

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	iii
ABSTRACT.....	iv
TABLE OF CONTENTS.....	v
LIST OF ILLUSTRATIONS.....	vii
ABBREVIATIONS USED.....	viii
Chapter I.....	1
INTRODUCTION.....	1
Synaptic plasticity – functional and structural.....	2
Adhesion molecules at the synapse.....	2
Chapter II.....	4
LITERATURE REVIEW.....	4
Cadherins.....	5
<i>Cadherin structure and adhesive function</i> .....	7
<i>Neuronal cadherins</i> .....	9
β-Catenin.....	12
Synaptic plasticity.....	15
The importance of Ca <sup>2+</sup> ion.....	17
<i>Cadherins and Ca<sup>2+</sup></i> .....	17
<i>Ca<sup>2+</sup> in the extracellular space</i> .....	18
<i>Ca<sup>2+</sup> and LTP</i> .....	19
FRET as a tool for studying protein dynamics.....	20
Chapter III.....	22
ANALYSIS OF β-CATENIN DYNAMICS IN NEURONS.....	22
Chapter IV.....	28
DEVELOPMENT OF CADHERIN FRET REPORTERS.....	28
<i>Epitope tagging approaches</i> .....	29
<i>Fluorescent protein approaches</i> .....	31
<i>Transposon-mediated functional insertion of fluorescent proteins in N-cadherin</i> ... 32	
<i>Monitoring cadherin interactions via acceptor bleaching (adFRET)</i> .....	38
<i>Visualizing changes in cadherin-cadherin interactions induced by changes in extracellular Ca<sup>2+</sup> concentration</i> .....	43
Chapter V.....	49
DISCUSSION AND FUTURE DIRECTIONS.....	49
<i>Construction of cadherin FRET reporters</i> .....	50
<i>Geometry of cadherins in cell junctions</i> .....	52
<i>Visualizing changes in cadherin-cadherin interactions induced by synaptic activity</i> .....	54
<i>Experiments made possible with cadherin FRET reporters</i> .....	58
WORK CITED.....	62
APPENDIX.....	69
MATERIALS AND METHODS.....	69
<i>Constructs, viruses and antibodies</i> .....	69
<i>Cultured hippocampal neurons</i> .....	69
<i>Cell culture and transfection</i> .....	70

<i>Immunoprecipitation</i> .....	70
<i>L cell aggregation assay</i> .....	71
<i>Microscopy and image analysis</i> .....	72