4), (2) time taken for the correct final tests (Figure 4.5) and (3) total testing time (Figure 4.6).   It is appeared from these graphs that no differences were evident between the unaffected and affected dogs and this was confirmed through Mann-Whitney U tests (Table 4.4).

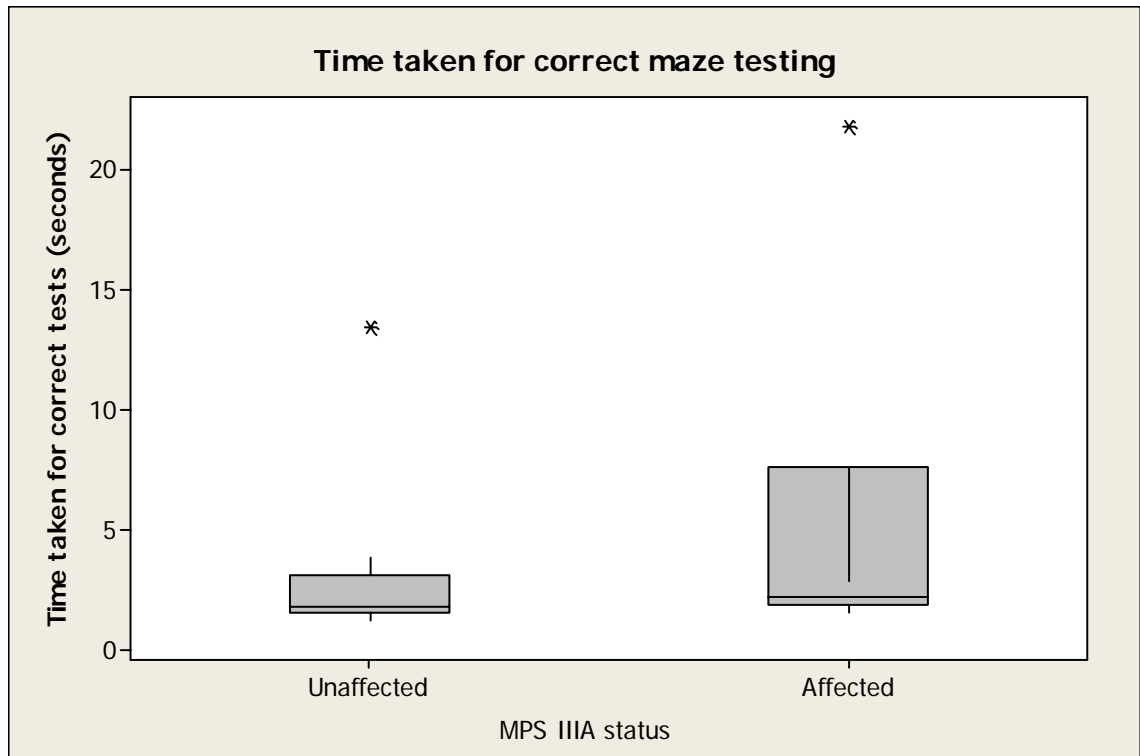**Figure 4.4** **Boxplot comparing the time taken to perform the correct tests for MPS IIIA affected and unaffected dogs**

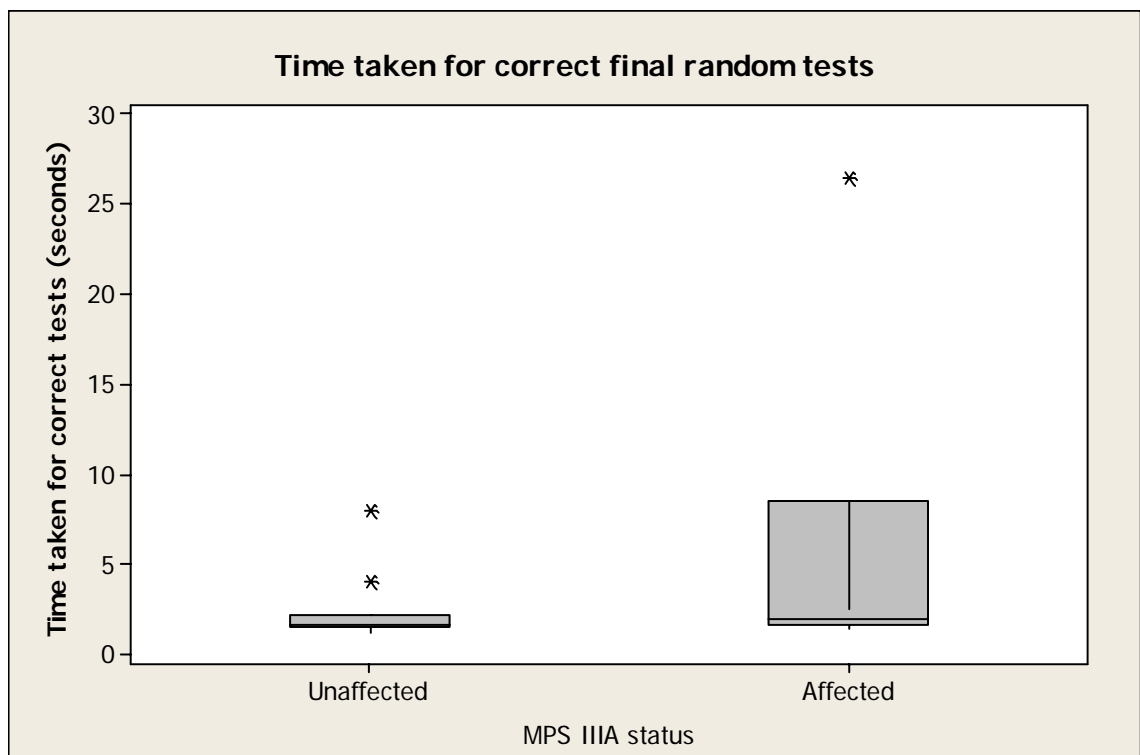**Figure 4.5** **Boxplot comparing the time taken for the correct final tests for MPS IIIA affected and unaffected dogs**

Figure 4.6 **Boxplot comparing the total testing time for MPS IIIA affected and unaffected dogs**
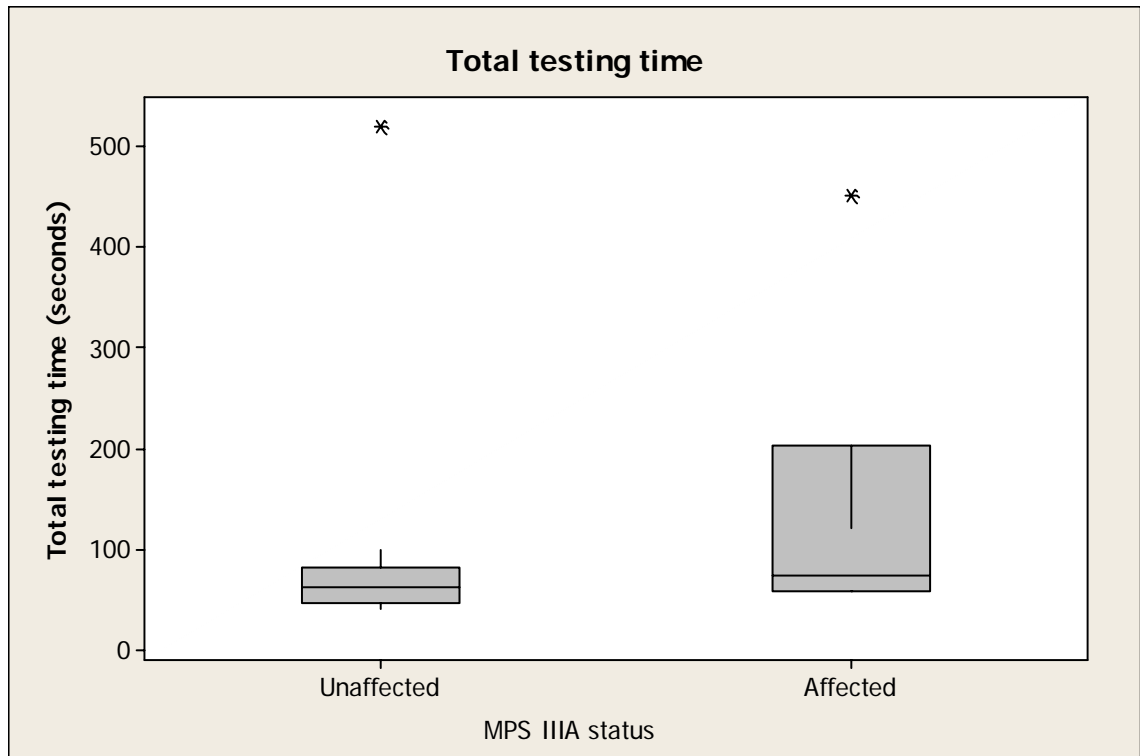

Table 4.4 **Mann-Whitney *U* tests comparing unaffected and MPS IIIA affected dogs for the time taken for correct maze tests, total maze tests and correct final maze tests**

	Number of dogs		Median		
Time taken for:	**Unaffected**	**affected**	**Unaffected**	**affected**	**P Value**
Correct tests (seconds)	14	6	1.83	2.2	0.3865
Total testing (seconds)	14	6	62	75.1	0.2655
Correct final tests (seconds)	14	6	1.71	2.05	0.2317

significance level 0.05

4.3.3 Results for first two days of testing

Descriptive statistics were calculated on the tabulated data in Table 4.8 to show the general data trends (Table 4.5). Standard deviations were high for the first two days of testing indicating a wide spread to the data. For the left item testing (performed using the item indicating to go down the left arm of the maze) the standard deviation for the unaffected dogs was 3.079 which suggests a large variability in the dogs' responses to the test.

Analysing left and right symbol testing for the first two days of testing was performed to look for signs of left or right bias. The left or right item was either the road cone or the brick for each dog (randomly allocated). On one of the first two testing days the dogs were presented with their left symbol. For this testing day the number of correct tests per dog out of ten was calculated and the results graphed (Figure 4.7). Examining the graph there was not a large difference between the results for the affected and unaffected dogs. In terms of distribution affected dog results were mostly five or six correct tests whereas for unaffected dogs the results were more widely distributed. When a Mann-Whitney U test was performed it showed that the medians of the affected and unaffected dogs were very statistically similar (Table 4.6). This was shown by a p-value of 0.8025 adjusted for ties. A Mann-Whitney U test was also performed which showed no statistically significant difference between the performance of the male or female dogs when locating the left symbol (p = 1).

To analyse the data for the dogs using their right symbol a boxplot was devised, and Mann-Whitney U test calculated. Figure 4.8 showed that the MPS IIIA affected dogs did not appear to have performed as well on this task as on the left symbol testing whereas the unaffected dogs appear to have performed better. The Mann-Whitney U test had a p-value of 0.0854 which although not demonstrating a significant difference between the affected and unaffected dogs is very different to the result for the left symbol (Table 4.6). A Mann-Whitney U test showed no statistically significant difference between the performance of male or female dogs when locating the right symbol (p = 0.4562 adjusted for ties).

The first two days of testing were then analysed. A graph of the first day of testing showed that MPS IIIA affected dogs appeared to perform slightly worse than the unaffected dogs (Figure 4.9). A Mann-Whitney U test however showed no significant difference in the medians (p =0.2779 adjusted for ties) (Table 4.6). The second day of testing was also graphed to examine the general trend (Figure 4.10). On the second day of testing the MPS IIIA affected dogs appeared to perform better than on the previous day and the unaffected dogs had a more variable response. A Mann-Whitney U test however showed that there was not a significant difference in the median score between the affected and unaffected dogs (p = 0.7382 adjusted for ties) (Table 4.6).

Table 4.5   **Descriptive statistics for the maze testing comparing the MPS IIIA affected and unaffected dogs**

Variable	Mean	SE Mean	Standard Deviation
	(number of correct tests)		
Unaffected: **14 dogs**			
Left symbol	5.643	0.823	3.079
Right symbol	5.571	0.754	2.821
First day of testing	4.786	0.720	2.694
Second day of testing	6.429	0.789	2.954
Final day of testing	5.429	0.359	1.342
MPS IIIA affected: **6 dogs**			
Left symbol	6.333	0.760	1.862
Right symbol	3.500	0.992	2.429
First day of testing	3.500	0.992	2.429
Second day of testing	6.333	0.760	1.862
Final day of testing	4.000	0.447	1.095

Figure 4.7   Boxplot comparing testing using the left symbol for MPS IIIA affected and unaffected dogs
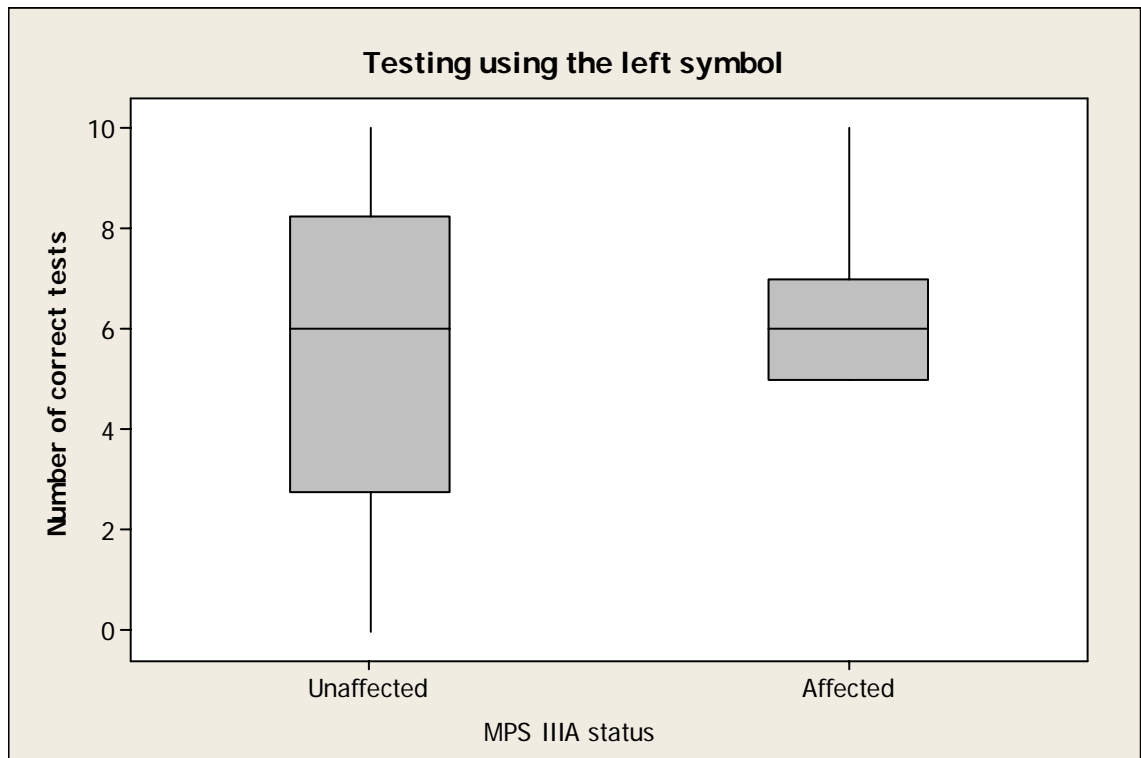

Figure 4.8   Boxplot comparing testing using the right symbol for MPS IIIA affected and unaffected dogs
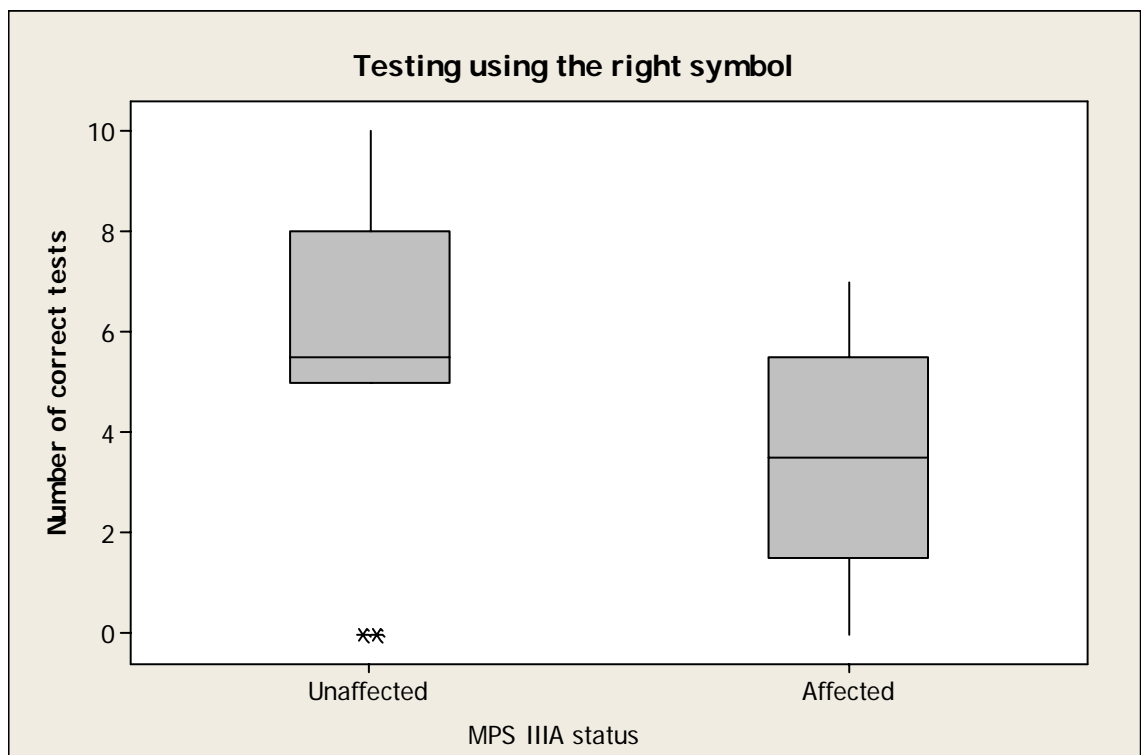
Figure 4.9 **Boxplot comparing the first day of maze testing for MPS IIIA affected and unaffected dogs**
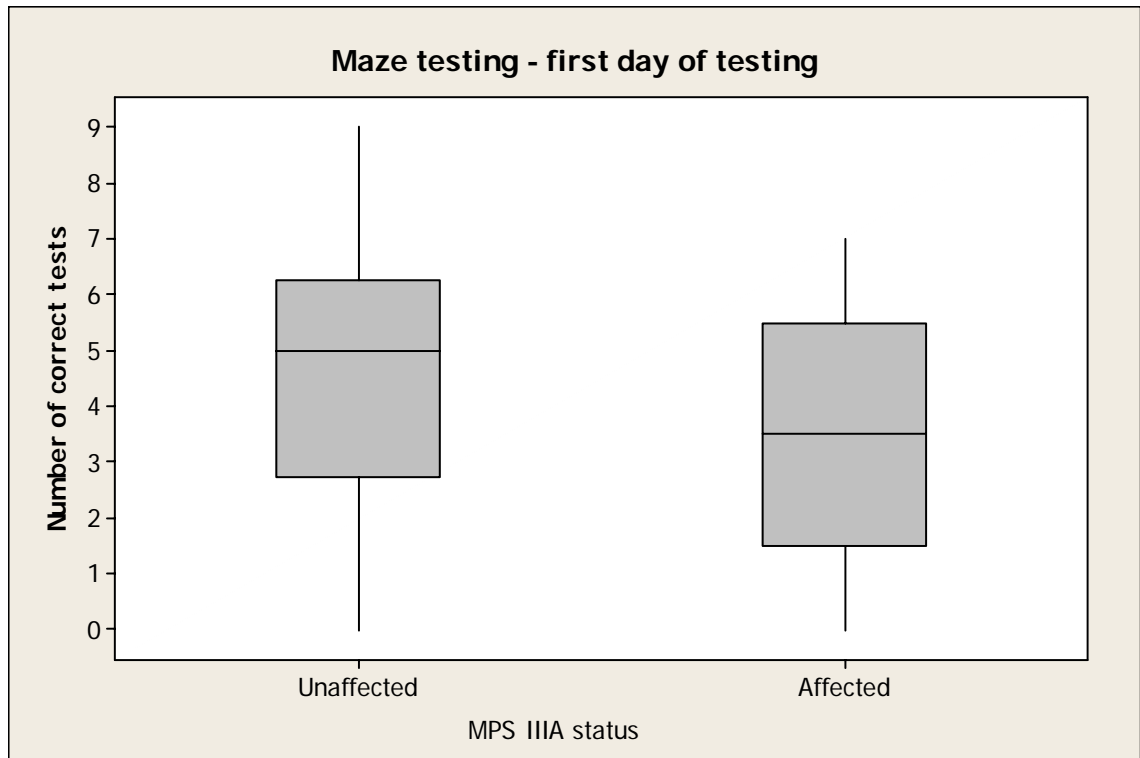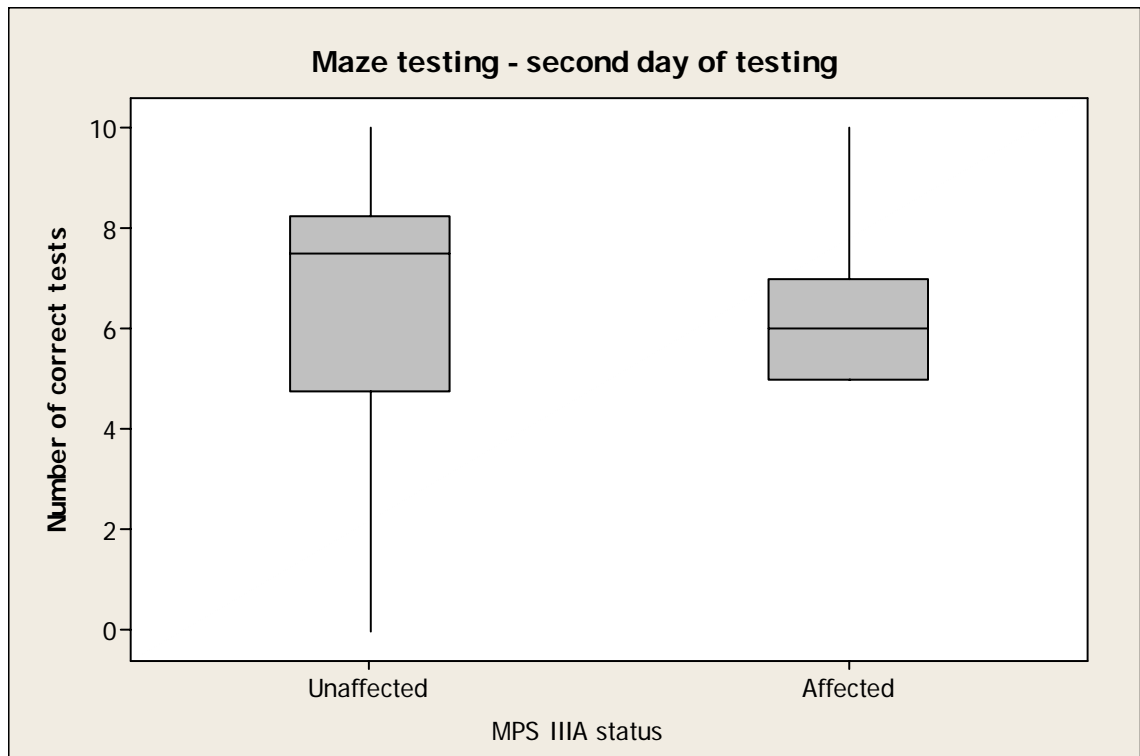

Figure 4.10 **Boxplot comparing the second day of maze testing for MPS IIIA affected and unaffected dogs**

4.3.4 Final day of testing

The final day of testing involved ten trials through the maze using randomly allocated left or right symbol presentations. Figure 4.11 and Table 4.8 displays the results. This test looked at the dogs' recognition of the two symbols and it appeared that the MPS affected dogs performed more poorly than the unaffected dogs did.

These results should be even slightly more left skewed because as mentioned earlier one of the affected dogs was unable to complete the training as it was not able to achieve the target of two days of 90% correct trials. In a total of sixty trials it only reached the correct location once. Thus it did not move on to complete the testing and so did not have any final results. It can be assumed that since it could not complete the training it should have had a zero score. Thus a boxplot with the zero result was also devised (Figure 4.12).

On examination of the graphical display of the maze testing data it appeared that the distribution of the data was not normal. The number of correct tests for the affected dogs had a left skew and the number of correct tests for the unaffected dogs had a right skew. Thus non-parametric analyses were performed. To check that the assumptions of equal variances were maintained for the Mann-Whitney U test a Levene's test was carried out (Figure 4.13). The p-value of 0.709 suggested that the variances were similar.

Thus the Mann-Whitney U test was performed (Table 4.6). A p-value of 0.0351 (adjusted for ties) showed that there was a statistically significant difference between the median number of successful tests on the final day of testing. When this was recalculated including the dog that could not complete the training the p-value was then 0.0147 adjusted for ties.

Figure 4.11   Boxplot comparing the results of the final day of maze testing for MPS IIIA affected and unaffected dogs
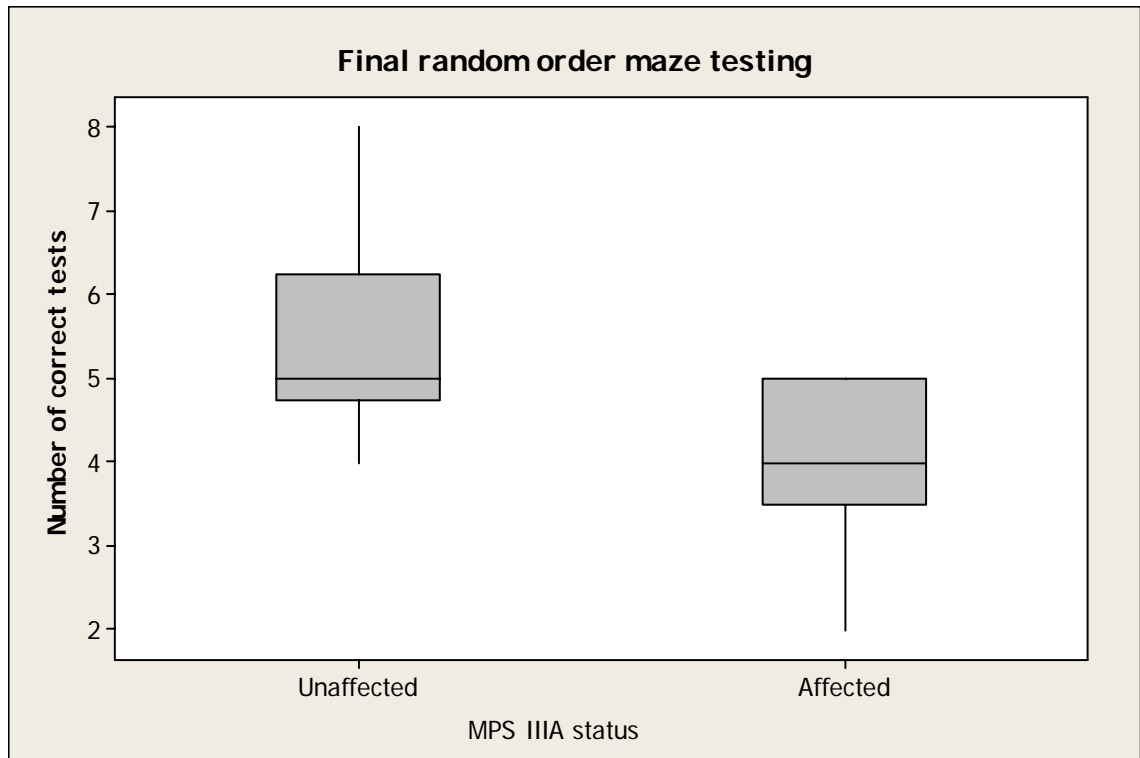

Figure 4.12 Boxplot comparing the final results for MPS IIIA affected and unaffected dogs including the affected dog that was unable to complete training
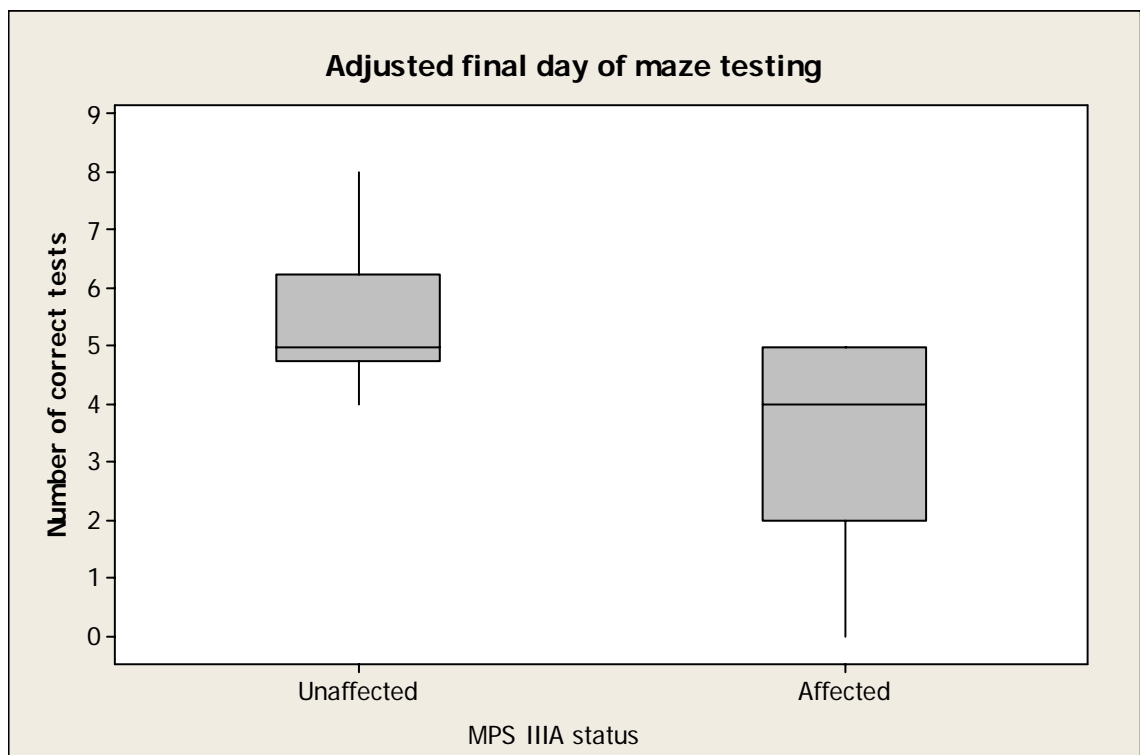
Figure 4.13   Levene's test for equal variances for final test
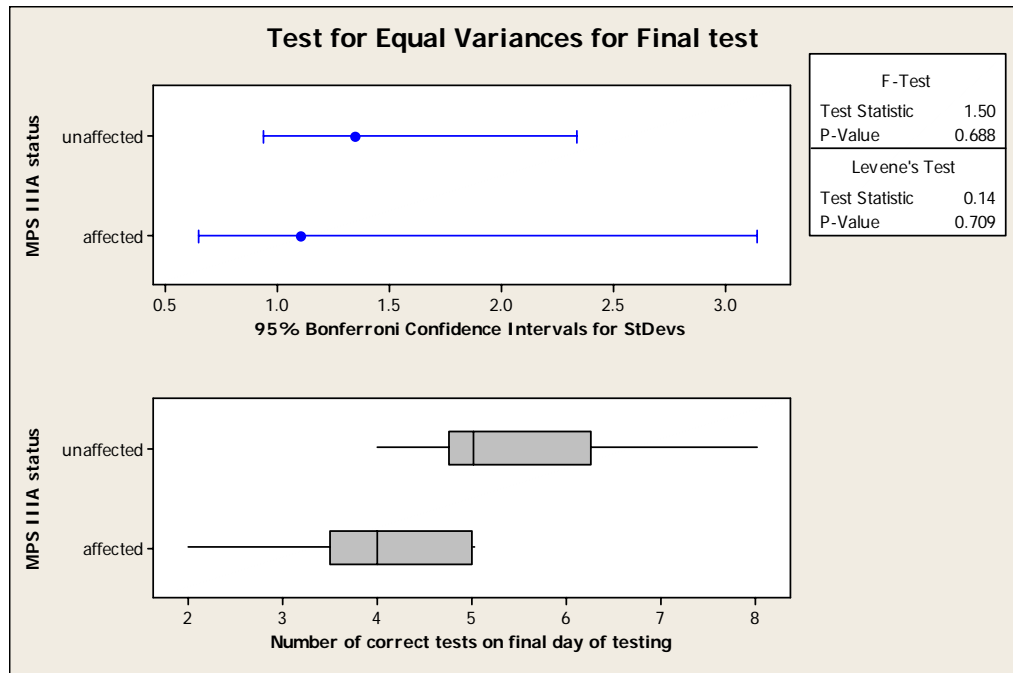
Table 4.6   Mann-Whitney *U* tests comparing the maze test medians for the MPS IIIA affected and unaffected dogs

	Median		W	P Value	P Value *	Confidence Interval
	Unaffected	**Affected**				
Final Test #	5	4	185.5	0.0207	0.0147	0.001 to 4
Final Test	5	4	171.5	0.0478	0.0351	0.001 to 3
Left symbol	6	6	143.5	0.8046	0.8025	-4.002 to 3
Right symbol	5.5	3.5	168	0.0909	0.0854	0 to 4.998
1st day testing	5	3.5	160.5	0.2836	0.2779	-2 to 4.001
2nd day testing	7.5	6	151.5	0.7415	0.7382	-2.001 to 3

#includes a dog that could not complete
training
* adjusted for ties
Significance level =0.05

4.3.5 Power analysis

According to the post hoc power analysis (Figure 4.14) this test shows that based on the statistics gathered for the final maze test then if the test had been two groups of six the power of the study would have been 0.53. However it was actually slightly more powerful since the control group had fourteen individuals.

It is important to note that the higher the power the greater the probability of getting a statistically significant result. In this case the power of the study was low and thus there was a high probability of having a false negative result. This suggests that the positive result which was gained is more likely to be accurate. The difference between the affected and unaffected dogs can be clearly seen in Figure 4.11.

To determine how likely it was that the significant Mann-Whitney U test for the final day of testing (Table 4.6) was accurately detecting a significant difference in the medians a two-sample t-test was performed. The two-sample t-test (p =0.034) (Table 4.7) supported the Mann-Whitney U test findings (p =0.0351). This suggests that there is a significant difference between the means of the unaffected and MPS IIIA affected dogs in terms of their performance on the final Maze test. Since the confidence interval for the final test borders zero this is considered a marginally significant result.

Figure 4.14 Power curve for the two-sample *t*-test for the final maze test
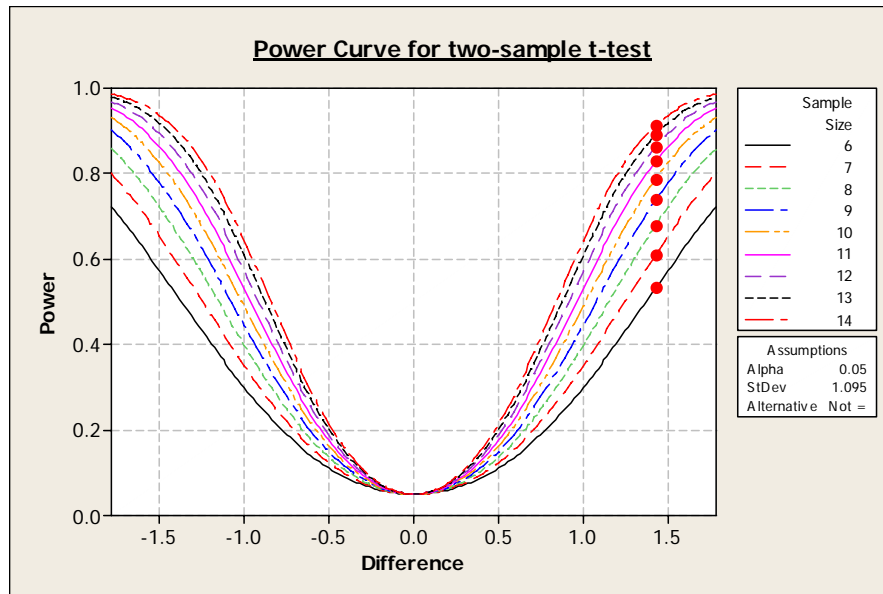
Table 4.7   Two-sample *t*-test comparing the final maze test medians for MPS IIIA affected and unaffected dogs

	N	Mean	Standard Deviation	SE Mean
Unaffected dogs	14	5.43	1.34	0.36
MPS IIIA affected dogs	6	4.00	1.10	0.45

Difference = mu (unaffected dogs) - mu (affected dogs)

Estimate for difference: 1.429

95% CI for difference: 0.118 to 2.739

T-Test of difference = 0 (vs not =):   T-Value = 2.29

P = 0.034   DF = 18

Table 4.8   Correct results for each day of training and testing for the MPS IIIA affected and unaffected dogs

Dog Number		Direction	1st symbol training (Training days – Number of correct trials)						Direction	2nd symbol training (Training days – Number of correct trials)							Testing – 1st Symbol (Testing Days 1, Correct Trials)	Testing – 2nd Symbol (2, Correct trials)	Testing – Random Symbol (3, Correct Trials)
			1	2	3	4	5	6		1	2	3	4	5	6	7			
1	*	Left	1	0	0	0	0	0	Right	DNC							3	5	5
2		Left	5	10					Left	2	9	10					6	5	5
3		Right	4	10	10				Left	6	9	9					5	4	5
4	*	Right	8	10	10				Left	5	9	10					0	5	5
5		Left	8	9	10				Right	1	10	10					7	10	5
6	*	Right	9	10					Left	3	9	10					2	6	4
7	*	Right	9	10					Right	5	10	10					6	6	5
8		Left	8	10	10				Left	6	9	10					5	8	7
9		Right	10	10					Left	6	10	10					5	2	4
10		Left	9	10					Right	10	10						6	8	4
11		Right	10	10					Left	6	10	9					9	8	5
12		Left	4	10					Right	7	10	10					7	5	8
13		Right	10	10					Left	2	10	9					4	6	4
14	*	Right	10	10					Left	0	1	7	9	10			5	5	8
15		Right	4	9	10				Left	4	10	10					0	9	5
16		Left	0	1	5	10	10		Right	1	8	5	10	10			2	10	4
17		Left	10	9					Right	0	0	0	0	1	9	10	0	8	2
18	*	Right	10	10					Left	0	0	2	0	8	10	10	5	10	6
19		Right	7	5	10	10			Right	4	4	9	10				3	7	4
20	*	Right	9	10					Right	0	8	10					8	6	5
21		Left	9	9					Left	1	0	4	9	9				0	5

*MPS IIIA affected dog   DNC –did not complete training

4.4 Discussion

The major findings from this study were:

1. The MPS IIIA affected dogs performed significantly worse (p=0.0351 adjusted for ties) than the unaffected dogs in the final maze test. The significance of this result was reduced due to the confidence interval having one border near zero (CI = 0.001 to 3).

2. There were no significant differences between the unaffected and MPS IIIA affected dogs in terms of the number of training days or mean number of correct training exercises per day per dog.

3. The number of correct tests following the left and right symbols per dog showed no significant difference between the unaffected and MPS IIIA affected dogs.

4. The total time for testing, time taken to perform the correct tests and time taken to perform correct training trials per dog showed no significant difference between the unaffected and MPS IIIA affected dogs.

4.4.1 Decreased performance in MPS IIIA affected dogs on the final day of testing

This is the first study in which a decline in cognitive function has been detected in MPS IIIA affected dogs. The affected dogs in this study performed significantly worse in a cognitive task than did the unaffected control dogs. This is the result which would be expected if cognitive function was declining due to the accumulation of glycosaminoglycans in the frontal lobe of their brains.

The difference between the medians of the affected and unaffected dogs' performance was significant (p= 0.0351 adjusted for ties). The confidence interval did however have one border near zero (CI = 0.001 to 3) which reduced the result's significance. Several findings did support the significance of this finding. The point estimate for the Mann-Whitney U test was 1 which supports there being a significant difference in the two populations. Also to further assess the spread of the data a two-sample t-test was performed. This showed a significant difference between the results of the affected and unaffected dogs (p = 0.034), and the result overall was more significant in that the

confidence interval did not include zero (CI = 0.118 to 2.739). Also this test estimated that the difference between the mean numbers of correct tests was 1.429. The post hoc power analysis for the final maze test showed that the power of this study was reasonably low. On a two-sample *t*-test power analysis the power was 0.53. This suggests that if more dogs had been in the study the power would have been higher and a more significant difference may have been detected. It should be noted that one of the MPS IIIA affected dogs was unable to achieve the targets for training and thus did not enter the testing phase. If it is assumed that this dog would have obtained a zero on the testing then the adjusted p-value for the difference between the affected and unaffected dogs on the final testing is 0.0147 (adjusted for ties).

Other studies have not detected any cognitive function decline in MPS IIIA affected dogs and thus this study's finding are in contrast to previous research (Fischer et al., 1998; Jolly et al., 2000). These previous studies lead us to suspect that if present, any decline in cognitive functioning was unlikely to be easily detectable, and this is supported by the subtle alteration in function detected. In a dog with MPS IIIA in a pet situation this degree of decline may not be detectable without specific testing.

Specific cognitive function testing has more routinely been performed in the murine model of MPS IIIA (Chang et al., 1993). In a Morris water maze affected mice were poor at remembering a platform's location in the maze and deficient at developing means of locating the platform. In dogs, T-shaped mazes or other tests of allocentric spatial learning are commonly used to test cognitive function (Ikeda-Douglas et al., 2005; Kelley & Lepine, 2005; Kelley et al., 2004). In this study the T-shaped maze was effective at detecting an alteration in cognitive function in the MPS IIIA affected dogs.

4.4.2   Training and testing using the T-shaped maze

There were two main components to the use of the T-shaped maze, training and testing. Since this was a cognitive task the training gives an indication of the cognitive functioning of the dog. If the affected dog is slower than the unaffected dogs at learning the task then this may indicate reduced learning. In terms of the number of training days needed for the dogs there was no difference between the MPS IIIA affected or unaffected dogs. This suggests that there was no large or obvious difference between

the learning speeds of the dogs. Latency to perform a cognitive function task can be used to measure cognitive function, however differences in the time taken to perform these tests or training were not detected in this study (Nippak et al., 2003; Nippak et al., 2006). The graphs of the time taken for the maze testing showed no difference between MPS IIIA affected and unaffected dogs, and most dogs were fast to achieve their task. This suggests that the dogs understood the task and moved quickly to attain the food reward. Apart from cognitive function two other factors are involved in how fast a dog completes a task. Dogs have differing individual latencies performing tasks and also there is a factor dependant on the difficulty of the task. Thus the more difficult the task the longer the latency (Nippak & Milgram, 2005).

To compensate for the large variation in the number of training days per dog the mean number of correct training exercises per day was calculated. There was a small difference between the means but it was not statistically significant. It was found that the affected dogs had slightly less correct training runs per day but this potential trend was not statistically significant. Larger numbers of dogs in the study may have given a clearer result.

The testing and the training were examined in concert with each other. The final random testing can be examined in isolation but the earlier testing may relate to the training which was carried out on the days prior to it. The procedure used for training, namely two days at 90% correct before moving onto the next task, is more stringent than similar dog training tasks which have been performed and is likely to have extended the training time (Meyer & Ladewig, 2008). It may have been satisfactory to have used a lower success at training rate such as eighty percent and to have had the training completed in a shorter time, however this may have reduced the ability of the test to detect cognitive function alterations.

The level of cognitive difficulty varied according to the stage of the study. Training was characterised by being repetitive, teaching the dog to recognise the symbol and direction in the maze association. In contrast testing involved the dog applying the information learned. The final day of testing was especially cognitively challenging since it was the first time that the ten trials for the day were not on the same symbol. There was therefore a large increase in difficulty in cognitive processing. This is

supported by the finding that it was only on this final day of testing that a difference between the affected and unaffected dogs was detected.

For the first two days of testing there are several reasons why a difference between the unaffected dogs and affected dogs was not detected. The power of the study was reasonably low and thus the chance of false negative results is increased. Alternatively it may have been that there was no cognitive decline to detect, however this is unlikely since final testing did find a difference between affected and unaffected dogs. Also training occurred over a long time whereas testing was mainly on consecutive days. This may have led to the first two testing days being an extension of training, priming the dogs for the final test. Another possibility is that the first two days of testing may not have provided a large enough learning challenge to detect cognitive decline. Thus it would only have been on the final day that the dogs' results would differentiate between the different levels of cognitive functioning in the dogs.

This can be examined in more detail by analysing the first two days of testing. The distribution of the data points when the food was to the left showed that the affected dogs mostly got six of the tests right whereas the unaffected dogs had a wider general individual distribution. In contrast when the food was to the right the MPS affected dogs didn't appear to have as many correct tests as they had when going left or as the unaffected dogs had. The difference in performance between the affected and unaffected dogs was not statistically significant but the trend suggests that there may have been a slight bias to the left for affected dogs or to the right for unaffected dogs or perhaps affected dogs behave in some way differently to the unaffected dogs. Research has inconsistently shown a possible left paw use preference for male dogs, (Batt et al., 2008; Wells, 2003) however analysis using Mann-Whitney *U* tests showed no significant differences between the male or female dogs' performance when going left or right during the testing. This suggests that there is not sex-related directionality for test performance in the maze testing.

On the first day of testing the dogs are tested on the symbol they first learnt which means that they are not presenting the behaviour that last got them a reward. The MPS IIIA affected dogs on the first day of testing had a very variable response whereas the unaffected dogs appeared to be more consistently correct, however there was not a

statistically significant difference. On the second day of training the MPS IIIA affected dogs did consistently better than they had on the first day and the unaffected dogs did better than they did on the first day of testing overall too. This suggests that the dogs all used their previous learning to change their behaviours and that the affected dogs may have been worse at doing this.

4.4.3   Limitations of the maze training and testing

As many of the sources of bias were mitigated as possible and unavoidable sources were examined to determine whether they affected the results. The main potential sources of bias were: researcher knowledge of status, weather, left or right bias, order effect and age of the dogs. This potential bias was mitigated by having researchers blinded as to the dogs' status, training and having a random order of testing.

Age was a potential point of bias since there was a variable length to training and thus age variation of the dogs. The dogs were all however still young in contrast to many of the other cognitive function studies where adult or aged dogs were tested (Milgram et al., 2002a; Milgram et al., 2002b; Nippak et al., 2007). To check whether age affected the results a Spearman's rank correlation coefficient was performed which showed no effect of age on the final testing results.

There are however several limitations to this study the main one of which is the low number of MPS IIIA affected dogs available for study. These low numbers meant that the power of the study was low also. To address this issue future study should test larger numbers of dogs. More testing days would have increased the significance of the results also especially more random symbol testing. Another limitation was that the cognitive testing was only performed while the dogs were young. Continuing to test as the dogs got older would have enabled repeated measurements of possible cognitive function decline as brain function deteriorated.

4.4.4   Standardisation of maze training and testing

To standardise the maze testing it would have been good to have preselected dogs so that the dogs could achieve to a minimum level before carrying out the testing as has

been carried out in previous studies (McCune et al., 2008). This did not occur in this test due to the low numbers of dogs already in the study. One potential selection point that was carried out in this testing was that the dogs had physical examinations within 48 hours of their birth and regular weight checks were performed. No dogs were removed from the study due to the results of these checks. It should be noted however that one of the MPS IIIA affected dogs was unable to achieve the targets for training and thus did not enter the testing phase.

Standardisation of the training phase could also be increased by having the T-shaped maze under shelter so that testing could proceed in any weather. This would allow set intervals between training sessions and more consistency in training each dog. A recent study has shown that training a few times a week gives better learning than training every day thus future studies may utilise this in the training plan (Meyer & Ladewig, 2008). As expected training measures were not as accurate as testing since the measures were blunt and dependant on the stage of training.

4.4.5   Conclusion

From this discussion it is evident that the MPS IIIA affected dogs' decline in cognitive performance on the final day of testing is likely to be significant. There is potential for future use of this testing with the standardization and adjustments discussed. Further testing with larger numbers of dogs may enable identification of which specific cognitive aspects of the MPS IIIA affected dogs deteriorate.

For future development of the MPS IIIA dog model this study provides an important new finding. The ability to measure decline in cognitive function before cerebellar signs are seen in these dogs provides an excellent opportunity for the development of future therapies.

CHAPTER 5

General Discussion

5.1 Introduction

Currently there are no treatments to stop the progressive neurodegeneration caused by MPS IIIA. The development of treatments will depend on the use of murine and canine MPS IIIA models. The dogs used in this study are from the only MPS IIIA dog colony in the world and this is the first work investigating behavioural and cognitive dysfunction caused by this disease. There were three objectives to this study: (1) measure the behaviours of MPS IIIA dogs and control dogs during the first year of their lives, (2) determine whether behavioural alteration or (3) cognitive dysfunction caused by MPS IIIA can be detected early in affected dogs' lives. These objectives were fulfilled and provided novel information on MPS IIIA in dogs.

5.2 Summary of the results

The first trials in this study were performed in dogs at six and eight weeks of age and no statistically significant behavioural differences were detected between MPS IIIA affected and unaffected dogs. This study suggests that clinical behavioural changes due to MPS IIIA do not occur at a young age.

The second trial was the C-BARQ at three, six and twelve months of age. There were no significant differences between the unaffected and MPS IIIA affected dogs which supports the findings of the previous study. On closer examination of several individual questions in the C-BARQ the only significant differences were that MPS

IIIA affected dogs retrieved more than unaffected dogs at three months of age, and at twelve months of age affected dogs were less distractible than unaffected dogs. In terms of clinical significance it is difficult to assume that there are important findings. These may be just spurious findings or there may be some unexpected form of brain function alteration in affected dogs. More research with larger numbers of dogs would be needed to determine whether this is a significant clinical finding in affected dogs.

The third trial in this study was the use of a T-shaped maze to test the cognitive ability of the affected and unaffected dogs. MPS IIIA affected dogs performed significantly worse than the unaffected colony dogs in the final cognitive maze test (p= 0.0351 adjusted for ties). The Mann-Whitney U test significance was reduced due to the confidence interval having one border near zero. Overall however these results suggest that there was a significant decline in the cognitive performance of the MPS IIIA affected dogs. This is the first study to identify cognitive function alteration in MPS IIIA affected dogs and as such it provides an opportunity for future study leading to the development of new therapies.

5.3   Limitations in this study

MPS IIIA detection in young colony dogs before the onset of classic ataxia and other neurological signs at one and a half years of age proved to be as difficult as would be expected. The two main limitations to this study were the low number of MPS IIIA affected dogs available for testing and the performance of only one round of cognitive testing. The low number of affected dogs reduced the power of the statistical analyses and increased the probability of sampling error. The chance of type II error (false negatives) is especially likely to be increased. This may have hidden some significant differences between affected and unaffected dogs in these tests.

Repeated measurements and tests of the dogs' behaviours were made but cognitive maze testing was only carried out once. The poorer performance of the affected dogs in the final maze testing was very interesting and marginally significant but retesting when the dogs were older would have helped to confirm that this test was detecting the decline in cognitive function due to the accumulation of glycosaminoglycans. If

repeated measurements showed a progressive decline in cognitive function this would have confirmed that the test was detecting the effects of MPS IIIA.

There were several other limitations to the trials. The length of time taken for the maze training was variable due to time constraints and the need for relatively fine weather. There were inconsistencies in the six and eight week test procedures which are likely to have reduced the accuracy of the behavioural testing. The tester knew the dogs in the test well prior to the testing and during the tests there was some inconsistency in the attention given to each puppy. This would have reduced the accuracy of the tests. For future testing using an independent tester would reduce this problem. Having another tester measuring behaviours at the same time would improve the reliability of the data measurement. The validity of the C-BARQ in this study was reduced due to some questions being irrelevant for this population of dogs. Thus alterations were made to some factor calculations and some factors could not be calculated due to excessive irrelevant questions.

5.4   Importance and novelty of findings

All three measures used in this study provided important information on MPS IIIA in dogs. The six and eight weeks test results reiterated that behavioural changes are not evident at these ages which suggests that behavioural measurement of response to therapies is not possible at such a young age. The C-BARQ results also mainly showed that MPS IIIA in dogs does not lead to behavioural changes in the first year of life and that it would be difficult to measure a behavioural response to therapy in the first year of the dogs' lives. The new finding was that affected dogs retrieved more than unaffected dogs at three months of age, and were less distractible at twelve months of age.

The findings of the cognitive function test were novel. Prior to this testing, research on MPS IIIA in dogs has not measured cognitive changes in affected dogs. This use of a T-shaped maze showed a decline in the cognitive ability of these MPS IIIA affected dogs. It provides a foundation for further investigation of MPS IIIA disease progression and potentially therapy effects. It also suggests that this form of testing is

relatively sensitive for detecting cognitive change and thus may have additional applications.

5.5   Future research

Future research would build on these findings to investigate cognitive decline in MPS IIIA dogs. This testing may be able to differentiate between affected and unaffected dogs earlier than is currently possible detecting cerebellar symptoms. It may also be used to detect therapy response in affected dogs. To continue this work the first step would be to develop a plan for maze testing MPS IIIA affected dogs and control dogs. Higher numbers of MPS IIIA affected dogs would be recruited and the maze training and initial testing should be carried out from 20 weeks of age and then the testing repeated at monthly or two monthly intervals until two years of age. Depending on findings the testing may be continued to investigate the degree of alteration over the entire course of the disease.

To enable this intensive testing to be performed procedures would need to be standardised as much as possible. The maze should be located under cover so that inappropriate weather does not prevent training. If enough affected dogs were available then optimally pre-selecting of dogs so that they achieved above a minimum level would be useful.

Future testing could also retest the dogs using the C-BARQ to investigate the repeatability and thus significance of the findings on retrieving and distractibility in the affected dogs.

5.6   Model for other diseases

The detection of a decline in cognitive function in diseased dogs through the use of symbol recognition in a maze has not been previously performed in dogs. Most measurements of cognitive function have been based around the examination of the aged dog model and this is new testing examining the difference in brain function between healthy and diseased dogs. The significant finding suggests that it may be

possible to expand this research into the investigation of other disease states in dogs. For example epilepsy in dogs has not been considered to affect the cognitive ability of affected dogs over time but this has not been quantitatively assessed. Maze testing would be a valid means of investigating epileptic and non-epileptic dogs. It may also be useful to measure affected dogs' cognitive function over a long time period and to test at a decided time point after a seizure to compare with test ability before the seizure. Many other diseases would lend themselves to this type of testing such as diabetes, or pre and post surgeries for hepatic shunts.

5.7   Conclusions

The objectives of this study were fulfilled by measuring and describing the behaviours seen in the MPS IIIA affected dogs in their first year of life and by performing measurements of cognitive function. MPS IIIA affected dogs' behaviours were mainly not significantly different from unaffected dogs but it was found that the MPS IIIA affected dogs retrieved more at three months of age and were less distractible at twelve months of age. This finding would be worth investigating to decide whether it suggests a subtle alteration in brain functioning. The decline in cognitive function detected through maze testing in affected dogs was a novel finding.

This research provides new knowledge on cognitive decline detection in MPS IIIA dogs which may lead to future developments measuring therapy response and disease progression. The T-shaped maze testing may also be of value in future research on cognitive function in dogs with other diseases such as epilepsy. Thus this research provides valuable information on canine MPS IIIA and provides a platform for further disease investigations.

References

Adams, B., Chan, A., Callahan, H., & Milgram, N. W. (2000). The canine as a model of human cognitive aging: recent developments. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 24*(5), 675-692.

Anson, D. S., & Fletcher, J. M. (2007). Gene therapy for disorders affecting children, progress and potential. *Journal of Paediatrics and Child Health, 43*, 323-330.

Araujo, J. A., & Milgram, N. W. (2004). A novel cognitive palatability assessment protocol for dogs. *Journal of Animal Science, 82*, 2200-2206.

Aronovich, E. L., Johnston, J. M., Wang, P., Giger, U., & Whitley, C. B. (2001). Molecular basis of mucopolysaccharidosis type IIIB in emu (Dromaius novaehollandiae): an avian model of Sanfilippo syndrome type B. *Genomics, 74*(3), 299-305.

Auclair, D., Hopwood, J. J., Brooks, D. A., Lemontt, J., & Crawley, A. C. (2003). Replacement therapy in mucopolysaccharidosis type VI: advantages of early onset of therapy. *Molecular Genetics and Metabolism, 78*, 163-174.

Batt, L., Batt, M., Baguley, J., & McGreevy, P. (2008). Stability of motor lateralisation in maturing dogs. *Laterality, 13*(5), 468-479.

Beaudet, R., Chalifoux, A., & Dallaire, A. (1994). Predictive value of activity level and behavioral-evaluation on future dominance in puppies. *Applied Animal Behaviour Science, 40*(3-4), 273-284.

Beesley, C., Moraitou, M., Winchester, B., Schulpis, K., Dimitriou, E., & Michelakakis, H. (2004). Sanfilippo B syndrome: molecular defects in Greek patients. *Clinical Genetics, 65*, 143-149.

Bhattacharyya, R., Gliddon, B., Beccari, T., Hopwood, J. J., & Stanley, P. (2001). A novel missense mutation in lysomal sulfamidase is the basis of MPS IIIA in a spontaneous mouse mutant. *Glycobiology, 11*, 99-103.

Bhaumik, M., Muller, V. J., Rozaklis, T., Johnson, L., Dobrenis, K., Bhattacharyy, R., et al. (1999). A mouse model for mucopolysaccharidosis type IIIA (Sanfilippo syndrome). *Glycobiology, 9*, 1389-1396.

Birkenmeier, E. H., Davisson, M. T., Beamer, W. G., Ganschow, R. E., Vogler, C. A., Gwynn, B., et al. (1989). Murine mucopolysaccharidosis Type-VII - characterization of a mouse with beta-glucuronidase deficiency. *Journal of Clinical Investigation, 83*(4), 1258-1266.

Burman, O. H. P., Parker, R., Paul, E. S., & Mendl, M. (2008). A spatial judgement task to determine background emotional state in labratory rats, *Rattus norvegicus*. *Animal Behaviour, 76*, 801-809.

Callahan, H., Ikeda-douglas, C., Head, E., Cotman, C. W., & Milgram, N. W. (2000). Development of a protocol for studying object recognition memory in the dog. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 24*(5), 693-707.

Ceuterick, C., Martin, J. J., Libert, J., & Farriaux, J. P. (1980). Sanfilippo - a disease in the fetus - comparison with prenatal and postnatal cases. *Neuropadiatrie, 11*(2), 176-185.

Chang, P., Lambert, D., & Pisa, M. (1993). Behavioural abnormalities in a murine model of a human lysosomal storage disease. *Neuroreport, 4*(5), 507-510.

Clarke, L. A., Russell, C. S., Pownall, S., Warrington, C. L., Borowski, A., Dimmick, J. E., et al. (1997). Murine mucopolysaccharidosis type I: targeted disruption of the murine alpha-L-iduronidase gene. *Human Molecular Genetics, 6*(4), 503-511.

Cowell, K. R., Jezyk, P. F., Haskins, M. E., & Patterson, D. F. (1976). Mucopolysaccharidosis in a cat. *Journal of the American Veterinary Medical Association, 169*(3), 334-339.

Crawley, A. C., Muntz, F. H., Haskins, M., Jones, B. R., & Hopwood, J. J. (2003). Prevalence of mucopolysaccharidosis type VI mutations in Siamese cats. *Journal of Veterinary Internal Medicine, 17*, 495-498.

De Meester, R. H., De Bacquer, D., Peremans, K., Vermeire, S., Planta, D. J., Coopman, F., et al. (2008). A preliminary study on the use of the socially acceptable behavior test as a test for shyness/confidence in the temperament of dogs. *Journal of Veterinary Behavior: Clinical Applications and Research, 3*(4), 161-170.

Denenberg, V., & Morton, J. (1962). Effects of environmental complexity and social groupings upon modification of emotional behaviour. *Journal of Comp and Physiol Psychol, 55*(2), 242-246.

Diederich, C., & Giffroy, J. M. (2006). Behavioural testing in dogs: a review of methodology in search for standardisation. *Applied Animal Behaviour Science, 97*, 51-72.

Dombrowski, D. C. S., Carmichael, K. P., Wang, P., O'Malley, T. M., Haskins, M. E., & Giger, U. (2004). Mucopolysaccharidosis type VII in a German Shepherd Dog. *Journal of the American Veterinary Medical Association, 224*(4), 553-557.

Downie, C., HughJones, K., & Hobbs, J. R. (1995). Up to fourteen years after bone marrow transplantation for Hurler's syndrome. *Correction of Genetic Diseases by Transplantation Iii*, 16-24.

Ellinwood, N. M., Wang, P., Skeen, T., Sharp, N. J. H., Cesta, M., Decker, S., et al. (2003). A model of mucopolysaccharidosis III B (Sanfilippo syndrome type III B): N-acetyl-alpha-D-glucosaminidase deficiency in Schipperke dogs. *Journal of Inherited Metabolic Diseases, 26*, 489-504.

Evers, M., Saftig, P., Schmidt, P., Hafner, A., McLoghlin, D. B., Schmahl, W., et al. (1996). Targeted disruption of the arylsulfatase B gene results in mice resembling the phenotype of mucopolysaccharidosis VI. *Proceedings of the National Academy of Sciences of the United States of America, 93*(16), 8214-8219.

Fischer, A., Carmichael, K. P., Munnell, J. F., Jhabvala, P., Thompson, J. N., Matalon, R., et al. (1998). Sulfamidase deficiency in a family of Dachshunds: a canine model of mucopolysaccharidosis IIIA (Sanfilippo A). *Pediatric Research, 44*(1), 74-82.

Fox, M. W. (1978). *The dog: its domestication and behaviour*. USA: Garland STPM Press.

Fox, M. W., & Stelzner, D. (1966). Behavioural effects of differential early experience in dogs. *Animal Behaviour, 14*(2-3), 273-281.

Frank, D., Minero, M., Cannas, S., & Palestrini, C. (2007). Puppy behaviours when left home alone: a pilot study. *Applied Animal Behaviour Science, 104*(1-2), 61-70.

Fraser, J., Gason, A., Wraith, J. E., & Delatycki, M. (2005). Sleep disturbance in Sanfilippo syndrome: a parental questionnaire study. *Archives of Disease in Childhood, 90*, 1239-1242.

Freedman, D. G., Elliot, O., & King, J. A. (1961). Critical period in social development of dogs. *Science, 133*, 1016-1017.

Fretwell, L. K., McClune, S., Fone, J. V., & Yates, D. J. (2006). The effect of supplementation with branched-chain amino acids on cognitive function in active dogs. *The Journal of Nutrition, 136*, 2069s-2071s.

Gabrielli, O., Coppa, G. V., Bruni, S., Villani, G. R. D., Pontarelli, G., & Di Natale, P. (2005). An adult Sanfilippo type a patient with homozygous mutation R206P in the sulfamidase gene. *American Journal of Medical Genetics Part A, 133A*(1), 85-89.

Gabrielli, O., Polonara, G., Regnicolo, L., Petroni, V., Scarabino, T., Coppa, G. V., et al. (2004). Correlation between cerebral MRI abnormalities and mental retardation in patients with mucopolysaccharidoses. *American Journal of Medical genetics, 125*, 224-231.

Galan-Gomez, E., Guerrero-Rico, A., Caceres-Marzal, C., Zambrano-Castano, M., Moreno-Tejero, M. L., Grande-Tejada, A. M., et al. (2008). Early response to idursulfase treatment in a 3 year-old boy affected of Hunter syndrome. *European Journal of Medical Genetics, 51*(3), 268-271.

Garcia, A., DaCosta, J., Pan, J., Muenzer, J., & Lamsa, J. (2007). Preclinical dose ranging studies for enzyme replacement therapy with idursulfase in a knock out mouse model of MPS II. *Molecular Genetics and Metabolism, 91*, 183-190.

Gliddon, B. L., & Hopwood, J. J. (2004). Enzyme-replacement therapy from birth delays the development of behavior and learning problems in mucopolysaccharidosis type IIIA mice. *Pediatric Research, 56*(1), 65-72.

Goddard, M. E., & Beilharz, R. G. (1982). Genetic and environmental-factors affecting the suitability of dogs as guide dogs for the blind. *Theoretical and Applied Genetics, 62*(2), 97-102.

Goddard, M. E., & Beilharz, R. G. (1983). Genetics of traits which determine the suitability of dogs as guide-dogs for the blind. *Applied Animal Ethology, 9*(3-4), 299-315.

Goddard, M. E., & Beilharz, R. G. (1984a). A Factor-Analysis of Fearfulness in Potential Guide Dogs. *Applied Animal Behaviour Science, 12*(3), 253-265.

Goddard, M. E., & Beilharz, R. G. (1984b). The relationship of fearfulness to, and the effects of, sex, age and experience on exploration and activity in dogs. *Applied Animal Behaviour Science, 12*(3), 267-278.

Goddard, M. E., & Beilharz, R. G. (1985). Individual Variation in Agonistic Behavior in Dogs. *Animal Behaviour, 33*(NOV), 1338-1342.

Goddard, M. E., & Beilharz, R. G. (1986). Early prediction of adult behavior in potential guide dogs. *Applied Animal Behaviour Science, 15*(3), 247-260.

Gosling, S. D., & Vasire, S. (2002). Are we barking up the right tree? Evaluating a comparative approach to personality. *Journal of Research in Personality, 36*, 607-614.

Grabowski, G. A. (2008). Lysosomal storage disease 1 - Phenotype, diagnosis, and treatment of Gaucher's disease. *Lancet, 372*(9645), 1263-1271.

Haley, S. M., Fragala Pinkham, M. A., Dumas, H. M., Ni, P., Skrinar, A. M., & Cox, G. F. (2006). A physical performance measure for individuals with mucopolysaccharidosis type I. *Developmental Medicine and Child Neurology, 48*, 576-581.

Haskins, M., Casal, M., Ellinwood, N. M., Melniczek, J., Mazrier, H., & Giger, U. (2002). Animal models for mucopolysaccharidoses and their clinical significance. *Acta Paediatrica Supplement, 439*, 88-97.

Haskins, M. E., Desnick, R. J., Diferrante, N., Jezyk, P. F., & Patterson, D. F. (1984). Beta-glucuronidase deficiency in a dog - a model of human mucopolysaccharidosis-VII. *Pediatric Research, 18*(10), 980-984.

Head, E. (2001). Brain aging in dogs: parallels with human brain aging and Alzheimer's disease. *Vet Therapy, 2*, 247-260.

Head, E., Callahan, H., Muggenburg, B., Cotman, C. W., & Milgram, N. W. (1998). Visual-discrimination learning ability and beta-amyloid accumulation in the dog. *Neurobiology of Aging, 19*(5), 415-425.

Head, E., Cotman, C. W., & Milgram, N. W. (2000). Canine cognition, aging and neuropathology. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 24*(5), 671-673.

Hemsley, K. M., Beard, H., King, B. M., & Hopwood, J. J. (2008). Effect of high dose, repeated intra-cerebrospinal fluid injection of sulphamidase on neuropathology in mucopolysaccharidosis type IIIA mice. *Genes Brain and Behavior, 7*(7), 740-753.

Hemsley, K. M., & Hopwood, J. J. (2005). Development of motor deficits in a murine model of mucopolysaccharidosis type III A (MPS -III A). *Behavioural Brain Research, 158*, 191-199.

Hemsley, K. M., King, B., & Hopwood, J. J. (2007). Injection of recombinant human sulphamidase into the CSF via the cerebellomedullary cistern in MPS III A mice. *Molecular Genetics and Metabolism, 90*, 313-328.

Hennessy, M. B., Voith, V. L., Mazzei, S. J., Buttram, J., Miller, D. D., & Linden, F. (2001). Behavior and cortisol levels of dogs in a public animal shelter, and an exploration of the ability of these measures to predict problem behavior after adoption. *Applied Animal Behaviour Science, 73*(3), 217-233.

Hsu, Y. Y., & Serpell, J. A. (2003). Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. *Journal of the American Veterinary Medical Association, 223*(9), 1293-1300.

Hughjones, K., Barrett, A. J., Byrom, N., Chambers, D., Henry, K., James, D. C. O., et al. (1982). Reversal of clinical features of Hurler disease after treatment by bone-marrow transplant. *Journal of Medical Genetics, 19*(1), 65-65.

Ikeda-Douglas, C. J., De Rivera, C., & Milgram, N. W. (2005). Pharmaceutical and other commercial uses of the dog model. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29*, 355-360.

Ikeda-Douglas, C. J., Zicker, S. C., Estrada, J., Jewell, D. E., & Milgram, N. W. (2004). Prior experience, antioxidants, and mitochondrial cofactors improve cognitive function in aged beagles. *Veterinary Therapeutics, 5*(1), 5-16.

Jolly, R. D., Allan, F. J., Collett, M. G., Rozaklis, T., Muller, V. J., & Hopwood, J. J. (2000). Mucopolysaccharidosis IIIA (Sanfilippo syndrome) in a New Zealand Huntaway dog with ataxia. *New Zealand Veterinary Journal, 48*(5), 144-148.

Jolly, R. D., Ehrlich, P. C., Franklin, R. J. M., Macdougall, D. F., & Palmer, A. C. (2001). Histological diagnosis of mucopolysaccharidosis IIIA in a wire-haired dachshund. *Veterinary Record, 148*(18), 564-567.

Jolly, R. D., Johnstone, A. C., Norman, E. J., Hopwood, J. J., & Walkley, S. U. (2007). Pathology of mucopolysaccharidosis IIIA in Huntaway dogs. *Veterinary Pathology, 44*, 569-578.

Kakkis, E., Lester, T., Yang, R., Tanaka, C., Anand, V., Lemontt, J., et al. (2004). Successful induction of immune tolerance to enzyme replacement therapy in canine mucopolysaccharidosis I. *Proceedings of the National Academy of Sciences of the United States of America, 101*(3), 829-834.

Karolewski, B. A., & Wolfe, J. H. (2006). Genetic correction of the fetal brain increases the lifespan of mice with the severe multisystemic disease mucopolysaccharidosis type VII. . *Molecular Therapy, 14*(1), 14-24.

Kelley, R. L., & Lepine, A. J. (2005). *Improving puppy trainability through nutrition.* Paper presented at the Iams Clinical Nutrition Symposia, Seville, Spain.

Kelley, R. L., Lepine, A. J., Burr, J. R., Shyan-Norwalt, M., & Reinhart, G. A. (2004, June 2004). *Effect of dietary fish oil on puppy traininability.* Paper presented at

the International Society for the Study of Fatty Acids and Lipids (ISSFAL) congress, Brighton, UK.

Knol, B. W., Roozendaal, C., Vandenbogaard, L., & Bouw, J. (1988). The suitability of dogs as guide dogs for the blind - criteria and testing procedures. *Veterinary Quarterly, 10*(3), 198-204.

LeBowitz, J. H. (2005). A breach in the blood-brain barrier. *Proceedings of the National Academy of Sciences of the United States of America, 102*(41), 14485-14486.

Ley, J. (2008). Measuring personality in dogs. *Journal of Veterinary Behavior: Clinical Applications and Research, 3*(4), 182.

Li, H. H., Yu, W. H., Rozengurt, N., Zhao, H. Z., Lyons, K. M., Anagnostaras, S., et al. (1999). Mouse model of Sanfilippo syndrome type B produced by targeted disruption of the gene encoding alpha-N-acetylglucosaminidase. *Proceedings of the National Academy of Sciences of the United States of America, 96*(25), 14505-14510.

Martin, D. C., Atmuri, V., Hemming, R. J., Farley, J., Mort, J. S., Byers, S., et al. (2008). A mouse model of human mucopolysaccharidosis IX exhibits osteoarthritis. *Human Molecular Genetics, 17*(13), 1904-1915.

Martin, P., & Bateson, P. (2005). *Measuring behaviour. an introductory guide*. Cambridge: Press Syndicate of the University of Cambridge.

McCune, S., Stevenson, J., Fretwell, L. K., Thompson, A., & Mills, D. S. (2008). Aging does not significantly affect performance in a spatial learning task in the domestic cat (*Felis silvestris catus*). *Applied Animal Behaviour Science, 112*, 345-356.

Meikle, P. J., Grasby, D. J., Dean, C. J., Lang, D. L., Bockmann, M., Whittle, A. M., et al. (2006). Newborn screening for lysosomal storage disorders. *Molecular Genetics and Metabolism, 88*, 307-314.

Meikle, P. J., Hopwood, J. J., Clague, A. E., & Carey, W. F. (1999). Prevalence of lysosomal storage disorders. *Journal of the American Medical Association, 281*(3), 249-254.

Menon, K. P., Tieu, P. T., & Neufeld, E. F. (1992). Architecture of the canine idua gene and mutation underlying canine mucopolysaccharidosis I. *Genomics, 14*(3), 763-768.

Merk, T., Wibmer, T., Schumann, C., & Kruger, S. (2009). Glycogen storage disease type II (Pompe disease) - influence of enzyme replacement therapy in adults. *European Journal of Neurology, 16*(2), 274-277.

Meyer, A., Kossow, K., Gal, A., Muhlhausen, C., Ullrich, K., Braulke, T., et al. (2007a). Scoring evaluation of the natural course of Mucopolysaccharidosis type IIIA (Sanfilippo syndrome type A). *Pediatrics, 120*(5), E1255-E1261.

Meyer, A., Kossow, K., Gal, A., Muhlhausen, C., Ullrich, K., Braulke, T., et al. (2007b). Study on the natural course of mucopolysaccharidosis type IIIA. *Journal of Inherited Metabolic Disease, 30*, 99-99.

Meyer, I., & Ladewig, J. (2008). The relationship between number of training sessions per week and learning in dogs. *Applied Animal Behaviour Science, 111*(3-4), 311-320.

Milgram, N. W., Head, E., Muggenburg, B., Holowachuk, D., Murphey, H., Estrada, J., et al. (2002a). Landmark discrimination learning in the dog: effects of age, an antioxidant fortified food, and cognitive strategy. *Neuroscience and Biobehavioral Reviews, 26*(6), 679-695.

Milgram, N. W., Zicker, S. C., Head, E., Muggenburg, B. A., Murphey, H., Ikeda-Douglas, C. J., et al. (2002b). Dietary enrichment counteracts age-associated cognitive dysfunction in canines. *Neurobiology of Aging, 23*(5), 737-745.

Mills, D. S. (2003). Medical paradigms for the study of problem behaviour: a critical review. *Applied Animal Behaviour Science, 81*(3), 265-277.

Muenzer, J., & Fu, H. (1999). Targeted disruption of the mouse iduronate sulfatase gene. *American Journal of Human Genetics, 65*(4), 2419.

Muenzer, J., Wraith, J. E., & Clarke, L. A. (2009). Mucopolysaccharidosis I: management and treatment guidelines. *Pediatrics, 123*(1), 19-29.

Murphy, J. A. (1998). Describing categories of temperament in potential guide dogs for the blind. *Applied Animal Behaviour Science, 58*(1-2), 163-178.

Neer, T. M., Dial, S. M., Pechman, R., Wang, P., Oliver, J. L., & Giger, U. (1995). Mucopolysaccharidosis-VI in a Miniature Pinscher. *Journal of Veterinary Internal Medicine, 9*(6), 429-433.

Nelson, J. (1997). Incidence of the mucopolysaccharidoses in Northern Ireland. *Human Genetics, 101*(3), 355-358.

Netto, W. J., & Planta, D. J. U. (1997). Behavioural testing for aggression in the domestic dog. *Applied Animal Behaviour Science, 52*(3-4), 243-263.

Neufeld, E. F., & Muenzer, J. (2001). *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw-Hill.

Nippak, P. M. D., Chan, A. D. F., Campbell, Z., Muggenburg, B., Head, E., Ikeda-Douglas, C. J., et al. (2003). Response latency in Canis familiaris: mental ability or mental strategy? *Behavioral Neuroscience, 117*(5), 1066-1075.

Nippak, P. M. D., Ikeda-Douglas, C., & Milgram, N. W. (2006). Extensive spatial training does not negate age differences in response latency. *Brain Research, 1070*(1), 171-188.

Nippak, P. M. D., Mendelson, J., Muggenburg, B., & Milgram, N. W. (2007). Enhanced spatial ability in aged dogs following dietary and behavioural enrichment. *Neurobiology of Learning and Memory, 87*(4), 610-623.

Nippak, P. M. D., & Milgram, N. W. (2005). An investigation of the relationship between response latency across several cognitive tasks in the beagle dog. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29*(3), 371-377.

Paroz, C., Gebhardt-Henrich, S., & Steiger, A. (2008). Reliability and validity of behaviour tests in Hovawart dogs. *Applied Animal Behaviour Science, 115*(1-2), 67-81.

Pfaffenberger, C. J., Scott, J. P., Fuller, J. L., Ginsburg, B. E., & Bielfelt, S. W. (1976). *Guide dogs for the blind: Their selection, development and training.* Amsterdam, Oxford, N.Y.: Elsevier Scientific Pub. Co.

Piotrowska, E., Jakóbkiewicz-Banecka, J., Barańska, S., Tylki-Szymańska, A., Czartoryska, B., Węgrzyn, A., et al. (2006). Genistein-mediated inhibition of glycosaminoglycan synthesis as a basis for gene expression-targeted isoflavone therapy for mucopolysaccharidoses. *European Journal of Human Genetics, 14*(7), 846-852.

Planta, J. U. D., & De Meester, R. (2007). Validity of the socially acceptable behavior (SAB) test as a measure of aggression in dogs towards non-familiar humans. *Vlaams Diergeneeskundig Tijdschrift, 76*(5), 359-368.

Poorthuis, B., Wevers, R. A., Kleijer, W. J., Groener, J. E. M., de Jong, J. G. N., van Weely, S., et al. (1999). The frequency of lysosomal storage diseases in The Netherlands. *Human Genetics, 105*(1-2), 151-156.

Randall, D. C., Billman, G. E., Knapp, C. F., & Evans, J. M. (1980). An examination of the possible role of pain and anxiety in the canine response to coronary-occlusion. *Pavlovian Journal of Biological Science, 15*(2), 89-89.

Reolon, G. K., Braga, L. M., Camassola, M., Luft, T., Henriques, J. A. P., Nardi, N. B., et al. (2006). Long-term memory for aversive training is impaired in idua -/- mice, a genetic model of mucopolysaccharidosis type I. *Brain Research*(1076), 225-230.

Rohrbach, M., & Clarke, J. T. R. (2007). Treatment of lysosomal storage disorders - progress with enzyme replacement therapy. *Drugs, 67*(18), 2697-2716.

Roudebush, P., Zicker, S. C., Cotman, C. W., Milgram, N. W., Muggenburg, B., & Head, E. (2005). Nutritional management of brain aging in dogs. *Journal of the American Veterinary Medical Association, 227*(5), 722-728.

Savas, P. S., Hemsley, K. M., & Hopwood, J. J. (2004). Intracerebral injection of sulphamidase delays neuropathology in murine MPS III A. *Molecular Genetics and Metabolism, 82*, 273-285.

Scriver, C. R., Beaudet, A. L., Sly, W. S., & Valle, D. (2001). *The molecular and metabolic basis of inherited disease - volume 1* (7 ed.): McGraw-Hill Inc.

Serpell, J. A., & Hsu, Y. (2001). Development and validation of a novel method for evaluating behaviour and temperament in guide dogs. *Applied Animal Behaviour Science, 72*, 347-364.

Siwak, C. T., Tapp, P. D., & Milgram, N. W. (2001). Effect of age and level of cognitive function on spontaneous and exploratory behaviors in the beagle dog. *Learning and Memory, 8*(6), 317-325.

Siwak, C. T., Tapp, P. D., & Milgram, N. W. (2003). Adrafinil disrupts performance on a delayed nonmatching-to-position task in aged beagle dogs. *Pharmacology Biochemistry and Behavior, 76*(1), 161-168.

Slabbert, J. M., & Rasa, O. A. E. (1997). Observational learning of an acquired maternal behaviour pattern by working dog pups: an alternative training method? *Applied Animal Behaviour Science, 53*(4), 309-316.

Studzinski, C. M., Christie, L., Araujo, J. A., McIntyre Burnham, W., Head, E., Cotman, C. W., et al. (2006). Visuospatial function in the beagle dog: an early marker of cognitive decline in a model of human aging and dementia. *Neurobiology of Learning and Memory, 86*, 197-204.

Svartberg, K. (2002). Shyness-boldness predicts performance in working dogs. *Applied Animal Behaviour Science, 79*(2), 157-174.

Svartberg, K. (2005). A comparison of behaviour in test and in everyday life: evidence of three consistent boldness-related personality traits in dogs. *Applied Animal Behaviour Science, 91*(1-2), 103-128.

Tapp, D. P., Silwak, C. T., Estrada, J., Holowachuk, D., & Milgram, N. W. (2003a). Effects of age on measures of complex working memory span in the beagle dog (Canis familiaris) using two versions of a spatial list learning paradigm. *Learning and Memory, 10*, 148-160.

Tapp, P. D., Siwak, C. T., Estrada, J., Head, E., Muggenburg, B. A., Cotman, C. W., et al. (2003b). Size and reversal learning in the beagle dog as a measure of executive function and inhibitory control in aging. *Learning and Memory, 10*(1), 64-73.

Taylor, K. D., & Mills, D. S. (2006). The development and assessment of temperament tests for adult companion dogs. *Journal of Veterinary Behavior: Clinical Applications and Research, 1*(3), 94-108.

Tessitore, A., Faella, A., O'Malley, G., Doria, M., Kunieda, T., Matarese, G., et al. (2008). Biochemical, pathological, and skeletal improvement of mucopolysaccharidosis VI after gene transfer to liver but not to muscle. *Molecular Therapy, 16*(1), 30-37.

Thompson, J. N., Jones, M. Z., Dawson, G., & Huffman, P. S. (1992). N-Acetylglucosamine 6-Sulfatase deficiency in a Nubian goat - a model of Sanfilippo syndrome type-D (Mucopolysaccharidosis IIID). *Journal of Inherited Metabolic Disease, 15*(5), 760-768.

Traas, A., Wang, P., Ma, X., O_Donnell, P., Sleeper, M., Aguirre, G., et al. (2007). Neonatal gene therapy reduces clinical manifestations in MPS I dogs. *Molecular Genetics and Metabolism, 92*, S11-S34.

Valstar, M. J., Ruijter, G. J. G., van Diggelen, O. P., Poorthuis, B. J., & Wijburg, F. A. (2008). Sanfilippo syndrome: a mini-review. *Journal of Inherited Metabolic Disease, 31*(2), 240-252.

Vanderborg, J. A. M., Netto, W. J., & Planta, D. J. U. (1991). Behavioral-testing of dogs in animal shelters to predict problem behavior. *Applied Animal Behaviour Science, 32*(2-3), 237-251.

Wagner, K. R. (2007). Enzyme replacement for infantile Pompe disease - The first step toward a cure. *Neurology, 68*(2), 88-89.

Weiss, E., & Greenberg, G. (1997). Service dog selection tests: effectiveness for dogs from animal shelters. *Applied Animal Behaviour Science, 53*(4), 297-308.

Wells, D. L. (2003). Lateralised behaviour in the domestic dog, Canis familiaris. *Behavioural Processes, 61*(1-2), 27-35.

White, B., & Castle, P. W. (1964). Visual exploratory behaviour following postnatal handling of human infants. *Perceptual and Motor skills, 18*, 497-502.

Wilkerson, M. J., Lewis, D. C., Marks, S. L., & Prieur, D. J. (1998). Clinical and morphologic features of mucopolysaccharidosis type II in a dog: naturally occurring model of Hunter syndrome. *Veterinary Pathology, 35*(3), 230-233.

Wilsson, E. (1984). The social-interaction between mother and offspring during weaning in German Shepherd dogs - individual differences between mothers and their effects on offspring. *Applied Animal Behaviour Science, 13*(1-2), 101-112.

Wilsson, E., & Sundgren, P. E. (1997). The use of a behaviour test for selection of dogs for service and breeding. 2. Heritability for tested parameters and effect of selection based on service dog characteristics. *Applied Animal Behaviour Science, 54*(2-3), 235-241.

Wilsson, E., & Sundgren, P. E. (1998a). Behaviour test for eight-week old puppies - heritabilities of tested behaviour traits and its correspondence to later behaviour. *Applied Animal Behaviour Science, 58*(1-2), 151-162.

Wilsson, E., & Sundgren, P. E. (1998b). Effects of weight, litter size and parity of mother on the behaviour of the puppy and the adult dog. *Applied Animal Behaviour Science, 56*(2-4), 245-254.

Yogalingam, G., Pollard, T., Gliddon, B., Jolly, R. D., & Hopwood, J. J. (2002). Identification of a mutation causing mucopolysaccharidosis type IIIA in New Zealand Huntaway dogs. *Genomics, 79*(2), 150-153.

Yoshida, M., Ikadai, H., Maekawa, A., Takahashi, M., & Nagase, S. (1993). Pathological characteristics of mucopolysaccharidosis-VI in the rat. *Journal of Comparative Pathology, 109*(2), 141-153.

Appendix One: DVD-ROM of research data

Appendix Two:

Canine Behavioural Assessment and Research
Questionnaire and accompanying cover form

General Questions:

Date: _____

Name of Dog: _____

Sex: _____   Desexed?   Yes / No

Age of dog: _____

ID Code: [   ]

Canine Behavioral Assessment & Research Questionnaire (C-BARQ)

The following questions are designed to allow you to describe how your dog has been behaving in the recent past (i.e. during the last few months).

Please try to answer all of the questions. Only leave a question blank if you cannot answer it for some reason (for instance, if you have never observed the dog in the situation described).

SECTION 1: Training and obedience

Some dogs are more obedient and trainable than others. By checking the appropriate boxes, please indicate how trainable or obedient your dog has been in each of the following situations in the recent past:

	Never	Seldom	Sometimes	Usually	Always
1. When off the leash, returns immediately when called.	☐	☐	☐	☐	☐
2. Obeys the "sit" command immediately.	☐	☐	☐	☐	☐
3. Obeys the "stay" command immediately.	☐	☐	☐	☐	☐
4. Seems to attend/listen closely to everything you say or do.	☐	☐	☐	☐	☐
5. Slow to respond to correction or punishment; 'thick-skinned'.	☐	☐	☐	☐	☐
6. Slow to learn new tricks or tasks.	☐	☐	☐	☐	☐
7. Easily distracted by interesting sights, sounds or smells.	☐	☐	☐	☐	☐
8. Will 'retrieve' or attempt to retrieve sticks, balls, or objects.	☐	☐	☐	☐	☐

SECTION 2: Aggression

Some dogs display aggressive behavior from time to time. Typical signs of moderate aggression in dogs include barking, growling and baring teeth. More serious aggression generally includes snapping, lunging, biting, or attempting to bite.

By circling or underlining a number on the following 5-point scales (0= No aggression, 4= Serious aggression), please indicate your own dog's recent tendency to display aggressive behavior in each of the following contexts:

9. When verbally corrected or punished (scolded, shouted at, etc) by you or a household member.

	Moderate aggression:	
No aggression:	growling/barking —baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

10. When approached directly by an unfamiliar adult while being walked/exercised on a leash.

	Moderate aggression:	
No aggression:	growling/barking —baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

11. When approached directly by an unfamiliar child while being walked/exercised on a leash.

	Moderate aggression:	
No aggression:	growling/barking —baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

12. Toward unfamiliar persons approaching the dog while s/he is in your car (at the gas station for example).

	Moderate aggression:	
No aggression:	growling/barking —baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

13. When toys, bones or other objects are taken away by a household member.

	Moderate aggression:	
No aggression:	growling/barking —baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

14. When bathed or groomed by a household member.

No aggression: No visible signs of aggression	Moderate aggression: growling/barking —baring teeth	Serious aggression: Snaps, bites or attempts to bite.
0..............1..............2..............3..............4		

15. When an unfamiliar person approaches you or another member of your family at home.

No aggression: No visible signs of aggression	Moderate aggression: growling/barking —baring teeth	Serious aggression: Snaps, bites or attempts to bite.
0..............1..............2..............3..............4		

16. When unfamiliar persons approach you or another member of your family away from your home.

No aggression: No visible signs of aggression	Moderate aggression: growling/barking —baring teeth	Serious aggression: Snaps, bites or attempts to bite.
0..............1..............2..............3..............4		

17. When approached directly by a household member while s/he is eating.

No aggression: No visible signs of aggression	Moderate aggression: growling/barking —baring teeth	Serious aggression: Snaps, bites or attempts to bite.
0..............1..............2..............3..............4		

18. When mailmen or other delivery workers approach your home.

No aggression: No visible signs of aggression	Moderate aggression: growling/barking —baring teeth	Serious aggression: Snaps, bites or attempts to bite.
0..............1..............2..............3..............4		

19. When his/her food is taken away by a household member.

No aggression: No visible signs of aggression	Moderate aggression: growling/barking —baring teeth	Serious aggression: Snaps, bites or attempts to bite.
0..............1..............2..............3..............4		

20. When strangers walk past your home while your dog is in the yard.

No aggression: No visible signs of aggression	Moderate aggression: growling/barking —baring teeth	Serious aggression: Snaps, bites or attempts to bite.
0..............1..............2..............3..............4		

4

21. When an unfamiliar person tries to touch or pet the dog.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0............1............2............3............4		attempts to bite.

22. When joggers, cyclists, rollerbladers or skateboarders pass your home while your dog is in the yard.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0............1............2............3............4		attempts to bite.

23. When approached directly by an unfamiliar male dog while being walked/exercised on a leash.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0............1............2............3............4		attempts to bite.

24. When approached directly by an unfamiliar female dog while being walked/exercised on a leash.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0............1............2............3............4		attempts to bite.

25. When stared at directly by a member of the household.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0............1............2............3............4		attempts to bite.

26. Toward unfamiliar dogs visiting your home.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0............1............2............3............4		attempts to bite.

27. Toward cats, squirrels or other animals entering your yard.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

28. Toward unfamiliar persons visiting your home.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

29. When barked, growled, or lunged at by another (unfamiliar) dog.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

30. When stepped over by a member of the household.

	Moderate aggression:	
No aggression:	growling/barking — baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

31. When you or a household member retrieves food or objects stolen by the dog.

	Moderate aggression:	
No aggression:	from growling/barking to snarling/baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

32. Towards another (familiar) dog in your household (leave blank if no other dogs).

	Moderate aggression:	
No aggression:	from growling/barking to snarling/baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

33. When approached at a favorite resting/sleeping place by another (familiar) household dog (leave blank if no other dogs).

	Moderate aggression:	
No aggression:	from growling/barking to snarling/baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0.............1.............2.............3.............4		attempts to bite.

34. When approached while eating by another (familiar) household dog (leave blank if no other dogs).

	Moderate aggression:	
No aggression:	growling/barking —baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0..............1..............2..............3..............4		attempts to bite.

35. When approached while playing with/chewing a favorite toy, bone, object, etc., by another (familiar) household dog (leave blank if no other dogs).

	Moderate aggression:	
No aggression:	growling/barking —baring teeth	Serious aggression:
No visible signs		Snaps, bites or
of aggression 0..............1..............2..............3..............4		attempts to bite.

Are there any other situations in which your dog is sometimes aggressive? If so, please describe:

_____——

_____——

SECTION 3: Fear and Anxiety

Dogs sometimes show signs of anxiety or fear when exposed to particular sounds, objects, persons or situations. Typical signs of mild to moderate fear include: avoiding eye contact, avoidance of the feared object; crouching or cringing with tail lowered or tucked between the legs; whimpering or whining, freezing, and shaking or trembling. Extreme fear is characterized by exaggerated cowering, and/or vigorous attempts to escape, retreat or hide from the feared object, person or situation.

Using the following 5-point scales (0=No fear, 4=Extreme fear), please indicate your own dog's recent tendency to display fearful behavior in each of the following circumstances:

36. When approached directly by an unfamiliar adult while away from your home.

No fear/anxiety:	Mild—Moderate fear/anxiety	Extreme fear:
No visible		cowers; retreats or
signs of fear 0..............1..............2..............3..............4		hides, etc.

37. When approached directly by an unfamiliar child while away from your home.

No fear/anxiety:	Mild—Moderate fear/anxiety	Extreme fear:
No visible		cowers; retreats or
signs of fear 0..............1..............2..............3..............4		hides, etc.

38. In response to sudden or loud noises (e.g. vacuum cleaner, car backfire, road drills, objects being dropped, etc.).

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:
    No visible   cowers; retreats or
    signs of fear   0.............1.............2.............3.............4   hides, etc.

39. When unfamiliar persons visit your home.

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:
    No visible   cowers; retreats or
    signs of fear   0.............1.............2.............3.............4   hides, etc.

40. When an unfamiliar person tries to touch or pet the dog.

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:
    No visible   cowers; retreats or
    signs of fear   0.............1.............2.............3.............4   hides, etc.

41. In heavy traffic

    No fear: Mild—Moderate fear   Extreme fear:
    No visible   cowers; retreats or
    signs of fear   0.............1.............2.............3.............4   hides, etc.

42. In response to strange or unfamiliar objects on or near the sidewalk (e.g. plastic trash bags, leaves, litter, flags flapping, etc.

    No fear: Mild—Moderate fear   Extreme fear:
    No visible   cowers; retreats or
    signs of fear   0.............1.............2.............3.............4   hides, etc.

43. When examined/treated by a veterinarian.

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:
    No visible   cowers; retreats or
    signs of fear   0.............1.............2.............3.............4   hides, etc.

44. During thunderstorms.

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:
    No visible   cowers; retreats or
    signs of fear   0.............1.............2.............3.............4   hides, etc.

45. When approached directly by an unfamiliar dog of the same or larger size.

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:
   No visible   cowers; retreats or
   signs of fear   0.............1.............2.............3.............4   hides, etc.

46. When approached directly by an unfamiliar dog of a smaller size.

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:
   No visible   cowers; retreats or
   signs of fear   0.............1.............2.............3.............4   hides, etc.

47. When first exposed to unfamiliar situations (e.g. first car trip, first time in elevator, first visit to veterinarian, etc.)

   No fear: Mild—Moderate fear   Extreme fear:
   No visible   cowers; retreats or
   signs of fear   0.............1.............2.............3.............4   hides, etc.

48. In response to wind or wind-blown objects.

   No fear: Mild—Moderate fear   Extreme fear:
   No visible   cowers; retreats or
   signs of fear   0.............1.............2.............3.............4   hides, etc.

49. When having claws clipped by a household member.

   No fear: Mild—Moderate fear   Extreme fear:
   No visible   cowers; retreats or
   signs of fear   0.............1.............2.............3.............4   hides, etc.

50. When groomed or bathed by a household member.

   No fear: Mild—Moderate fear   Extreme fear:
   No visible   cowers; retreats or
   signs of fear   0.............1.............2.............3.............4   hides, etc.

51. When stepped over by a member of the household.

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:
   No visible   cowers; retreats or
   signs of fear   0.............1.............2.............3.............4   hides, etc.

52. When having his/her feet toweled by a member of the household.

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:

No visible   cowers; retreats or

signs of fear 0...............1...............2...............3...............4   hides, etc.

53. When unfamiliar dogs visit your home.

No fear: Mild—Moderate fear   Extreme fear:

No visible   cowers; retreats or

signs of fear 0...............1...............2...............3...............4   hides, etc.

54. When barked, growled, or lunged at by an unfamiliar dog.

No fear/anxiety: Mild—Moderate fear/anxiety   Extreme fear:

No visible   cowers; retreats or

signs of fear 0...............1...............2...............3...............4   hides, etc.

SECTION 4: Separation-related behavior.

Some dogs show signs of anxiety or abnormal behavior when left alone, even for relatively short periods of time. Thinking back over the recent past, how often has your dog shown each of the following signs of separation-related behavior when left, or about to be left, on its own (check appropriate boxes):

	Never	Seldom	Sometimes	Usually	Always
55. Shaking, shivering or trembling.	☐	☐	☐	☐	☐
56. Excessive salivation.	☐	☐	☐	☐	☐
57. Restlessness/agitation/pacing.	☐	☐	☐	☐	☐
58. Whining.	☐	☐	☐	☐	☐
59. Barking.	☐	☐	☐	☐	☐
60. Howling.	☐	☐	☐	☐	☐
61. Chewing/scratching at doors, floor, windows, curtains, etc.	☐	☐	☐	☐	☐
62. Loss of appetite.	☐	☐	☐	☐	☐

Are there any other situations in which your dog is fearful or anxious? If so, please describe:

_____ — –

_____ — –

SECTION 5: Excitability

Some dogs show relatively little reaction to sudden or potentially exciting events and disturbances in their environment, while others become highly excited at the slightest novelty. Signs of mild to moderate excitability include increased alertness, movement toward the source of novelty, and brief episodes of barking. Extreme excitability is characterized by a general tendency to over-react. The excitable dog barks or yelps hysterically at the slightest disturbance, rushes towards and around any source of excitement, and is difficult to calm down.

Using the following 5-point scales (0=Calm, 4=Extremely excitable), please indicate your own dog's recent tendency to become excitable in each of the following circumstances:

63. When you or other members of the household come home after a brief absence.

Calm: little or no special reaction	Mild—Moderate excitability	Extremely excitable: over-reacts, hard to calm down.
0..............1..............2..............3..............4		

64. When playing with you or other members of your household.

Calm: little or no special reaction	Mild—Moderate excitability	Extremely excitable: over-reacts, hard to calm down.
0..............1..............2..............3..............4		

65. When doorbell rings.

Calm: little or no special reaction	Mild—Moderate excitability	Extremely excitable: over-reacts, hard to calm down.
0..............1..............2..............3..............4		

66. Just before being taken for a walk.

Calm: little or no special reaction	Mild—Moderate excitability	Extremely excitable: over-reacts, hard to calm down.
0..............1..............2..............3..............4		

67. Just before being taken on a car trip.

Calm: little or no special reaction	Mild—Moderate excitability	Extremely excitable: over-reacts, hard to calm down.
0..............1..............2..............3..............4		

68. When visitors arrive at your home.

Calm: little or no special reaction	Mild—Moderate excitability	Extremely excitable: over-reacts, hard to calm down.
	0..............1..............2..............3..............4	

Are there any other situations in which your dog sometimes becomes over-excited? If so, please describe:

_____

_____

SECTION 6: Attachment and Attention-seeking.

Most dogs are strongly attached to their people, and some demand a great deal of attention and affection from them. Thinking back over the recent past, how often has your dog shown each of the following signs of attachment or attention-seeking.

	Never	Seldom	Sometimes	Usually	Always
69. Displays a strong attachment for one particular member of the household.	☐	☐	☐	☐	☐
70. Tends to follow you (or other members of household) about the house, from room to room.	☐	☐	☐	☐	☐
71. Tends to sit close to, or in contact with, you (or others) when you are sitting down.	☐	☐	☐	☐	☐
72. Tends to nudge, nuzzle or paw you (or others) for attention when you are sitting down.	☐	☐	☐	☐	☐
73. Becomes agitated (whines, jumps up, tries to intervene) when you (or others) show affection for another person.	☐	☐	☐	☐	☐
74. Becomes agitated (whines, jumps up, tries to intervene) when you (or others) show affection for another dog or animal.	☐	☐	☐	☐	☐

SECTION 7: Miscellaneous

Dogs display a wide range of miscellaneous behavior problems in addition to those already covered by this questionnaire. Thinking back over the recent past, please indicate how often your dog has shown any of the following behaviors:

	Never	Seldom	Sometimes	Usually	Always
75. Chases cats (given the chance).	☐	☐	☐	☐	☐
76. Chases birds (given the chance).	☐	☐	☐	☐	☐
77. Chases squirrels, rabbits, etc. (given the chance).	☐	☐	☐	☐	☐
78. Escapes from home or yard, and roams (given the chance).	☐	☐	☐	☐	☐
79. Rolls in own or other animals' droppings or feces, or other 'smelly' substances.	☐	☐	☐	☐	☐
80. Eats own or other animals' droppings or feces.	☐	☐	☐	☐	☐
81. Chews inappropriate objects.	☐	☐	☐	☐	☐
82. 'Mounts' objects, furniture, or people.	☐	☐	☐	☐	☐
83. Begs persistently for food when people are eating.	☐	☐	☐	☐	☐
84. Steals food.	☐	☐	☐	☐	☐
85. Nervous or frightened of going up or down stairs.	☐	☐	☐	☐	☐
86. Pulls excessively hard when on the leash.	☐	☐	☐	☐	☐
87. Urinates against objects/ furnishings in your home.	☐	☐	☐	☐	☐
88. Urinates when approached, petted, handled or picked up.	☐	☐	☐	☐	☐
89. Urinates when left alone at night, or during the daytime.	☐	☐	☐	☐	☐
90. Defecates when left alone at night, or during the daytime.	☐	☐	☐	☐	☐
91. Hyperactive, restless, has trouble settling down.	☐	☐	☐	☐	☐

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	Never	Seldom	Sometimes	Usually	Always
92. Playful, puppyish, boisterous.	☐	☐	☐	☐	☐
93. Active, energetic, always on the go.	☐	☐	☐	☐	☐
94. Stares intently at nothing visible.	☐	☐	☐	☐	☐
95. Snaps at (invisible) flies.	☐	☐	☐	☐	☐
96. Chases own tail/hind end.	☐	☐	☐	☐	☐
97. Chases/follows shadows.	☐	☐	☐	☐	☐
98. Barks excessively.	☐	☐	☐	☐	☐
99. Licks him/herself excessively.	☐	☐	☐	☐	☐
100. Licks people or objects excessively.	☐	☐	☐	☐	☐
101. Displays other bizarre, strange, or repetitive behavior(s) *	☐	☐	☐	☐	☐

* Please describe: _____

_____