

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

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

A thesis presented  
in partial fulfilment of the requirements  
for the degree of Master of Veterinary Science  
At Massey University,  
Palmerston North  
New Zealand

**Vicki Heather Erceg**

**2009**

---

## 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.

---

## Acknowledgements

---

I would firstly like to thank Professor Kevin Stafford for all the years of support, help and great humour. I always knew when I heard you laughing down the corridor that it was going to be a good day.

Thanks also to Liz Norman for your unfailing calmness, perseverance and great knowledge when teaching me about mucopolysaccharidosis or statistics. It was greatly appreciated.

I would like to thank the staff out at the MPS colony. You were always so willing to help and you looked after the dogs so well. Thanks especially to Emma Holloway for all your hours of work and care. The dogs learnt such a lot from you and their lives are the better for it.

Thank you also to Professor Bob Jolly. Your dedication to advancing the study of these dogs is amazing and your strong work ethic is an inspiration.

Thanks to the team at the Lysosomal Disease Research Unit for your support.

Special thanks to my Mum and Dad for your love and support.

This thesis is dedicated to my husband Michael, daughter Sophie and son Nicholas without whom this thesis would not be here.

Thank you Michael for being you, and for all the years of love, patience, support and encouragement.

Thanks also to my children Sophie Rose and Nicholas Samuel for being your wonderful loving selves.

---

# Table of Contents

---

<b>Abstract.....</b>	<b>ii</b>
<b>Acknowledgements.....</b>	<b>iv</b>
<b>Table of Contents .....</b>	<b>v</b>
<b>List of Tables .....</b>	<b>ix</b>
<b>List of Figures.....</b>	<b>xi</b>
<b>Introduction.....</b>	<b>1</b>
<b>1 Literature Review.....</b>	<b>3</b>
1.1 Introduction.....	3
1.2 Lysosomal Storage Diseases and Mucopolysaccharidoses.....	3
1.3 Clinical signs and diagnosis.....	5
1.3.1 MPS III.....	6
1.3.2 MPS IIIA.....	7
1.4 Progress in treatment of MPS.....	9
1.4.1 Use of animal models.....	9
1.4.2 Current therapies.....	12
1.5 Detection of behavioural and cognitive alterations in MPS IIIA dogs.....	14
1.5.1 Behaviour monitoring.....	15
1.5.2 Measuring behaviour at different ages.....	16
1.5.3 Standardisation of behavioural measurements.....	17
1.5.4 Environmental and maternal influences on puppy behaviours.....	21
1.6 Measurement of cognitive function.....	21
1.7 Summary.....	23

1.8	Objectives of Thesis.....	24
<b>2</b>	<b>Behavioural Tests at Six and Eight Weeks of Age Comparing MPS IIIA Affected and Unaffected Dogs.....</b>	<b>25</b>
2.1	Introduction.....	25
2.2	Materials and methods.....	26
2.2.1	Aim.....	26
2.2.2	Subjects.....	26
2.2.3	Procedure.....	27
2.3	Data collection and statistical analysis.....	31
2.4	Results.....	32
2.4.1	Tests for normality.....	32
2.4.2	Descriptive statistics.....	35
2.4.2.1	Summary statistics for the six week tests.....	35
2.4.2.2	Summary statistics for the eight week tests.....	39
2.4.3	Comparing the six and eight week tests.....	43
2.4.4	Interobserver reliability.....	46
2.4.5	Spearman's rank correlation coefficient.....	46
2.5	Discussion.....	49
2.5.1	Correlations.....	49
2.5.2	Limitations of these tests.....	50
2.5.3	Comparison between the six week and eight week findings.....	50
2.5.4	Areas for improvement in future testing.....	51
2.5.5	Six and eight week test behaviours.....	51
<b>3</b>	<b>Canine Behavioural Assessment and Research Questionnaire to Measure Behaviour in MPS IIIA Affected and Unaffected Dogs.....</b>	<b>53</b>
3.1	Introduction.....	53
3.2	Materials and methods.....	54
3.3	Statistical analysis.....	54
3.4	Results.....	55
3.4.1	Descriptive statistics.....	56

3.4.2	Mann-Whitney <i>U</i> tests.....	66
3.5	Discussion.....	71
<b>4</b>	<b>Cognitive Function Measurement – Maze Testing Comparing between MPS IIIA Affected and Unaffected Dogs.....</b>	<b>75</b>
4.1	Introduction.....	75
4.2	Materials and methods.....	76
4.2.1	Subjects.....	76
4.2.2	Experimental design.....	77
4.2.3	Data collection and statistical analysis.....	79
4.3	Results.....	79
4.3.1	Training results.....	79
4.3.2	Time taken for testing.....	84
4.3.3	Results for first two days of testing.....	87
4.3.4	Final day of testing.....	92
4.3.5	Power analysis.....	95
4.4	Discussion.....	98
4.4.1	Decreased performance in MPS IIIA affected dogs on the final day of testing .....	98
4.4.2	Training and testing using the T-shaped maze.....	99
4.4.3	Limitations of the maze training and testing.....	102
4.4.4	Standardisation of maze training and testing.....	102
4.4.5	Conclusion.....	103
<b>5</b>	<b>General Discussion.....</b>	<b>104</b>
5.1	Introduction.....	104
5.2	Summary of the results.....	104
5.3	Limitations in this study.....	105
5.4	Importance and novelty of findings.....	106
5.5	Future research.....	107
5.6	Model for other diseases.....	107
5.7	Conclusions.....	108

**References..... 109**

**Appendix One: DVD-ROM of research data.....125**

**Appendix Two: Canine Behavioural Assessment and Research Questionnaire  
and accompanying cover form.....126**

---

## List of Tables

---

<b>Table 1.1</b>	Mucopolysaccharidosis types and corresponding enzyme deficiencies.....	4
<b>Table 1.2</b>	Animal Models of Mucopolysaccharidosis.....	11
<b>Table 2.1</b>	Puppy testing methodology.....	29
<b>Table 2.2</b>	Anderson- Darling test for normality for six week tests in MPS IIIA affected and unaffected puppies.....	33
<b>Table 2.3</b>	Anderson-Darling test for normality for eight week tests in MPS IIIA affected and unaffected puppies.....	34
<b>Table 2.4</b>	Summary statistics for six week tests for MPS IIIA affected and unaffected puppies.....	36
<b>Table 2.5</b>	Summary statistics for eight week tests for MPS IIIA affected and unaffected puppies.....	40
<b>Table 2.6</b>	Mann-Whitney <i>U</i> six week test results comparing MPS IIIA affected and unaffected puppies.....	44
<b>Table 2.7</b>	Mann-Whitney <i>U</i> eight week test results comparing the MPS IIIA affected and unaffected puppies.....	44
<b>Table 2.8</b>	Spearman's rank correlation - Six week unaffected puppies.....	47
<b>Table 2.9</b>	Spearman's rank correlation - Six week MPS IIIA affected puppies.....	47
<b>Table 2.10</b>	Spearman's rank correlation - Eight week Unaffected puppies.....	48
<b>Table 2.11</b>	Spearman's rank correlation -Eight week MPS IIIA affected puppies.....	48
<b>Table 3.1</b>	Number of questionnaires completed for the MPS IIIA affected and unaffected dogs at three, six and twelve months.....	55
<b>Table 3.2</b>	C-BARQ scoring method and formulae calculations for the C-BARQ Factors.....	57
<b>Table 3.3</b>	Reductions and alterations to the original C-BARQ factors formulae Due to irrelevant questions causing missing data.....	58
<b>Table 3.4</b>	Summary statistics for the C-BARQ factors.....	59
<b>Table 3.5</b>	Mann-Whitney <i>U</i> comparing the MPS IIIA affected and unaffected dogs for the C-BARQ factors at three, six and twelve months of age.....	67
<b>Table 3.6</b>	Mann-Whitney <i>U</i> – C-BARQ Results for questions one, two, three, four, five, seven, eight and ninety-one comparing the MPS IIIA affected and unaffected dogs.....	68

<b>Table 4.1</b>	Mann-Whitney <i>U</i> test comparing the median number of training days for the MPS IIIA affected and unaffected dogs.....	80
<b>Table 4.2</b>	Mann-Whitney <i>U</i> test comparing the median number of correct training exercises for MPS IIIA affected and unaffected dogs per day.....	82
<b>Table 4.3</b>	Spearman's rank correlation coefficient for the number of successful final test results correlated to the age of the dogs.....	83
<b>Table 4.4</b>	Mann-Whitney <i>U</i> tests comparing unaffected and MPS IIIA affected dogs for the time taken for correct maze tests, total maze tests and correct final maze tests.....	86
<b>Table 4.5</b>	Descriptive statistics for the maze testing comparing the MPS IIIA affected and unaffected dogs.....	89
<b>Table 4.6</b>	Mann-Whitney <i>U</i> tests comparing the maze test medians for the MPS IIIA affected and unaffected dogs.....	94
<b>Table 4.7</b>	Two-sample t-test comparing the final test medians for MPS IIIA affected and unaffected dogs.....	96
<b>Table 4.8</b>	Correct results for each day of training and testing for the MPS IIIA affected and unaffected dogs.....	97

---

## List of Figures

---

<b>Figure 2.1</b>	Floor plan of the site for the six and eight week tests.....	28
<b>Figure 2.2</b>	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.....	37
<b>Figure 2.3</b>	Histograms comparing MPS IIIA affected and unaffected puppies for the six week ordinal qualitative tests; large ball, contact one, retrieve and tug of war.....	38
<b>Figure 2.4</b>	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.....	41
<b>Figure 2.5</b>	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.....	42
<b>Figure 2.6</b>	Boxplots comparing the results of the six and eight week test items.....	45
<b>Figure 3.1</b>	Box plots of C-BARQ factors at three months of age comparing the unaffected and MPS IIIA affected dogs.....	63
<b>Figure 3.2</b>	Box plots of C-BARQ factors at six months of age comparing the unaffected and MPS IIIA affected dogs.....	64
<b>Figure 3.3</b>	Box plots of C-BARQ factors at twelve months of age comparing the unaffected and MPS IIIA affected dogs.....	65
<b>Figure 3.4</b>	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. ....	70
<b>Figure 4.1</b>	Diagram of the T-shaped maze used to test the cognitive function of MPS IIIA affected and unaffected dogs.....	77
<b>Figure 4.2</b>	Boxplot comparing the number of days of training carried out by MPS IIIA affected and unaffected dogs.....	80
<b>Figure 4.3</b>	Boxplot comparing the mean number of correct training exercises for MPS IIIA affected and unaffected dogs per training day.....	82

<b>Figure 4.4</b>	Boxplot comparing the time taken to perform the correct tests for MPS IIIA affected and unaffected dogs.....	85
<b>Figure 4.5</b>	Boxplot comparing the time taken for the correct final tests for MPS IIIA affected and unaffected dogs.....	85
<b>Figure 4.6</b>	Boxplot comparing the total testing time for MPS IIIA affected and unaffected dogs.....	86
<b>Figure 4.7</b>	Boxplot comparing testing using the left symbol for MPS IIIA affected and unaffected dogs.....	90
<b>Figure 4.8</b>	Boxplot comparing testing using the right symbol for MPS IIIA Affected and unaffected dogs.....	90
<b>Figure 4.9</b>	Boxplot comparing the first day of maze testing for MPS IIIA affected and unaffected dogs.....	91
<b>Figure 4.10</b>	Boxplot comparing the second day of maze testing for MPS IIIA affected and unaffected dogs.....	91
<b>Figure 4.11</b>	Boxplot comparing the results of the final day of maze testing for MPS IIIA affected and unaffected dogs.....	93
<b>Figure 4.12</b>	Boxplot comparing the final results for MPS IIIA affected and unaffected dogs including the affected dog that was unable to complete training.....	93
<b>Figure 4.13</b>	Levene's test for equal variances for final test.....	94
<b>Figure 4.14</b>	Power curve for the two-sample t-test for the final maze test.....	96

**Ethics Approval:**

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