Role for the Cadherin Family of Cell Adhesion Molecules in Synaptic Function in the Adult Hippocampus

Thesis by

Lixin Tang

In Partial Fulfillment of the Requirements
for the Degree of
Doctor of Philosophy

California Institute of Technology
Pasadena, California
1999
(Submitted March 29, 1999)

To my beloved family

Acknowledgments

Scientific research requires constant dedication, intelligent experimental designs, broad spectrum of knowledge, and in-depth sights into various phenomena of the nature. My 5-6 years of Ph.D. thesis research is such a valuable, exciting, and enjoyable training process. Good tutelage and insightful advice on the research project is the critical factor for the graduate success. In this regard, I have been so fortunate to have Professor Erin M. Schuman as my thesis advisor. Owing to her continuous guidance, support and encouragement throughout these many years, I have been able to conduct and finish this enjoyable thesis research. Besides many techniques of modern biology, Erin has taught me how to define a most challenging scientific problem, how to design elegant experiments to address the question, and how to present scientific discoveries and share many ideas with colleagues in a very efficient way. Moreover, I have learned from Erin how to conduct an independent research as well as how to collaborate with other scientists. I'd thus like to express my sincere gratitude to Erin for so much help she has given to me.

I would also like to thank my wife for her understanding and everlasting support to me these years. She shared with me all my excitements of discoveries as well as frustrations and confusions during my graduate study. She has been always with me. My beloved parents have also given me much distant support from China so that I was able to focus on the research.

Special thanks go to the thesis committee members - Profs. Henry A. Lester, Paul H. Patterson, Mary B. Kennedy and Pamela Bjorkman. During the course of this study, they gave me many helpful suggestions and comments that greatly guided my research.

Finally I'd like to thank Dr. Sachiko Murase for her help on the application of FM 1-43, Ms. Joanna L. Jankowsky for her detailed help on the *in situ* hybridization experiments, and Leslie Schenker for her techniques on immunohistochemistry. I'd also like to thank Ms. Dong-Hong Ju for her effort of detailed work of constructing the recombinant adenovirus, Dr. Mary B. Kennedy for providing the synapsin I antibody, Michael Tsung and Sheri McKinney for cultured hippocampal neurons. All the Schuman lab members also deserve my thanks for sharing a great deal of insightful ideas and very pleasant collaborations.

Abstract

The cadherins are a family of type I single-pass integral membrane glycoproteins that span intercellular junctions and mediate Ca^{2+}-dependent homophilic intercellular interactions. The highly conserved cytoplasmic C termini of cadherins interact with the catenins and the cytoskeleton. The extracellular domain is composed of five repeats with the most distal repeat, especially a region containing the highly conserved His-Ala-Val (HAV) sequence, being critical for homophilic binding.

I first examined the expression of cadherins, especially the neural- (N-) and epithelial- (E-) subtypes, in the adult hippocampus. *In situ* hybridization experiments indicated the presence of mRNAs for both N- and E- cadherins in the adult hippocampus. Immunoblot analysis revealed the expression of cadherin proteins in the hippocampal synaptosome fraction. Immunofluorescent staining indicated that cadherins and catenins are expressed at synaptic sites.

I investigated the possible role of cadherins in synaptic plasticity at the CA1 synapses in the adult hippocampus. Preincubation of hippocampal slices with function-blocking cadherin antibodies or HAV-containing antagonistic peptides greatly reduced long-term potentiation (LTP) whereas basal synaptic properties including input-output relations, and paired-pulse facilitation were normal. The HAV peptides inhibited LTP in a concentration-dependent and LTP induction protocol-independent manner.

A decrease in the extracellular Ca^{2+} associated with LTP induction may increase the vulnerability of Ca^{2+}-sensitive cadherin bonds to cadherin inhibitory reagents. In support

of this hypothesis, I found that doubling of the extracellular Ca^{2+} abolished the inhibition of LTP by HAV peptides. Moreover, HAV peptides delivered in a lower Ca^{2+} solution reduced previously potentiated responses, suggesting a role for cadherins in both the induction and expression of LTP.

A recombinant adenovirus containing a dominant-inhibitory cadherin cDNA was constructed. I found that hippocampal slices infected with this virus exhibited normal synaptic properties but less LTP than adjacent slices infected with an adenovirus containing a reporter gene.

I also examined the effect of HAV peptides on presynaptic vesicle exocytosis in hippocampal cultures using the fluorescent membrane dye FM 1-43. HAV peptides do not affect the dye release following stimulation, suggesting cadherin function is not required for normal exocytosis.

Taken together, these data suggest cadherins make important contributions to synaptic plasticity in the adult hippocampus.

Table of Contents

ACKNOWLEDGMENTS ..... **IV**

ABSTRACT ..... **VI**

TABLE OF CONTENTS ..... **VIII**

TABLE OF FIGURES ..... **XII**

CHAPTER 1. INTRODUCTION **1**

1.1 LEARNING AND MEMORY, SYNAPTIC PLASTICITY AND LTP 1

1.1.1 Memory and the hippocampus 1

1.1.2 Synapses, plasticity and LTP 2

1.1.3 LTP and memory 4

1.1.4 Mechanisms of LTP 7

1.1.4.1 Structural changes 7

1.1.4.2 Presynaptic vs. postsynaptic debate 9

1.1.4.3 Quantal analysis 10

1.1.4.4 Complexity of central synapses 11

1.1.4.5 Silent synapses 14

1.1.4.6 Retrograde messengers 16

1.1.4.7 Diffusion of potentiation 17

1.1.5 Proposed role of cell adhesion molecules in LTP 18

1.2 CELL ADHESION MOLECULES 21

1.2.1 IgSF 21

1.2.2 Integrins 23

1.2.3 Cadherins 27

1.2.3.1 General structure 29

1.2.3.1.1 Extracellular domain 29

1.2.3.1.2 Cytoplasmic domain and catenins 32

1.2.3.2 Cadherin interactions and the junctional complex 35

1.2.3.3 Cadherin clustering, adhesion, and regulation 38

1.2.3.4 Cadherin expression and function in animal morphogenesis 40

1.2.3.5 Cadherin in tumorigenesis 41

1.2.3.6 Cadherin in the formation of neural network in the CNS 42

CHAPTER 2. EXPRESSION OF CADHERINS IN YOUNG ADULT HIPPOCAMPUS 54

2.1 INTRODUCTION 54

2.2 RESULTS 55

2.2.1 *In situ* hybridization 55

2.2.2 Western blot analysis 56

2.2.3 Immunohistochemistry 56

2.2.3.1 Single immunofluorescence labeling in hippocampal slice sections 57

2.2.3.2 Dual immunofluorescence labeling in hippocampal slice sections 57

2.2.3.3 Single immunofluorescence labeling in cultured hippocampal neurons 58

2.2.3.4 Dual immunofluorescence labeling in cultured hippocampal neurons 58

2.3 DISCUSSION 59

CHAPTER 3. ROLES OF CADHERINS IN HIPPOCAMPAL SYNAPTIC PLASTICITY 74

3.1 INTRODUCTION 74

3.2 RESULTS 75

3.2.1 Function-blocking cadherin antibodies inhibit LTP 75

3.2.1.1 Ab penetration and persistence in slices 75

3.2.1.2 Function-blocking cadherin antibodies do not affect basal synaptic parameters 76

3.2.1.3 Function-blocking cadherin antibodies inhibit LTP 77

3.2.2 HAV peptides inhibit LTP 78

3.2.2.1 Introduction 78

3.2.2.2 HAV peptides inhibit LTP without disrupting normal synaptic function 80

3.2.2.3 Inhibition of LTP induction by HAV peptides 81

3.2.2.4 Dose-dependent inhibition of LTP by HAV peptides 82

3.2.2.5 HAV peptides also block LTP induced by other protocols 83

3.2.2.6 HAV peptides do not affect NMDA receptor-mediated responses 83

3.2.2.7 HAV peptides do not affect the postsynaptic neuron's ability to depolarize 84

3.2.3 The inhibition of LTP by cadherin inhibitory reagents: Dependence on $[Ca^{2+}]_o$ 85

3.2.3.1 Introduction 85

3.2.3.2 High Ca^{2+} protects LTP from inhibition by cadherin inhibitory reagents 86

3.2.3.3 Low Ca^{2+} manipulation reveals cadherin-dependency during LTP maintenance 86

3.3 DISCUSSION 87

CHAPTER 4. RECOMBINANT AD-VIRUS CONTAINING MUTANT CADHERIN DNA 123

4.1 INTRODUCTION 123

4.2 CONSTRUCTION OF AD-cN390Δ VIRUS 124

4.3 INFECTION OF SLICES BY AD-cN390Δ VIRUS 124

4.4 LTP IS REDUCED IN AD-CN390Δ VIRUS INFECTED SLICES 125
4.5 DISCUSSION 125

CHAPTER 5. PRESYNAPTIC VESICLE EXOCYTOSIS 133

5.1 INTRODUCTION 133
5.2 FM 1-43 STAINING AND DESTAINING 134
5.3 EFFECT OF HAV PEPTIDES ON THE DESTAINING OF FM 1-43 135
5.4 DISCUSSION 136

CHAPTER 6. GENERAL DISCUSSION: CELL ADHESION MOLECULES IN THE MODULATION OF SYNAPTIC TRANSMISSION AND PLASTICITY 145

6.1 ROLE OF OTHER CELL ADHESION MOLECULES IN SYNAPTIC PLASTICITY 145
6.1.1 Ig family members in synaptic plasticity 145
6.1.2 Integrins in synaptic plasticity 149
6.1.3 Neuronal activity regulates the expression of CAMs 151
6.2 ROLE OF CADHERINS IN SYNAPTOGENESIS 152
6.3 ROLE OF CADHERINS IN ADULT SYNAPSES 153
6.3.1 Thesis summary 153
6.3.2 Multifunctional cadherins 154
6.3.3 Cadherins in synaptic function 155
6.3.4 Concluding remarks 157

APPENDIX A. CENTRIFUGAL FORCE-BASED CELL ADHESION ASSAY 158

APPENDIX B. SIMILARITIES BETWEEN DIFFERENT CADHERIN TYPES ACROSS MAJOR DIFFERENT SPECIES 163

APPENDIX C. MATERIALS AND METHODS 164

1. Antibodies 164
2. Peptides 165
3. cDNA clones 165
4. Hippocampal slice preparation and storage 166
5. Electrophysiological recordings 166
6. Antibody and peptide introduction 167
7. Recombinant adenovirus vectors and slice infection 167
8. Western blotting 168
9. *In situ* hybridization 168

10. Immunofluorescent staining and confocal microscopy 171
11. FM1-43 staining, destaining, and measurement of fluorescence intensity 171
12. Data analysis 172
13. Centrifugal force-based cell adhesion assay 172

REFERENCES 175

Table of Figures

Figure 1-1. Induction of LTP in hippocampal synapses *in vitro*. 46

Figure 1-2. Possible role of cell adhesion molecules in LTP. 48

Figure 1-3. Neuronal cell adhesion molecules. 50

Figure 1-4. Cadherin junction complex and dimer interfaces. 52

Figure 2-1. *In situ* hybridization for N- and E-cadherin in hippocampal sections. 62

Figure 2-2. Immunoblot analysis indicates the presence of cadherins in adult hippocampus 64

Figure 2-3. Immunohistochemical labeling of cadherins and catenins in the CA1 region of the adult hippocampus. 66

Figure 2-4. Dual immunolabeling suggests synaptic localization of cadherins in adult hippocampus 68

Figure 2-5. Immunohistochemical labeling of cadherins and catenins in cultured hippocampal neurons 70

Figure 2-6. Dual immunolabeling suggests synaptic localization of cadherins and catenins in hippocampal neurons 72

Figure 3-1. Full penetration and persistence of N-cadherin antibodies in hippocampal slices. 91

Figure 3-2. Persistence of various antibodies in hippocampal slices for at least 60 minutes following perfusion with normal ACSF. 93

Figure 3-3. Cadherin antibodies do not alter basal properties of synaptic transmission in hippocampal slices. 95

Figure 3-4. Adhesion-blocking antibodies raised against the extracellular domains of either N- or E-cadherin attenuate LTP. 97

Figure 3-5. Sequence alignment of the EC1 domain of cadherins showing the highly conserved HAV motifs. 99

Figure 3-6. HAV peptides do not alter basal properties of synaptic transmission in hippocampal slices. 101

Figure 3-7. Slices incubated in HAV peptides exhibit diminished LTP. 103

Figure 3-8. Two-pathway experimental design with HAV peptides 105

Figure 3-9. HAV peptides perturb the induction of LTP. 107

Figure 3-10. Concentration-dependent inhibition of LTP by HAV peptides. 109

Figure 3-11. HAV peptides also inhibit LTP induced by other protocols. 111

Figure 3-12. HAV peptides do not affect NMDA receptor-mediated responses. 113

Figure 3-13. HAV peptides do not inhibit PPF, or postsynaptic depolarization mechanisms. 115

Figure 3-14. Hypothetical model of cadherin action during LTP induction. 117

Figure 3-15. Elevated extracellular Ca^{2+} prevents the block of LTP by cadherin inhibitory reagents. 119

Figure 3-16. HAV peptides delivered in low Ca^{2+} ACSF reverse LTP. 121

Figure 4-1. Construction of Ad-cN390Δ virus containing domain-negative mutant N-cadherin cDNA. 127

Figure 4-2. Slices infected with Ad-cN390Δ virus exhibit attenuated LTP..... 129

Figure 4-3. Basal synaptic properties in slices infected with Ad-cN390Δ virus or control Ad-lacZ virus..... 131

Figure 5-1. Staining and destaining of FM 1-43 labeled synaptic terminals in cultured hippocampal neurons..... 139

Figure 5-2. Destaining of synaptic terminals in HAV peptide-treated hippocampal neurons. 141

Figure 5-3. HAV peptides do not appear to affect the release of FM 1-43..... 143

Figure A-1. Experiment design for centrifuge force-based cell adhesion assay. 159

Figure A-2. Summary graph of adhesion assay on L cells expressing E-cadherin. 161

Chapter 1. Introduction

1.1 Learning and Memory, Synaptic Plasticity and LTP

The brain, is without a doubt, the most sophisticated machine in the world that is specifically designed and dedicated to constant information processing, storage and retrieval. To fulfill these daunting tasks, the brain must be able to marshal and coordinate its individual brain cells (~10^{12} neurons plus a large number of glial cells in human brain, for example) in a precise and efficient way. One of the central challenges of neuroscience is to understand the mechanisms underlying learning and memory in the brain of humans and other animals.

1.1.1 Memory and the hippocampus

Towards the end of investigating the mechanisms for learning and memory, the hippocampus has received great attention from neuroscientists for about a century. One influential work involving memory in hippocampus is the study on patient H.M. in 1950s (Scoville and Milner, 1957). After bilateral removal of much of his medial temporal lobe (including hippocampal formation and adjacent cortical areas) responsible for his epileptic seizures, H.M. suffered a great inability to acquire new memory ("anterograde amnesia"). He knows little of world events or discoveries that have taken place over the last 30 years or so (Parkin, 1996). Subsequent studies in patients with damage restricted to hippocampal formation and studies using hippocampus-lesioned animals have further established the role for hippocampus in the memory formation (for a review, see Nadel and

Moscovitch, 1997). It is known that memories may be separated into declarative memory and non-declarative memory (Squire, 1992). Declarative (or explicit) memory affords the capacity for conscious recollections about facts and events whereas non-declarative (or implicit) memory involves unconscious recollections about skills, habits and some forms of classic conditioning. Humans and monkeys with hippocampus lesions were impaired on tasks of declarative memory, but fully intact at skills and habit learning and other tasks of non-declarative memory (Zola-Morgan and Squire, 1993). Moreover, the hippocampus is important for learning and remembering spatial information, as revealed by studies in hippocampus-damaged rodents and humans (for a review, see Barnes, 1988). In parallel to this large literature of behavioral studies in the hippocampus, the cellular and molecular mechanisms that may underlie learning and memory formation in the hippocampus have been under intensive investigation by neurobiologists.

1.1.2 Synapses, plasticity and LTP

In the 1890s, Cajal demonstrated that networks of neurons are not in cytoplasmic continuity but communicate with each other at specialized connection sites between two neuronal cells (Ramon y Cajal, 1894), which were later termed "synapses" by Sherrington (Sherrington, 1897). Electric signals are communicated from one cell to its partner cell at the synaptic site. This communication or "synaptic transmission" involves conversion of a presynaptic electric signal into a chemical signal: released neurotransmitters, which diffuse across synaptic cleft and bind to postsynaptic receptors. The binding of a ligand causes ion channels to open, thus resulting in electric signals in the postsynaptic cell. It is estimated that an average neuron receives about 1000 synaptic connections and that there are about 10^{14} synaptic connections in the brain (Kandel et al., 1991). Since

synapses are the most fundamental units of signal transmission, information processing and storage in the brain, the structure and function of synapses has been the focus of many studies in neurobiology.

Memories have long been speculated to result from changes in synaptic efficiency. This idea emerged about a century ago following Cajal's work (Ramon y Cajal, 1894). External stimuli are represented in the brain as spatio-temporal patterns of neural activity which must themselves lead to changes in synaptic efficiency. In the late 1940s, this idea was refined by Hebb (Hebb, 1949) and Konorski (Konorski, 1948). Hebb proposed a coincidence-detection rule (Hebbian rule) that when two cells are active at the same time, the synaptic connection between them is increased whereas Konorski introduced the term "synaptic plasticity" to describe activity-dependent changes in synaptic strength.

In 1973, Bliss et al. discovered that the first excitatory synapses made by perforant path fibers onto dentate granule cells of the hippocampus possess such Hebbian properties (Bliss and Lomo, 1973): A brief high frequency stimulation co-activating both pre- and postsynaptic neurons causes long-lasting enhancement of the efficiency of synaptic transmission, which is called long-term potentiation (LTP). Since then, LTP has been found in all excitatory pathways in the hippocampus and in a number of other brain areas as well. In the field of LTP studies, the most extensively studied synapses so far have been the synapses made by CA3 pyramidal cell axons onto CA1 pyramidal cell dendrites in the hippocampus. The *in vitro* experimental preparation is schematically depicted in Figure 1-1. In the past two decades or so, LTP in the hippocampus, especially at CA1 synapses, has served as the dominant model of activity-dependent synaptic plasticity in

the mammalian brain. It has been used as the most compelling experimental model for investigating the synaptic basis of learning and memory in vertebrates.

As any change has two directions, synaptic transmission has also been found to be able to undergo long-term depression (LTD): activity-dependent synaptic weakening. LTD can be elicited by low-frequency stimulation (for example, 900 pulses at 1-3 Hz) in the hippocampus *in vitro* and *in vivo*, and has been suggested to play an equally important role in the mnemonic operations of hippocampal neural networks (e.g., Heynen et al., 1996).

1.1.3 LTP and memory

LTP has interested both cellular and behavioral neuroscientists as the kind of synaptic change likely to underlie memory formation in the brain. Both LTP and memory share some common features including their association with hippocampal function, their rapid induction and the ability to endure for long periods following brief bursts of neuronal activity at specific synapses. These are all consistent with a natural memory mechanism. Extensive studies have thus been devoted to establish a link between LTP and memory formation. However, the definitive proof of a link is still missing.

Earlier studies reported that rodent explorations of an unfamiliar environment was associated with LTP-like increments in synaptic efficacy in the hippocampus (Green et al., 1990; Sharp et al., 1989). However, this was later explained by a rise in brain temperature and other factors associated with active exploratory behavior (Andersen and Moser, 1995; Moser and Andersen, 1994). Nonetheless, temperature-independent synap-

tic potentiation in the hippocampus during learning about the environment has been observed (Moser et al., 1993).

Another line of evidence comes from the "saturation" of hippocampal synapses by intense afferent stimulation. McNaughton et al. used single bilateral stimulating electrodes to deliver episodes of tetanic stimulation to axons of the perforant path until LTP was saturated (McNaughton et al., 1986; Castro et al., 1989). The tetanized rats had deficits in spatial memory tasks. However, attempts to replicate this in other laboratories were unsuccessful (e.g., Robinson, 1992). More recently, Moser et al. revisited this problem by some refinements of the experimental design such as reducing the volume of functional hippocampal tissue and increasing the likelihood of saturating the major part of the perforant path synapses (Moser et al., 1998). After analysis of the degree of saturation and performance in the Morris water maze, they found that the saturated animals were the poor learners and the unsaturated animals were the good learners, thus supporting a LTP-memory connection.

Other evidence supporting the LTP-memory connection came from targeted genetic manipulations that showed that blockade of the molecular cascades critical for LTP induction also causes severe memory deficits. Earlier pharmacological studies showed that a number of molecules in the postsynaptic neuron, such as the NMDA receptor, α subunit of CaMKII (αCaMKII) and the tyrosine kinase Fyn, are involved in the induction of LTP (Bliss and Collingridge, 1993). Homologous recombination in embryonic stem cells was used in mice to delete the genes encoding αCaMKII (Silva et al., 1992; Silva et al., 1992) and Fyn (Grant et al., 1992). In these initial genetic studies, deletion of the target gene caused a defect in LTP and an impairment in explicit spatial memory. Since then, dozens

of papers have published that utilize knockout mice to address the relationship between the function of a specific gene and synaptic plasticity or behavior (for examples, Abeliovich et al., 1993; Aiba et al., 1994; Aiba et al., 1994; Conquet et al., 1994; Sakimura et al., 1995, reviewed in Chen and Tonegawa, 1997). The use of the conventional genetic techniques to study synaptic function in adult animals, however, suffers from a number of limitations (Rose, 1995; Routtenberg, 1995; Gerlai, 1996; Zimmer, 1996). These limitations include that the gene under study is often altered in many brain regions (the lack of spatial specificity) and that the genetic alteration is present during the entire developmental history of the animal (lack of temporal specificity). The recent development of a second generation of knockout mouse technology, in which the expression of a genetic alteration in mice can be restricted both anatomically and temporally, has allowed a more precise examination of the role of LTP in memory formation (Tsien et al., 1996l Jiang and Gridley, 1997; Mayford et al., 1997). An example of this approach is the knockout of the NMDAR1 gene in only CA1 pyramidal cells of the hippocampus (McHugh et al., 1996; Tsien et al., 1996). Adult mutant mice lacked LTP in the CA1 synapses and exhibited impaired spatial memory. These data provide strong evidence supporting the notion that NMDAR-dependent synaptic plasticity at CA1 synapses is required for the formation of spatial memory.

One interesting study linking LTP and learning has been to use the marine invertebrate *Aplysia*. Synapses in *Aplysia* also exhibit short- and long-term synaptic plasticity that play different roles in behavior (Hawkins and Kandel, 1984). Synapses formed by sensory neurons onto the motor neurons responsible for withdrawal of the siphon are the site of learning during the withdrawal reflex. These synapses are able to undergo NMDA-

receptor-dependent LTP-like plasticity (Lin and Glanzman, 1994). Recently, Murphy and Glanzman showed that classic conditioning of the responses of these sensory-motor synapses is blocked by the NMDA receptor-antagonist AP5 (Murphy and Glanzman, 1997), strengthening the link between Hebbian plasticity and associative learning and suggesting a conservation of mechanisms among different species such as *Aplysia* and mammals.

1.1.4 Mechanisms of LTP

To many, the Holy Grail of memory research is the elucidation of learning and memory at the cellular and molecular levels. Because of the potential link between LTP and memory, the cellular and molecular mechanisms underlying hippocampal LTP has been one of the most actively researched areas in neuroscience. I will briefly summarize some of relevant studies on the mechanisms of LTP.

1.1.4.1 Structural changes

Over a century ago, it was proposed that memory storage in the brain is accompanied by changes in synapse number and/or synapse morphology (Tanzi, 1893). Considerable work has been devoted to the investigation of the structural correlate of activity-dependent plasticity and LTP. Structural changes in the dentate gyrus of the hippocampus *in vivo* following repeated electrical stimulation have been observed for many years. Swelling of dendritic spines (Van Harreveld and Fifkova, 1975), changes in numerical density of synaptic contacts, postsynaptic density and synaptic interface area (Desmond and Levy, 1986; Desmond and Levy, 1988; Desmond and Levy, 1990) have been reported within 30 minutes after LTP induction. An increase in the number of axospinous

synapses with segmented postsynaptic densities and partitioned transmission zones has also been seen hours or days after LTP induction in the dentate gyrus *in vivo* (Geinisman et al., 1991; Geinisman et al., 1992; Geinisman, 1993; Geinisman et al., 1993). To examine the structural changes in CA1 region of hippocampus following induction of plasticity, different techniques have been utilized. Buchs et al. developed a cytochemical method to detect a stimulation-induced, D-AP5-sensitive accumulation of calcium at the electron microscopic level (Buchs et al., 1994). Using this method to label activated synapses in CA1 hippocampus, they found dramatic ultrastructural modifications including an increase in perforated synapses associated with LTP induction (Buchs and Muller, 1996). Other single-section analyses also indicated structural changes following LTP induction (Lee et al., 1980; Chang and Greenough, 1984). Moreover, confocal microscopy in conjunction with microdrop application of DiI in living hippocampal CA1 pyramidal neurons in acute slices indicated (though not conclusively, see below) two forms of structural change occur in chemically induced LTP: growth of a subpopulation of small spines and angular displacement of spines (Hosokawa et al., 1995). However, recent 3-D reconstruction data from serial electron microscopy (EM) failed to indicate significant changes in total synapse number or synapse size following LTP in CA1 area, thus favoring the hypothesis that LTP involves the redistribution of synaptic weights among existing synapses (Sorra et al., 1998; Sorra and Harris, 1998). It is not clear how accurate these different measurements are due to certain limitations associated with each technique. For example, the heterogeneity of synapse size, shape and orientation may lead one to misidentify individual synapses in single-section analysis. The irregular shapes of synapses make it very hard to obtain the true 3-D values from single section

measurements. Confocal microscopy does not provide sufficient resolution for dimensional measurements, nor does it distinguish short dendritic spines from the overlapping shaft. Serial EM may also have problems in preserving synapse structure during preparation. Despite the inconsistency across these studies, it is an attractive hypothesis that structural changes either in existing synapses and/or the formation of new synapses are associated LTP induction. These structural changes in these synapses may underlie learning-associated synaptic plasticity.

1.1.4.2 Presynaptic vs. postsynaptic debate

The most prominent feature of LTP is the long-lasting enhancement of synaptic transmission. Although the trigger for LTP is generally agreed to occur in the postsynaptic cell, the site of expression of LTP has been the subject of intense investigation and much debate, ranging from pure presynaptic mechanisms to pure postsynaptic mechanisms to a mixture of both types of mechanisms.

Using radioactive labeling of neurotransmitter, increased transmitter release was observed following LTP in the CA1 region *in vitro* and dentate gyrus *in vivo* (Skrede and Malthe-Sorenssen, 1981; Dolphin et al., 1982). However, pharmacological dissections of different subtypes of glutamate receptors in the postsynaptic membrane revealed postsynaptic modifications of AMPA-type, not NMDA-type, glutamate receptor components of the EPSP following LTP, suggesting that increased postsynaptic sensitivity to neurotransmitter underlies LTP (Kauer et al., 1988; Muller et al., 1988). Yet another study provided for both pre- and post-synaptic changes, but presumably in a temporally distinct manner, during maintenance of hippocampal LTP (Davies et al., 1989).

The advent of patch-clamp techniques with improved signal-to-noise ratio has brought the analysis of synaptic transmission to the microscopic level. Quantal analysis, originally used for synaptic transmission at the neuromuscular junction (NMJ), has thus been utilized to gain insights into plastic changes of synaptic transmission of central synapses, especially hippocampal synapses. This spurred an explosion in research characterizing the site of expression of LTP. However, due to indirect criteria and many problems applying quantal analysis to central synapses (see below), quantal studies on LTP have produced controversial conclusions about both pre- vs. postsynaptic expression of LTP (also see below).

1.1.4.3 Quantal analysis

The frog NMJ was established as the standard model for synaptic transmission by Katz and his colleagues (Katz, 1969). In this system, a number of critical assumptions were made in order to form a mathematical description of synaptic transmission.

(1) Neurotransmitter is prepackaged in discrete quantities of fixed size, called quanta or synaptic vesicles

(2) Each release site can release either zero or one quantum (binary release)

(3) Each quantum is released independently of the others

(4) The probability of release (P_r) is uniform at all release sites

(5) The postsynaptic response to each quantum is constant

Under these conditions, the distribution (binomial) of NMJ postsynaptic responses following presynaptic stimulation falls into a number of evenly spaced peaks (Boyd and Martin, 1956): the first peak at zero response represents failures and the next peak repre-

sents the unit potential, the smallest elicited response. In reality, each peak is slightly spread out in a Gaussian distribution, presumably reflecting the postsynaptic noise and the fact that the amount of transmitter in each quantum varies slightly and in a random fashion.

The above Katz theory can be extended further by assuming the following:

(6) There are a large number of release sites

(7) The probability of release at release sites is very low

With these assumptions, the binomial distribution of postsynaptic responses now approximates a Poisson distribution.

In general, the Katz theory remains an accurate description for neurotransmitter release at NMJ. The experimental verification of the predictions made in the theory provides strong support for the Katz stochastic view of neurotransmitter release (Stevens, 1993). However, due to the differences between NMJ and central synapses, the validity of using the Katz theory for central synapses and LTP studies has been strong challenged with most of above assumptions being questioned. In the following section I will summarize the differences between these two types of synapses.

1.1.4.4 Complexity of central synapses

Both physiological and anatomical studies have documented the heterogeneity of synapses in the hippocampus. Studies have shown that spontaneous miniature excitatory postsynaptic currents (mEPSCs) recorded in hippocampal CA1 neurons have a very broad and skewed distribution (Bekkers et al., 1990; Manabe et al., 1992). This high variability could result from the non-uniformity in the size of quantum (assumption #1),

as suggested by some studies (Stevens, 1993). Also possible is that mEPSCs are generated at different electrotonic distances from the soma. The other likely possibility is that synapses onto the same postsynaptic cell differ in their responsiveness to neurotransmitter (assumption #5) (Lisman and Harris, 1993).

Postsynaptic heterogeneity such as different spine shapes and volumes, and discrete aggregate of particles associated with the postsynaptic density (PSD) has been revealed by morphological studies of the central synapses using freeze-fracture EM and 3-D reconstruction from serial EM (Harris and Landis, 1986; Harris and Stevens, 1989). It is likely that synapses with different morphology and structural components have different synaptic efficacies.

Analysis of the progressive block of NMDA-receptor-mediated EPSCs by the irreversible open channel block MK-801 demonstrated that the release probability (assumption #4) from hippocampal neurons was non-uniform for terminals arising from single axon, with high P_r more likely to be affected by the activity-dependent modulation in LTP (Hessler et al., 1993; Rosenmund et al., 1993).

Tang et al. made an interesting observation that when two spontaneous miniature EPSCs (minis) are evoked in rapid succession, the amplitude of the second mini is smaller than that of the first, which suggests a high degree of receptor occupancy during the first mini (Tang et al., 1994). Many other studies have suggested that the degree of receptor occupancy can be affected by a number of factors such as the time course of transmitter clearance and the detailed anatomy of the synaptic cleft. In addition, as receptor occupancy probably varies different from one synapse to the next (Frerking and

Wilson, 1996), this certainly adds a degree of complexity to analyzing synaptic transmission in the context of synaptic plasticity.

Furthermore, both theoretical calculations and experimental evidence have suggested that the many transmitter molecules released by a single quantum nearly may saturate the postsynaptic receptors at central synapses (e.g., Redman, 1990; Larkman et al., 1991). The multi-peaked histograms of postsynaptic responses (see Liao et al., 1992) indicate that the postsynaptic response to a presynaptic action potential involves the linear summation of multiple quanta that presumably released at different synaptic sites between pre- and postsynaptic neurons.

Due to the above and other (for example, silent synapses, see below) complexities and many incorrect assumptions concerning the basic properties of synaptic transmission at central synapses, studies attempting to apply quantal analysis to synaptic transmission of central synapses have produced controversial conclusions which dance back and forth across the synapse (Korn and Faber, 1998; Korn and Faber, 1991; Lisman and Harris, 1993; Malinow, 1994; Stevens, 1993).

Attempts to avoid quantal analysis in the central synapses have thus been made. For example, analysis of the mEPSCs from CA1 pyramidal cells revealed an increase in amplitude after induction LTP, suggesting an increase in postsynaptic transmitter sensitivity (Manabe et al., 1992). Use of MK-801 failed to find change in the probability of transmitter release (P_r) (Manabe and Nicoll, 1994), also arguing for a pure postsynaptic modifications of LTP. However, under minimal stimulation conditions where weak near-threshold stimulation presumably activates only a few presynaptic fibers, changes in the fraction of failures but not the amplitude of the non-failure response were observed,

suggesting a pure presynaptic mechanism of LTP (Stevens and Wang, 1994). More recently, direct assessment of the presynaptic release of glutamate using glial cells was performed in hippocampal slices (Luscher et al., 1998). Glial cells respond to synaptically released glutamate by activation of AMPA-kainate type glutamate receptors (Linden, 1997) and by activation of electrogenic transporters (Sarantis et al., 1993; Bergles and Jahr, 1997); thus, these transporter currents may reflect the amount of transmitter released. Glial cell responses remained constant during LTP, arguing strongly for a postsynaptic expression mechanism for LTP (Luscher et al., 1998). These fundamentally different mechanisms suggested in these studies may reflect the complexities of central synapses as well as the different experimental conditions used in different laboratories such as animal ages and species, recording configurations and temperatures, methods of preparation.

1.1.4.5 Silent synapses

Silent synapses have recently been suggested to play a role in activity-dependent plasticity such as LTP (Isaac et al., 1995). The idea is that there is a rapid conversion of silent synapses to functional synapses following induction of LTP. Silent or ineffective synapses have long been found to exist in many systems such as spinal cord Crayfish NMJ. The idea that changes in silent synapses may contribute to LTP has also been long proposed (Voronin, 1983; Kullmann, 1994) but did not gain direct experimental support until recently. Note that synapses that are "functionally silent" can be attributed to either a failure of presynaptic terminal to release transmitter following an action potential (called "presynaptically silent") or lack of functional transmitter receptors in the postsynaptic cell (called "postsynaptically silent"). Hippocampal area CA3 synapses have been

found to be presynaptically silent and turned on during induction of mossy fiber LTP, which is NMDA receptor-independent (Tong et al., 1996). Silent synapses have also found to exist and play an important role in NMDA receptor-dependent LTP in CA1 region of hippocampus (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996). This is based on the observation that some synapses that yield no evoked EPSCs at resting membrane potentials exhibit AP5-sensitive EPSCs at depolarized potentials and thus contain NMDA receptors but no functional AMPA receptors. Furthermore, following pairing of low frequency stimulation with postsynaptic depolarization, previously undetected EPSCs become apparent. These data are consistent with the proposal that postsynaptically silent synapses are converted into synapses containing functional AMPA receptors. This mechanism may call for revisions of the interpretation for some LTP data such as an increase in mEPSC frequency and a decrease in synaptic failures previously attributed to presynaptic changes.

However, an alternative explanation is that the silent synapses are simply due to "spillover" of glutamate onto nearby synapses at a concentration that is able to activate NMDA receptors but not AMPA receptors (Kullmann et al., 1996; Asztely et al., 1997). Biochemically, NMDA receptors have a higher affinity for glutamate than AMPA receptors. This spillover explanation could account for the observation of small EPSCs mediated only by NMDA receptors. It requires, however, the existence of presumably presynaptically silent synapses to be converted into functional ones following LTP induction in order to explain the quick appearance of AMPA receptor-mediated EPSCs during the process. Interestingly, a recent imaging study in hippocampal CA1-CA3 neu-

ronal cultures showed that LTP involves the recruitment of pre-existing, presynaptically silent synapses (Ma et al., 1999).

One interesting finding is that the proportion of silent synapses in the hippocampus and some other systems tends to decrease during early postnatal development (Wu et al., 1996; Isaac et al., 1997). This is consistent with the long-held notion that similar mechanisms may be utilized both for the activity-dependent refinement of neural circuitry at early developmental stage and learning and memory formation at a later stage. This mechanism involves coordinated pre- and postsynaptic activity, LTP, and conversion of silent synapses into functional synapses.

1.1.4.6 Retrograde messengers

In summary, despite the controversial issue of the site of LTP expression, a general view is that there are at least some modifications of presynaptic cell, such as increase in transmitter release, together with postsynaptic changes. The involvement of presynaptic changes has led to the proposal that the postsynaptic cell must communicate back to the presynaptic terminals by producing and releasing a "retrograde" message, which diffuses across the synaptic cleft to the presynaptic terminal and initiates presynaptic changes associated with LTP. A handful of retrograde message candidates including arachidonic acid, nitric oxide (NO), carbon monoxide (CO), have been proposed and rigorously tested (Schuman and Madison, 1991; Bohme et al., 1991; O'Dell et al., 1991; Haley et al., 1992). Among these, evidence has been provided to suggest that NO may play an important role in LTP. LTP can be blocked by extracellular or intracellular application of NOS inhibitors or an extracellularly applied membrane-impermeant NO scavenger, consistent with the proposed action of NO in LTP (Schuman and Madison, 1991; O'Dell et al.,

1991). Biochemically, NO is produced from L-arginine by a Ca^{2+}/calmodulin dependent enzyme: nitric oxide synthase (NOS). Mice lacking neuronal NOS (nNOS) still exhibit NOS inhibitor-sensitive normal LTP, suggesting involvement of other NOS subtype(s) (O'Dell et al., 1994). Subsequent studies (Kantor et al., 1996; Arancio et al., 1996; Son et al., 1996) suggest that the membrane-associated endothelial subtype of NOS (eNOS) is necessary for LTP. Further, photoactivation of caged NO compound paired with a weak stimulation produced LTP in cultured hippocampal neurons (Arancio et al., 1996), supporting the role of retrograde messenger for NO in hippocampal LTP. Regarding the downstream effectors for the presynaptic changes, several candidates have been proposed. These include ADP-ribosyltransferase (Schuman et al., 1994) and PKG (Zhuo et al., 1994).

1.1.4.7 Diffusion of potentiation

The highly diffusible nature of some proposed retrograde messengers raises the possibility that such messengers may diffuse and influence the synapses of nearby neurons. Indeed, it was found that pairing-induced LTP in hippocampal slice cultures and LTP in hippocampal slices can spread to neighboring synapses that would otherwise exhibit no LTP (Bonhoeffer et al., 1989; Schuman and Madison, 1994). These studies challenged one of the basic properties of LTP – synapse specificity: only synapses undergo correlated pre- and postsynaptic activity express LTP. More recently, more evidence that LTP and LTD can spread to neighboring inactive synapses has been provided. Using a local perfusion method, researchers showed that LTP can diffuse to nearby synapses within a physical distance of ~ 70 μm (Engert and Bonhoeffer, 1997). This spread of plasticity occurs at synapses on the same postsynaptic neuron whereas the spread in earlier studies

occurs to synapses along the same axon. LTD was also found to spread over a distance and LTD in the neighboring synapses does not require NMDA receptor activation and postsynaptic Ca^{2+} influx (Scanziani et al., 1996). Moreover, LTD can spread more extensively in culture. This is suggested by studies of LTD spreading from triplets of neurons in dissociated hippocampal cell cultures (Fitzsimonds et al., 1997). The spread may utilize some unidentified intracellular messenger instead of an extracellular messenger. The proposed intracellular messenger can both travel back from axons to dendrites leading to LTP of synapses onto those dendrites (backward propagation) and travel along the axon to other synapses (lateral propagation) (Murthy, 1997).

1.1.5 Proposed role of cell adhesion molecules in LTP

It is known that multiple signaling pathways are utilized by synapses to sustain and modulate synaptic transmission. A number of studies devoted to examining the site of expression of LTP have suggested that certain local signaling molecules are necessary for hippocampal synapses to express LTP. I have discussed studies demonstrating the requirement for soluble retrograde messengers in synaptic plasticity in hippocampus (see above). Most signaling molecules are diffusible, and may thus potentiate the neighboring synapses within their diffusion domain during LTP induction. Moreover, distributed potentiation in nearby synapses depends on a number of factors that are local to the postsynaptic neuron (Schuman and Madison, 1994).

The spreading of LTP has challenged a long-held view for LTP, that is, the fundamental requirement for conjunctive activation of both pre- and postsynaptic cell. This input-specific property of LTP has been reported by numerous previous studies. The introduction of a requirement for local signaling molecules in synaptic plasticity may

serve to reconcile this controversy: local signaling molecules may take an active role in specifying the microdomain within which synapses undergo plasticity.

This idea has thus led to us to propose that cell adhesion molecules (CAMs) may act as local signaling molecules. CAMs, which are present in the synaptic cleft and plasma membranes, may play an important role in modulation of synaptic transmission and structural plasticity. In fact, in addition to mediating cell adhesion and maintenance of normal tissue architecture throughout the life of an adult organism, many of these cell adhesion molecules have well-characterized signal transduction machinery and are capable of transducing signals from the extracellular to the intracellular domain via second messenger pathways.

As shown schematically in Figure 1-2, synaptic cell adhesion molecules may participate in following aspects of activity-dependent synaptic modifications:

(1) Ultrastructural changes. One of the many consequences of activation of CAMs is the alteration in cytoskeleton structure and the strength of cell-cell interactions. Activation of CAMs may initiate signal transduction cascades and change the morphology of activated synapses such as size of synapses (diameters of dendrite spine, opposing area) as well as perforation of presynaptic terminals via dynamic changes in the cytoskeleton structures. Adhesive bonds in synaptic areas may undergo dynamic breakdown and reformation during these processes, thus assisting in synaptic modifications following induction of plasticity (Figure 1-2 D).

(2) Postsynaptic changes. Cell adhesion molecules may change the transmitter sensitivity of the postsynaptic membrane receptors by interacting their cytoplasmic domains with the biochemical network of protein kinases, phosphatases and other

enzymes that participate in the activity-dependent modifications of transmitter receptors with their cytoplasmic domains (Figure 1-2 B). A host of enzymes have been shown to interact with the cadherin family of CAMs and other family members (see below).

(3) Presynaptic changes. Synaptic cell adhesion molecules adhere pre- and postsynaptic neurons together and may hence provide a channel/bridge through which bidirectional communications between the two sides of neurons can be achieved by virtue of mechanical interactions or signal transduction of these CAMs. Additionally, CAMs may interact with retrograde messengers and/or growth factors involved in LTP, therefore providing additional controls over the degree of potentiation and the degree of LTP spreading. CAMs might also change presynaptic transmitter release by virtue of their signal transduction mechanisms (Figure 1-2 C).

Our hypothesis that cell adhesion molecules may modulate synaptic function has gained support from recent studies by other groups during the course of my thesis study (see Chapter 6): several immunoglobulin family members and integrins have been implicated in modulation of synaptic transmission. However, the role of cadherins, one major family of cell adhesion molecules, in modulating synaptic function and plasticity has not been thoroughly studied. The major part of this thesis has been centered on the role of cadherin family of cell adhesion molecules in hippocampal synaptic plasticity.

1.2 Cell Adhesion Molecules

A diverse system of cell adhesion molecules participates in orchestrating many vital biologic phenomena, for examples, embryogenesis, cell growth and differentiation, and wound repair. There are three major families of cell adhesion molecules that have been identified in the nervous system: the immunoglobulin superfamily (IgSF, Figure 1-3 B), the integrin family (Figure 1-3 C), and the cadherin superfamily (Figure 1-3 A). In brief, IgSF members mediate Ca^{2+}-independent cell adhesion in several different fashions (homophilic, heterophilic, and assisted heterophilic). Integrin family members form heterodimers and serve as receptors for extracellular matrix molecules (ECMs); they mediate Ca^{2+}-dependent cell-cell and cell-matrix interactions. Cadherin superfamily members mediate Ca^{2+}-dependent homophilic cell-cell interactions.

1.2.1 IgSF

Members of the immunoglobulin superfamily (IgSF) contain one or more copies of Ig-like domains in their extracellular domain. The Ig domain is 70-110 amino acids in length and usually has two cysteine residues approximately 55-75 residues apart. They can be fitted into one of four Ig sets: C1, C2, V and I set. Cell adhesion molecules of IgSF (Ig CAMs) are thought to be evolutionarily related by virtue of gene duplication and diversification (Hunkapiller and Hood, 1989). In the extracellular domain, most IgSF members also have fibronectin type III (FNIII) repeats, which are 90 amino acids long and structurally related to the domains found in fibronectin. The region between these two repeats is variable in structure due to alternative splicing. IgSF members either are integral membrane proteins or associate with the membrane via a glycosylphosphatidylinositol (GPI) anchor.

There are three types of adhesion mediated by IgSF members: homophilic, heterophilic or "assisted" homophilic (e.g., stable NCAM-L1 complex).

Neural cell adhesion molecule (N-CAM), the prototype of the IgSF, is expressed in multiple isoforms (180, 140, 120 kDa) due to alternative splicing. The extracellular part of all isoforms has 5 Ig domains upstream of 2 FNIII repeats. There is a specific glycosylation site for the unusual carbohydrate structure polysialic acid (PSA) that reduces the adhesiveness of N-CAM because of the high negative charges of the α2-8-linked N-acetylneuraminic acid units in PSA. L1, another well-studied IgSF member in the nervous system, has a molecular weight of 200-230 kDa and an extracellular domain consisting of 6 Ig-like domains and 5 FNIII repeats.

A wide range of mammalian neurons expresses many Ig CAMs. N-CAM and L1 are present on growth cones and neuritic shafts (Persohn and Schachner, 1987). Studies using different experimental paradigms have implicated a number of Ig CAMs including L1 and N-CAM in various aspects of the development of the nervous system, including axonal growth and guidance (for examples, Bixby et al., 1988; Lemmon et al., 1989; Drazba and Lemmon, 1990; Williams et al., 1992; Bastmeyer et al., 1995; Brittis et al., 1995). These experiments include direct examination of growth promoting activity by the molecule *in vitro*, perturbations by function-blocking antibodies, and investigation of the gain- and loss-of-function mutants (for a review, see Walsh and Doherty, 1997). The mechanisms underlying modulation of growth cone functions by Ig CAMs have also been studied. Tyrosine kinases and phosphatases of Ig CAMs have been suggested in the control of axonal growth and guidance. For example, it was found that soluble NCAM and L1-Fc chimeras can stimulate axonal growth responses from cerebellar neurons as

effectively as cell-expressed CAMs (Doherty et al., 1995; Saffell et al., 1997). This result suggests that these Ig CAMs can operate via signaling rather than by adhesion-based mechanisms. Further studies by Walsh and Doherty's group have indicated that a tyrosine kinase receptor (FGF receptor) is the key component in N-CAM, L1 and N-cadherin-mediated neurite outgrowth. The FGF receptor is activated following a *cis* interaction in the plane of the plasma membrane with N-CAM or L1. This leads to its binding to PLCγ, subsequent generation of DAG, which is converted to arachidonic acid (AA) by DAG lipase. AA likely acts on Ca^{2+} channels to produce a Ca^{2+} influx, which leads to down-stream events such as activation of CaM kinase, and the final neurite outgrowth responses (reviewed in Walsh and Doherty, 1997). Besides the FGF receptor, the non-receptor tyrosine kinases (some src family members), p90rsk serine/threonine kinase, and receptor-linked protein tyrosine phosphatases (RPTPs) are also implicated in interacting and affecting the functional status of Ig CAMs.

1.2.2 Integrins

The integrins are heterodimers and receptors of extracellular matrix molecules (ECMs); they mediate Ca^{2+}-dependent cell-cell and cell-matrix interactions. The integrins are integral membrane glycoproteins composed of two non-covalently associated subunits: α and β. There are about 16 known α subunits and 8 known β subunits. Heterodimerization of these subunits gives rise to about two dozen different integrins. Alternative splicing of these subunits adds further complexity. Integrins are major receptors by which cells attach to ECMs. Some integrins also mediate important cell-cell adhesion by binding to counter-receptors, leading to homo- or heterotypic aggregation. Integrin clustering by its ligands is important for the formation of focal adhesions where integrins connect to

cytoskeletal structure and actin filament bundles, activating intracellular responses. Integrin interactions are important in the regulation of many cellular functions, such as embryonic development, tumor cell growth, programmed cell death, leukocyte homing and activation, to name a few (reviewed in Clark and Brugge, 1995; Schwartz et al., 1995; Yamada and Miyamoto, 1995; Dedhar and Hannigan, 1996).

Most integrins are expressed on a wide variety of cells, and most cells express several integrins. Individual integrins can often bind to more than one ligand. Equally, individual ligands are often recognized by more than one integrin. Considerable efforts have been made in defining the integrin recognition site in the ligands and counter-receptors. The Arg-Gly-Asp (RGD) sequence that is present in many ECM proteins and recognized by some integrins represents one of those integrin-binding sites.

The cytoplasmic domain of the α and β subunits are coupled to cytoplasmic protein complexes containing cytoskeletal and catalytic signaling proteins. Studies have shown that β cytoplasmic domains are necessary and sufficient to target integrins to focal adhesions, independent of ligands, while the α cytoplasmic domains regulate the specificity of the ligand-dependent interactions (Kassner et al., 1994; Laflamme et al., 1994).

Two important features of integrins are that they exist in active and inactive states and that there are two signaling directions. An activated cell can transmit a signal from its cytoplasmic domain and alter the conformation of the extracellular domains of integrins on the cell membrane, thus changing the affinity of the integrins for their ligands and regulating the integrin function (reviewed in Schwartz et al., 1995; Dedhar and Hannigan, 1996). This "inside-out" signaling occurs, for example, when leukocytes are stimulated by bacterial peptides, and it rapidly increases the affinity of the leukocyte integrins for

members of the immunoglobulin family. The regions within integrin cytoplasmic domains involved in regulating inside-out signaling have recently been identified (e.g., Hughes et al., 1996). Moreover, a number of intracellular proteins such as calreticulin, serine/threonine kinases and phosphatases, and members of the small GTPase family have been implicated in this regulation (Hotchin and Hall, 1995; Schwartz et al., 1995; Zhang et al., 1996).

"Outside-in" signaling occurs following the binding of an integrin to its ligand (ECM components) and affects many fundamental cellular processes. These include cell survival and proliferation, cellular differentiation, morphogenesis and cell migration (Clark and Brugge, 1995; Roskelley et al., 1995; Yamada and Miyamoto, 1995). The attempt to understand the molecular basis of integrin-mediated "outside-in" signal transduction is an area of intense study. Integrin signaling requires ligand binding and GTPase Rho A-dependent integrin clustering. The activity of Rho A is also regulated by other growth factor receptors. Integrin occupation and clustering, together with the activity of Rho A results in the spatial organization of the focal adhesion plaque, a large complex of intracellular proteins composed of cytoskeletal and signaling molecules such as α-actinin, Src and cadherin-associated substrate (CAS). Phosphorylation of focal adhesion kinase (FAK) occurs following integrin occupation and clustering, and causes association of phosphorylated FAK with Grb-2 and subsequent activation of Ras by SOS. One of the consequences is the activation of MAPK and subsequent activation of cell cycle and regulation of gene expression. Ras may also activate p190 Rho-GAP, resulting in Rho A inactivation and presumably turning off the integrin-initiated signal transduction (see Dedhar and Hannigan, 1996 for more details).

The ECM consists of a mixture of macromolecules that are abundant in the CNS and PNS in spatial and temporal distribution patterns (Hay, 1991). Many *in vivo* and *in vitro* studies have identified the function of ECM in regulation of cell migration, growth, and differentiation. The approaches involved in these studies including injecting function-blocking antibodies into embryos or tissue slices, synthetic peptides mimicking individual structural domains of ECM proteins, and gene-knockout strategies (reviewed in Letourneau et al., 1994). Structurally, many ECM components are modular or mosaic proteins, composed of several polypeptide domains that can be differentially assembled as a consequence of alternative mRNA splicing.

The ECM can be grouped loosely into fiber-forming elements (such as collagen and elastin), glycoproteins (such as fibronectin, laminin, thrombospondin, and tenascin), and proteoglycans (PGs, such as aggrecan and syndecan, Figure 1-3 D) (Scott-Burden, 1994). Proteoglycans are a special class of glycoproteins that have a high content of glycosaminoglycans (GAGs). GAGs are highly negatively charged due to carboxyl and sulfate groups along the chain. There are four major groups of GAGs: hyaluronan, chondroitin sulfate, heparan sulfate, and keratan sulfate (Figure 1-3 D). PGs are present not only as ECM components, but also in cell surface, membrane-spanning, soluble, and intracellular forms. A diverse set of PGs is expressed in the rat CNS and is temporally and spatially regulated during development (Herndon and Lander, 1990). Interestingly, glypican, a GPI-anchored heparan sulfate proteoglycan, is predominantly expressed in the late embryonic and postnatal rat CNS including pyramidal cells in the hippocampus (Karthikeyan et al., 1994; Litwack et al., 1994). PGs have been implicated in the regulation of cell morphology, adhesion, migration, proliferation, differentiation, neuronal

polarity, growth factor binding and presentation, signal transduction, synapse stabilization, and neurological disorders (for a review, see Letourneau et al., 1994).

1.2.3 Cadherins

The cadherins are homophilic Ca^{2+}-dependent adhesion molecules that consist of an extracellular domain, a transmembrane domain and a cytoplasmic domain (an exception is truncated- (T-) cadherin, which associates with cell membrane via GPI anchor). Currently, about 40 cadherin and cadherin-related proteins have been reported in the brain. The cadherin superfamily can be divided up into at least four subclasses based upon their molecular structure:

(1) The classic cadherins. This class is defined by their highly conserved cytoplasmic domain that associates with catenins and $p120^{CAS}$. They are type I transmembrane glycoproteins. Three well-studied subtypes of the classic cadherins are neural- (N-, also called A-CAM), epithelial- (E-, also called uvomorulin for mouse), and placental- (P-) cadherins.

(2) The protocadherins. These cadherins represent the first non-classic subclass to be identified from vertebrate CNS using PCR (Sano et al., 1993). Their amino acid sequences are highly homologous to the sequences of the extracellular domains, but not the cytoplasmic domains, of the classic cadherins. Experiments using transfection of non-adherent mouse fibroblasts (L cells) or ectopic expression in embryos have shown that the protocadherins also exhibit cell adhesive properties (Bradley et al., 1998; Sano et al., 1993), and that the adhesion activity of the protocadherins requires unidentified cytoplasmic cofactors other than catenins (Bradley et al., 1998).

(3) The desmosomal cadherins. These N-glycosylated, type I transmembrane proteins are present in desmosome, the disc-shaped intercellular junctions in epithelia and cardiac muscle. The major desmosomal cadherins are desmocollins and desmogleins, each of which have 3 distinct isoforms with differential distribution between and within epithelia. They are linked to the intermediate filament cytoskeletal network (cytokeratins in epithelia and desmin filaments in heart) by several cytoplasmic plaque proteins, including the desmoplakins and plakoglobin. The desmosomal cadherins have high homology with the classic cadherins in their extracellular domains whereas their cytoplasmic domains are of heterogeneous sizes due to alternative splicing. They are believed to play a regulatory role in epithelial morphogenesis and differentiation (Koch and Franke, 1994; Garrod et al., 1996). Autoantibodies against some of these cadherins are causes of several autoimmune blistering diseases (Stanley, 1995).

(4) The numbered cadherins. These cadherins (for examples, cadherin-5 through cadherin-12) were isolated using PCR (Suzuki et al., 1991). They show low homology with classic cadherins although the overall structure of them is similar to that of classic cadherins.

(5) Other cadherin-related proteins. These include the proto-oncogene *ret* (a receptor tyrosine kinase) (Schneider, 1992), the *Drosophila* tumor suppressor gene *fat* (Mahoney et al., 1991), rat LI-cadherin (Berndorff et al., 1994), human HPT-1 antigen (Dantzig et al., 1994), and the recently identified cadherin-related neuronal receptor (CNR, Kohmura et al., 1998).

The following is a review of the known structure and function of cadherins, with an emphasis on classic cadherins.

1.2.3.1 General structure

The classic cadherins are a family of glycoproteins with a molecular weight of 120-130kDa. They have been identified in a variety of organisms, including mammals, *Xenopus*, *Drosophila* and *C. elegans*. Cadherins span many different intercellular junctions by forming Ca^{2+}-dependent homophilic bonds and participate in a variety of morphogenetic events in various developing tissues (Takeichi, 1990; Takeichi, 1991; Kemler, 1992; Gumbiner, 1996). The intercellular junctions where cadherins are concentrated are termed adherens junction, which include the familiar zonula adherens junctions found in epithelia.

There are about 20 subtypes of the classic cadherins identified within a single vertebrate species. The mature protein consists of 723-748 amino acids. The classic cadherins from different species are very similar in their overall primary structure. Amino acid sequences are conserved among the various cadherins with homologies in the range of 43-65% (Dalseg et al., 1993), except between chicken N- and Retinal- (R-) cadherin, where the similarity is as high as 74% (Inuzuka et al., 1991). A comparison of the domain similarities between some of the cadherins within or across species is shown in Appendix B.

1.2.3.1.1 Extracellular domain

The extracellular domain of classic cadherins can be divided into five internal repeats (EC1 through EC5, the most distal repeat is EC1), each of which is about 110 amino acids long. EC1 and EC2 have the highest degree of internal homology (Figure 1-3 A). Several putative N-linked glycosylation sites are found, but they are generally not con-

served among the cadherins (Takeichi, 1988) and the sugar moieties are not involved in the adhesion (Shirayoshi et al., 1986). The number of the extracellular repeats varies from cadherin subclass to subclass: classic and numbered cadherins have 4-5 repeats; proto-cadherins have 6-7 repeats; LI-cadherin has 6 repeats; and the *Drosophila* cadherin *fat* has as many as 34 repeats.

One important feature of the cadherin-cadherin bond is its subtype specificity: a cadherin molecule of one type binds preferentially to a like one at cell-cell boundaries. Extensive cell aggregation experiments have shown that transfected cells expressing exogenous cadherins of different subtypes sort out and aggregate depending on cadherin types and amount (e.g., Nagafuchi et al., 1987; Miyatani et al., 1989). The binding specificities of cadherin types seem to be conserved between species. Instances of heterophilic binding have also been reported (Volk et al., 1987; Inuzuka et al., 1991; Cepek et al., 1994; Karecla et al., 1996); however, homophilic binding is stronger and preferred.

The homophilic binding specificity of cadherins is determined by the EC1 domain: chimeric P- (EC1) and E-cadherins have been shown to bind to P-cadherin, not E-cadherin (Nose et al., 1990). Furthermore, site-directed mutations in the His-Ala-Val (HAV) sequence and its flanking amino acids of the EC1 repeat (Figure 1-3 A) result in loss of cadherin binding specificity (Nose et al., 1990). The HAV sequence in the EC1 repeat is highly conserved among the classic cadherins (Blaschuk et al., 1990; Blaschuk et al., 1990). Synthetic peptides containing HAV inhibit cadherin-mediated cell adhesion and cadherin-dependent processes including mouse embryo compaction, neurite out-growth, and osteoclast formation (Blaschuk et al., 1990; Blaschuk et al., 1990; Doherty et al., 1991; Mbalaviele et al., 1995; Willems et al., 1995). The N-terminal region in E-, N-,

and P-cadherin also harbors the epitopes of the adhesion-blocking antibodies (Nose et al., 1990). It is interesting to note that FGF-receptor (Williams et al., 1994) and extracellular super-oxide dismutase (Willems et al., 1995) also contain an "HAV" motif and may interact directly with cadherins. It has been speculated that the HAV motif may represent an evolutionarily conserved amino acid sequence functionally important in a wide variety of proteins (Byers et al., 1992).

The adhesive function of the classic cadherins is strongly dependent upon extracellular Ca^{2+} (Hyafil et al., 1981; Ringwald et al., 1987; Ozawa et al., 1990). First of all, E-cadherin expresses its adhesive function only in the presence of Ca^{2+}; Ca^{2+} protects E-cadherin from protease digestion (Hyafil et al., 1981). Certain monoclonal antibodies recognize E-cadherin only in the presence of Ca^{2+} suggesting Ca^{2+}-dependent conformation changes of E-cadherin (Hyafil et al., 1981; Vestweber and Kemler, 1985). Ringwald et al. have demonstrated direct binding of Ca^{2+} to E-cadherin and identified two sequence motifs, DQNDN and DADDD, at each tandem EC repeat with putative Ca^{2+}-binding properties (Ringwald et al., 1987). A synthetic peptide containing DADDD was found to bind Ca^{2+}, but a mutated version of this peptide (KADDD) lost its Ca^{2+}-binding activity (Ozawa et al., 1990). Moreover, mutated E-cadherin protein (with the same D→K mutation) are more susceptible to degradation and incapable of adhesive binding (Ozawa et al., 1990). Sequence comparisons and structure studies of EC1 repeat of N- and E-cadherin suggest that the Ca^{2+}-binding sites are between adjacent repeats. These Ca^{2+}-binding sites may stabilize the interface between successive EC repeats to give a stiff, rod-like molecule rather than direct participating in the intercellular adhesive interface (Overduin et al., 1995; Shapiro et al., 1995; Nagar et al., 1996). An electron-

microscopical analysis suggested that upon Ca^{2+} depletion, E-cadherin reversibly changes its conformation from a rod-like structure to a more globular assembly of the five subdomains (Pokutta et al., 1994).

1.2.3.1.2 Cytoplasmic domain and catenins

The cytoplasmic (C-terminus) domain of the classic cadherins is about 160 amino acids. This domain is highly conserved among different cadherin subtypes. It connects to cytoskeletal filaments via catenins, which are members of the armadillo family of proteins; this family includes the armadillo protein in *Drosophila*, and β-catenin, γ-catenin (plakoglobin) and $p120^{CAS}$ in vertebrates (see below, Figure 1-3 A). Linkage of cadherins to the cytoskeleton via catenins is a key contributor to stable cell adhesion.

(1) α-catenin: α-catenin is homologous to vinculin (Nagafuchi et al., 1991), and links to actin either directly, or through β-catenin (Knudsen et al., 1995; Rimm et al., 1995). α-catenin does not bind to cadherin directly (Jou et al., 1995). It is suggested that α-catenin turnover is stabilized by cadherin molecules because α-catenin protein was shown to be greatly enhanced after transfection of L cells with cadherin cDNAs but not with mutant cadherin cDNAs lacking the catenin-binding site (Nagafuchi et al., 1991).

(2) β-catenin: β-catenin was first isolated as a 92 kDa protein associated with the cytoplasmic domain of E-cadherin in adherens junction (McCrea and Gumbiner, 1991; McCrea et al., 1991). It directly associates with the cadherin cytoplasmic domain and with α-catenin. It is homologous to the *Drosophila* segment polarity gene *armadillo*. X-ray crystallography of β-catenin suggested that 12 copies of armadillo repeats in the conserved central "core" region form a superhelix of helices with a positively charged

groove for cadherins, T-cell factors (TCFs), and Adenomatous Polyposis Coli (APC) to interact (Huber et al., 1997).

β-catenin is a multi-functional protein that can affect both cell adhesion and gene expression (reviews, Hinck et al., 1994; Barth et al., 1997). It is a key mediator between cadherins, APC protein and different signal transduction pathways. The key evidence can be summarized as follows.

i) β-catenin is subject to dynamic regulation by tyrosine kinases and phosphatases. Phosphorylation of β-catenin on tyrosine residues plays a role in controlling cadherin-catenin association, and therefore, cadherin function (Lilien et al., 1997). Increased tyrosine phosphorylation in v-src transformed cells correlates with decreased cell-cell adhesion (Matsuyoshi et al., 1992; Behrens et al., 1993; Hamaguchi et al., 1993). Activation of EGF also leads to the similar effects (Hoschuetzky et al., 1994). β-catenin is found to bind to the EGF receptor and this binding is mediated by the conserved central "core' region of β-catenin (Hoschuetzky et al., 1994). Moreover, protein tyrosine phosphatases (PTPs) have been found to bind to β-catenin, which may counteract the activity of tyrosine kinases in regulating adhesion (Bradykalnay et al., 1995; Fuchs et al., 1996; Kypta et al., 1996; Cheng et al., 1997). For example, when PTP1B is phosphorylated, it binds to cytoplasmic domain of N-cadherin and removes tyrosine-bound phosphate residues from β-catenin, thus maintaining the cadherin-actin connection (Balsamo et al., 1996; Balsamo et al., 1998).

ii) β-catenin and armadillo are components of the Wnt/Wingless pathway, which is important for cell-fate decisions and pattern formation during development (Peifer, 1995; Miller and Moon, 1996). Many studies have suggested the following model of β-catenin

regulation in the Wnt pathway: when a Wnt protein binds to its cognate receptor, β-catenin fails to be phosphorylated and thus prevents itself from entering the ubiquitin-proteasome pathway. Instead, unphosphorylated β-catenin accumulates in the cytoplasm, enters the nucleus and interacts with TCF. This interaction alleviates TCF's repression of the downstream genes and provides a transcriptional activation domain (for a review, see Barth et al., 1997).

iii) β-catenin may mediate signals between cadherins and the Wnt/Wingless signaling pathways. It seems that β-catenin's role in cell adhesion and gene expression are separable as *in vivo* mutational analysis of armadillo showed that β-catenin mutants defective in cell adhesion are still capable of transducing a Wnt signal (Orsulic and Peifer, 1996). However, overexpression of cadherins antagonizes the signaling function of β-catenin (Fagotto et al., 1996; Sanson et al., 1996; Torres et al., 1996), suggesting the interaction between cadherin-mediated cell adhesion and Wnt/Wingless signaling.

iv) Mutations in β-catenin and APC protein result in constitutive activation of β-catenin signaling and are correlated with cancer formation (Korinek et al., 1997; Morin et al., 1997; Rubinfeld et al., 1997).

v) β-catenin may regulate the function of APC in organizing the microtubule cytoskeleton. In addition to binding β-catenin, plakoglobin, and the Discs Large tumor suppressor protein, APC interacts with microtubules in the C-terminus. APC has been suggested to stabilize microtubules and the formation of membrane protrusions (Nathke et al., 1996). Deletion of the amino terminus of β-catenin results in increased stability of β-catenin in APC protein clusters and decreased APC-dependent microtubule stabilization and formation of membrane extensions (Munemitsu et al., 1996; Pollack et al., 1997).

(3) γ-catenin: γ-catenin is identical to plakoglobin (Knudsen and Wheelock, 1992), and may associate with FGF receptors. γ-catenin interacts with both classic and desmosomal cadherins and may play a role in the sorting of desmosomal and adherens junction components.

(4) $p120^{CAS}$: $p120^{CAS}$ is a tyrosine kinase src substrate and binds directly to E-cadherin in both the E-cadherin-β-catenin and the E-cadherin-γ-catenin complexes but not to APC or α-catenin (Daniel and Reynolds, 1995). It may play a role in the regulation of cadherin-mediated adhesion by these signaling pathways (Aberle et al., 1996).

1.2.3.2 Cadherin interactions and the junctional complex

Cadherin-mediated cell adhesion is one of the most important and ubiquitous types of adhesion required for the maintenance of solid tissues. The structural basis of the cadherin-mediated homotypic adhesion interactions has become very important for our understanding of cadherin function. Over the past several years, the solution NMR structure of the N-terminal domain of E-cadherin (ECD1) and the crystal structure of two N-terminal extracellular domains of E-cadherin (ECD12) have been reported (Overduin et al., 1995; Nagar et al., 1996). The crystal structure of the N-terminal domain of N-cadherin (NCD1) has also been studied (Shapiro et al., 1995).

Shapiro et al. identified two dimer interfaces in murine NCD1 structure: the "strand" dimer and "adhesion" dimer interfaces (Figure 1-4, Shapiro et al., 1995). The twofold symmetrical strand dimer interface is formed by parallel alignment of two protomers from the same cell surface. This dimerization involves ~1,800 $Å^2$ surface area and mutual insertion of the side chain of Trp 2 into a hydrophobic pocket of the partner protomer

across the interface. This conserved strand dimer between NCD1 domains is likely also adopted by all other NCD extracellular domains, and thus contributes to lateral interaction of entire cadherin extracellular domain. On the other hand, the approximately two-fold symmetrical adhesion interface is formed by the anti-parallel alignment of protomers from opposing cell surfaces. It involves ~3,300 $Å^2$ surface area and the well-known HAV sequence. It is suggested that the adhesion dimer interface is weaker than the strand dimer interface as the water-mediated, long-range interactions in the adhesion dimer may be weaker than close-packed hydrophobic interactions in the strand dimer (Shapiro et al., 1995). Therefore, clustering many low affinity adhesion sites may be required to increase the effective affinity of the cadherin-cadherin interaction.

The structure of ECD12 is similar to NCD1 in many aspects such as a common tendency for a parallel alignment of the β barrels of two EC1 domains. However, a number of important questions and debates remain as to the dimerization details, the contributions of Ca^{2+} ions, and the overall architecture of the extracellular domains (Jones, 1996). Most notably, studies of the ECD1 and ECD12 structure have limited E-cadherin domain dimerization to only ECD1 domain (Nagar et al., 1996; Tomschy et al., 1996). It is thus necessary to further the structural studies on additional fragments of cadherin extracellular region in order to resolve whether there is any difference in the structural architecture of N- and E-cadherins. It is also unclear whether the packing arrangement of ECD12 is in the mode of adhesive interaction. Nonetheless, the ECD12 dimer can be incorporated within the proposed ribbon model for the NCD1 adhesion interface in that the interaction is mediated by many water molecules along the large surface area (1,500 $Å^2$). Moreover,

the conserved HAV sequence is located on the F strand of the N-terminal domain of both N- and E-cadherin, and adopts a similar conformation in both cadherin subtypes.

A recent study by Tamura et al. raises that possibility that classic cadherins stably exist in both dimeric and monomeric forms, and suggests that lateral dimerization is required for adhesive function (Tamura et al., 1998). This is based on their observation that adhesion is completely abolished by mutating residues located in the strand dimer interface including Trp 2. It is thus proposed that a monomeric form may be inactive for cell adhesion and the *cis* dimerization may be a key regulatory step to produce an active conformation. This notion is supported by an earlier study using recombinant extracellular domain of E-cadherin, where the *cis* pairing of EC1 domains takes place first, followed by *trans* interaction (Tomschy et al., 1996). In addition, it is found that cytoplasmic clustering as well as lateral interaction of EC1 contributes to the strengthening of adhesion (Yap et al., 1998).

It is interesting to note that the cadherin folding topology is similar to Ig-like domains and other "Greek key" β-sheet structures (Vaughn and Bjorkman, 1996). However, sequence similarities between cadherins and these other molecules are very low, and intron patterns are different. It is thus more likely that they come from an independent origin for a favorable folding topology rather than being evolutionarily divergent from a common ancestor (Shapiro et al., 1995).

The cadherins are the major adhesion receptors of various intercellular junctions, for example, the zonula adherens junctions of epithelia, where they colocalize with a prominent actin filament bundle. The zonula junction provides epithelia with strong contractile or mechanical forces for epithelial physiology or morphogenesis. However, cadherins

may also diffusely be distributed over the cell surface and yet mediate robust cell-cell adhesion (Gumbiner, 1996). The junctional localization of cadherins may represent stronger points of intercellular adhesion.

1.2.3.3 Cadherin clustering, adhesion, and regulation

Cadherin lateral dimerization and clustering may be necessary for cell adhesion. This notion has been supported by a number of studies *in vitro* (Adams et al., 1996; Yap et al., 1998) and *in vivo* (Yap et al., 1997). It was found that cells expressing mutant cadherins with the cytoplasmic juxtamembrane region exhibiting cadherin clustering activity, but without the β-catenin binding domain, showed significant cell-cell adhesion (Yap et al., 1998). This indicates that clustering of the cadherins may be sufficient for cell-cell adhesion. However, interactions of cadherin cytoplasmic domain with other intracellular proteins may also be necessary for lateral clustering of cadherins *in vivo*. For example, it has been known that disruption of cadherin binding to β-catenin causes decreased cell-cell adhesion (Chen et al., 1997). A recent study, however, suggested that binding of cadherin to $p120^{CAS}$, but not β-catenin, is necessary for cadherin clustering (Yap et al., 1997).

It is known that β-catenin links cadherin to the actin cytoskeleton via α-catenin. This linkage is believed to be important for regulating cadherin-mediated cell adhesion, as cell adhesion may be inhibited by a disruption of α-catenin binding to the cadherin-catenin complex (Gumbiner, 1996) or β-catenin binding to cadherin (Chen et al., 1997). In general, the catenin-cadherin complexes, catenin-APC complexes, and pools of free catenins exist in dynamic equilibrium within cells (Su et al., 1993; Rubinfeld et al., 1993; Hinck et

al., 1994; Nathke et al., 1994; Rubinfeld et al., 1995). Shifting this equilibrium due to changes in expression levels of cadherins and catenins have been shown to affect cell adhesion (Nose et al., 1988; Friedlander et al., 1989; Steinberg and Takeichi, 1994; Barth et al., 1997). Moreover, it was found that ectopic expression of dominant-negative *Xenopus* N-cadΔ, a mutant form of N-cadherin lacking most of its extracellular sequence, inhibits cell adhesion presumably by inhibition of the association of α-catenin to endogenous E-cadherin (Kintner, 1992). In adult chimeric mouse, the perturbation of cell adhesion by this mutant N-cadΔ causes an inflammatory bowel disease associated with changes in the amounts and intracellular distributions of β-catenin and E-cadherin in the intestinal epithelium (Hermiston and Gordon, 1995; Hermiston and Gordon, 1995). Taken together, shifting the equilibrium of free and bound cadherins and catenins can affect cell adhesion and functions such as morphogenesis and tumorigenesis.

Short-range and long-range diffusion and clustering of cadherins has been suggested to immobilize cadherin-catenin complexes within a cell-cell contact (Adams and Nelson, 1998). It is proposed that freely diffusing cadherin may associate with initially immobilized cadherin or other cytoplasmic proteins such as catenins or actin and therefore strengthen cell adhesion by increasing the local cadherin concentration at the contact. This is supported by the observation of homogeneous distribution of E-cadherin over the plasma membrane prior to cell-cell contact and accumulated E-cadherin within the contact after initiation of intercellular adhesion (McNeill et al., 1993; Adams et al., 1996). The distribution and assembly kinetics of cadherins and catenins at newly formed contact sites have also been analyzed in Madin-Darby canine kidney (MDCK) cells. During the initial stages of cell-cell adhesion, E-cadherin, α-, and β-catenin exist as uniformly sized

and spaced punctate clusters along the length of a cell-cell contact. Each of these puncta is associated with actin filaments. These discrete puncta are assembled along the contact at a constant average density of ~1 punctum per 1.5 μm of contact length (Adams et al., 1996). Diffusion of cadherin within the plasma membrane has been studied using single-particle-tracking and optical tweezers techniques (Sako et al., 1998). It was found that a small proportion of E-cadherin is attached to the cytoskeleton without cell-cell contacts. These E-cadherins may play a role in initiating the immobilization of E-cadherin at a developing contact site.

1.2.3.4 Cadherin expression and function in animal morphogenesis

To date, more than 20 subtypes of the classic cadherins have been identified within a single vertebrate species, most of which are expressed in the vertebrate CNS.

Each subtype of the classic cadherins has its unique spatial and temporal expression patterns. N-cadherin was originally characterized in the brain at the embryonic stage but is also found in cardiac muscle and lens cells (Hatta and Takeichi, 1986). E-cadherin is most abundant in liver, kidney, lung, intestine, and most epithelia (e.g., Damsky et al., 1983). E-cadherin's expression in the nervous system was described to be restricted to a subset of cells in sensory ganglia and spinal cord (Takeichi et al., 1990). P-cadherin was originally discovered in large amounts in mouse plancenta; it is not expressed in the nervous system (Nose and Takeichi, 1986; Shimoyama et al., 1989). R-cadherin was first described in chicken retina, but has later been found in several areas of the brain and spinal cord (Inuzuka et al., 1991; Inuzuka et al., 1991). T-cadherin is expressed in embryonic chicken brain and heart (Ranscht and Bronnerfraser, 1991; Ranscht and Dourszimmermann, 1991).

Extensive studies have been done on the developmental roles of cadherins in animal morphogenesis including studies of embryo compaction, cell migration, cell aggregation, segregation, neurite extension, axon pathfinding, and more recently, synaptogenesis (Takeichi, 1995). For example, studies using gene targeting approaches have indicated a critical role of cadherins in basic morphogenetic events in development. Mouse embryos homozygous for E-cadherin deletions die around the time of implantation. At the blastocyst stage, these embryos fail to form a trophectodermal epithelium or a blastocyst cavity (Larue et al., 1994). The N-cadherin homozygous mutant embryos die by day 10 of gestation. The mesodermal and endodermal cell layers of the yolk sac are separated in the N-cadherin mutants; myocytes dissociate and the heart tube fails to develop normally (Radice et al., 1997).

1.2.3.5 Cadherin in tumorigenesis

One of the early steps of cancer metastasis is the detachment of cells from the primary tumor mass. Many studies have suggested that cadherins (classic cadherins such as E- and P-cadherins, and the desmosomal cadherins) are involved in this process. It has been proposed that when cadherins lose their activity either due to suppression of cadherin gene expression or to a loss of function of cadherin protein, cadherin-expressing cells become less adherent to other cells facilitating their detachment from their parent colonies. In support of this, it has been observed that most human carcinomas have down-regulated cadherin expression and/or cadherin-mediated cell adhesion (Oka et al., 1993). To examine the correlation between loss of cadherin function and tumor progression, manipulation of cadherin expression has been performed and interesting results have been obtained. For example, maintaining E-cadherin expression in β-tumor cells results

in arrest of tumor expression at the adenoma stage, whereas expression of a dominant-negative form of E-cadherin induces early invasion and metastasis (Perl et al., 1998). This observation suggests that loss of E-cadherin-mediated cell adhesion is one rate-limiting step in the progression from well-differentiated adenoma to invasive carcinoma.

Since cadherins connect to cytoskeletal structures via catenins, perturbations of this connection may also affect cadherin function. Injection of mRNA encoding the N-cadherin cytoplasmic domain (dominant-negative) into *Xenopus* embryos causes segregation of cells in some tissues (Kintner, 1992) whereas exogenously expressed mutated non-functional N-cadherin (cN390Δ) disrupts endogeneous epithelial cell-cell adhesion (Fujimori and Takeichi, 1993). The phosphorylation of catenins on tyrosine residues has also been the focus of study. The adherens junction and N-cadherin largely disappear in v-src transformed chick lens cells (Volberg et al., 1991). Phosphorylation of catenins causes inactivation of cadherin-mediated cell aggregation (Matsuyoshi et al., 1992; Hamaguchi et al., 1993) or loss of epithelial differentiation and gain of invasiveness in MDCK cells (Behrens et al., 1993).

1.2.3.6 Cadherin in the formation of neural network in the CNS

The dynamic rearrangement of cells and wiring of neurons is required to form complex networks in the developing brain. For examples, the neural tube and epidermis need to be physically separated into two different domains during neural development; cell migration and clustering occur in differentiating neuroblasts to form specific "nuclei" or "layers"; axons undergo fasciculation before they migrate together, but later defasciculation upon reaching their targets; neurons need to make specialized synaptic connections

with their target cells. Cell adhesion molecules are believed to provide a mechanism to regulate these complex cell behaviors.

Cell lineage or migration is confined to the boundaries of neuromeres in the early neural tube. Takeichi and his colleagues found that a number of cadherins are expressed in restricted compartments or in their boundary regions (Espeseth et al., 1995; Redies and Takeichi, 1996). This provides indirect support for the idea that cadherin-mediated cell sorting may play a role in compartmentalization of early brains. Cadherin adhesion mechanisms may also be utilized by developing axons for pathfinding, migration and fasciculation. A genetic study on the *Drosophila* N-cadherin gene showed that null mutants for this gene displayed reduced or altered interactions between axon fascicles as well as misorientation of growth cones (Iwai et al., 1997).

More recently, the role of cadherins in synapse formation has become one of the actively researched areas in developmental neurobiology. Sanes and his colleagues studied synapse formation in developing chick optic tectum and found that N-cadherin and certain cell surface glycoconjugates are selectively associated with different "retinorecipient" laminae as synapses form (Yamagata et al., 1995). A function-blocking antibody to N-cadherin perturbed laminar selectivity (Inoue and Sanes, 1997).

In mammals, an earlier biochemical study suggested that N-cadherin is a major component of rat forebrain postsynaptic densities (Beesley et al., 1995). Using an adult mouse brain preparation, Uchida et al. found that α-catenin has a symmetrical distribution pattern over the pre- and post-synaptic membranes bordering the active zone and is absent in the transmitter release zones (Uchida et al., 1996). When N-cadherin distribution was examined in chick midbrain, N-cadherin was only synaptically localized to a subset of

neurons, suggesting that other cadherin types are expressed in the N-cadherin-negative neurons (Takeichi et al., 1997). A recent immunohistochemical study on the expression of N- and E-cadherin in mouse cerebellar synapses suggested that N- and E-cadherin are localized to mutually exclusive synapses (Fannon and Colman, 1996). This led Fannon et al. to propose a model in which the differential distribution of cadherins along the axonal and dendritic plasma membranes, and ultimately cadherin self-association, "locks in" nascent synaptic connections once neurites have been guided to the vicinity of their cognate targets. There are a number of other studies that illustrate that postnatal neurons express different cadherin subtypes in a complex mosaic pattern, with each cadherin subtype correlated with a pattern of known neuronal circuitry. Benson et al. used cultured hippocampal neurons as a model system and found that N-cadherin and β-catenin are present in axons and dendrites before synapse formation and then cluster at developing synapses between hippocampal neurons (Benson and Tanaka, 1998). More interestingly, N-cadherin was found to be expressed initially at all synaptic sites but rapidly becomes restricted to a subpopulation of excitatory synaptic sites. This suggests that N-cadherin is involved in stabilization of early synapses but these synapses may be functionally and spatially modeled to express a different cadherin at a later time. Take together, these studies suggest that the selective adhesion of cadherins may provide a mechanism by which presynaptic neurons connect with postsynaptic partners and thus form a functional neural network (Redies, 1997; Takeichi et al., 1997).

Most, if not all, studies on cadherin function have been centered on its role in animal morphogenetic events such as segregation and aggregation of tissues at early develop-

mental stages, in correct neural cell connections during development, and in invasion and metastasis of tumor cell lines, and more recently, in synapse formation and formation of functional neuron circuitry. This thesis focuses on the role of cadherins in modulation of synaptic transmission and activity-dependent synaptic plasticity in adult brain. Expression of cadherins in adult hippocampus, especially at synaptic sites, was first examined in this thesis. The role of synaptic cadherins in plasticity was then studied using a number of different approaches.

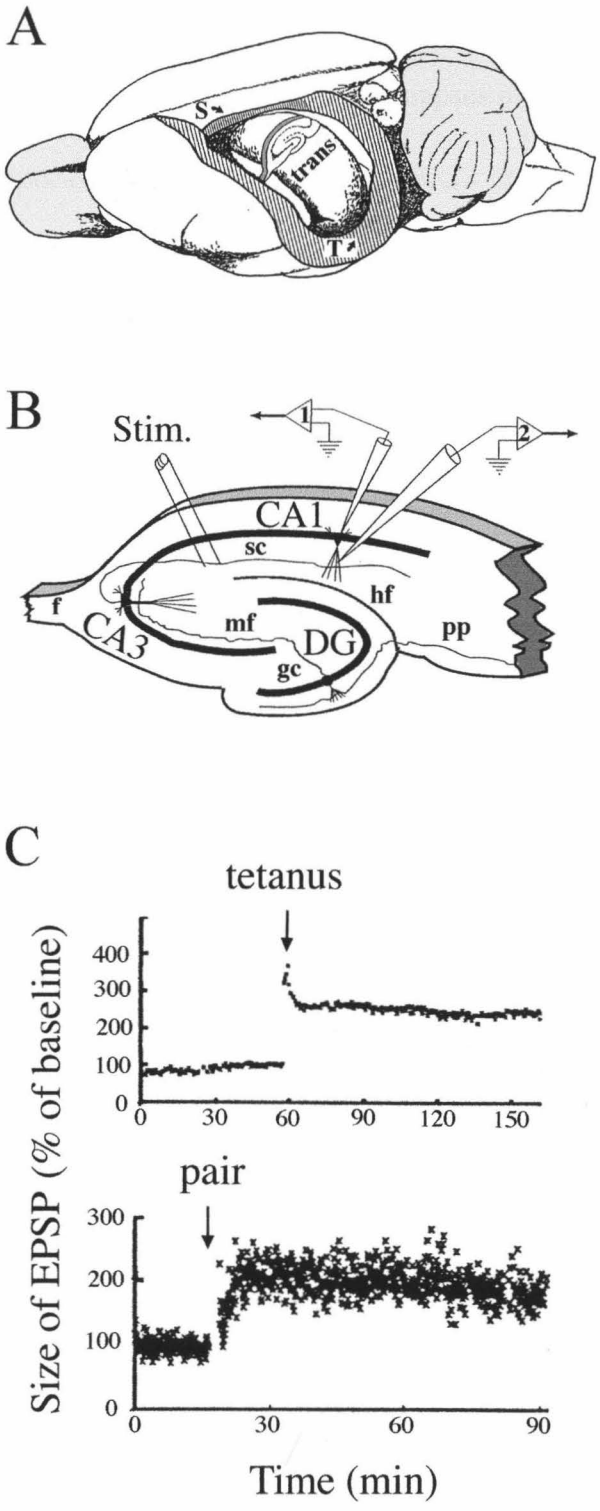

A
S
trans
T
B
Stim.
1
2
CA1
sc
hf
f
mf
CA3
DG
pp
gc
C
tetanus
400
300
200
100
0
0
30
60
90
120
150
pair
300
200
100
0
0
30
60
90
Size of EPSP (% of baseline)
Time (min)

Figure 1-1. Induction of LTP in hippocampal synapses *in vitro*.

Rat or mouse hippocampi are taken out of the brain (A), and dissected into ~500 μm thick transverse slices in which the tri-synaptic synaptic circuit is largely kept intact (B). Synaptic transmission is monitored by delivering electric stimulation through a stimulating electrode to presynaptic cells and recording electric responses from postsynaptic cells either intracellularly or extracellularly. The size of each response is represented as each dot over time (C). After a baseline period, if a brief high frequency stimulation "tetanus" (for example, 100 Hz for a second) is given through the stimulating electrode (C, upper), or a low frequency stimulation is paired with postsynaptic depolarization in the intracellular recording configuration (C, lower), enhanced synaptic transmission over the baseline can occur (C). This long-term potentiation (LTP) can last many hours *in vitro* or even days *in vivo*. In A, S: septal, T: temporal, trans: transverse. In B, sc: Schaffer-collateral, pp: perforant path, gc: granule cells, mf: mossy fiber, f: fimbria, hf: hippocampal fissure, DG: dentate gyrus. Modified from Madison and Schuman (1991).

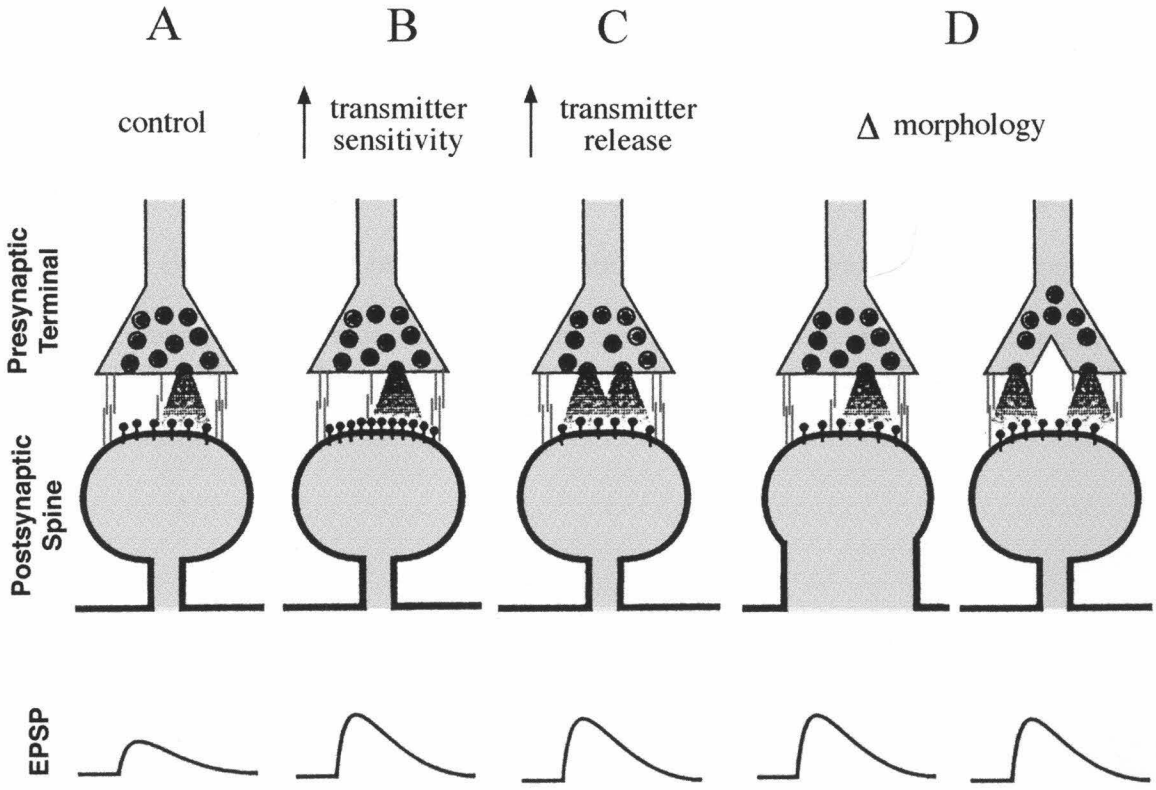
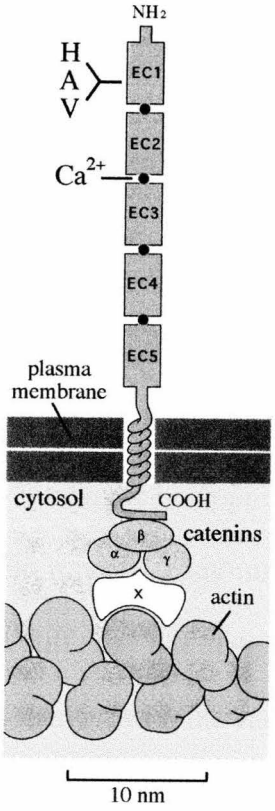

A
B
C
D
control
transmitter sensitivity
transmitter release
Δ morphology
Presynaptic Terminal
Postsynaptic Spine
EPSP

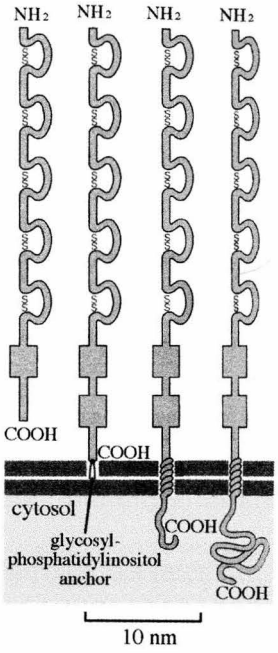
Figure 1-2. Possible role of cell adhesion molecules in LTP.

LTP has been reported to be associated with many or all of the following changes: post-synaptic changes such as increased transmitter sensitivity (B), presynaptic changes such as increased transmitter release (C), morphological changes such as change of spine geometry (D, left) and/or increased perforations (D, right). Synaptic cell adhesion molecules (represented as shaded short lines at synaptic cleft) may participate in many or all these processes (see text). Modified from Madison and Schuman (1991).

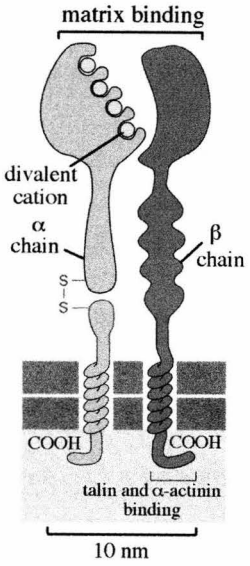
A **Cadherin Family**



B **Ig Family**



C **Integrins**



D **Proteoglycans**

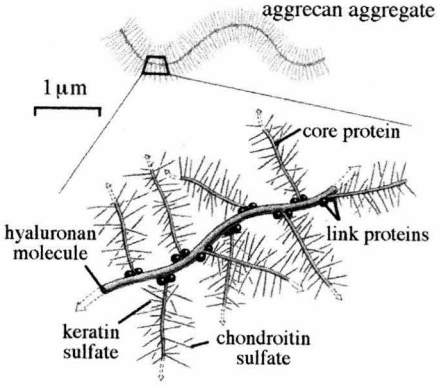


Figure 1-3. Neuronal cell adhesion molecules.

There are three major families of neuronal cell adhesion molecules: the cadherin superfamily (A), the immunoglobulin (Ig) superfamily (IgSF, B), and the integrin family (C). (modified from Alberts et al., 1994)

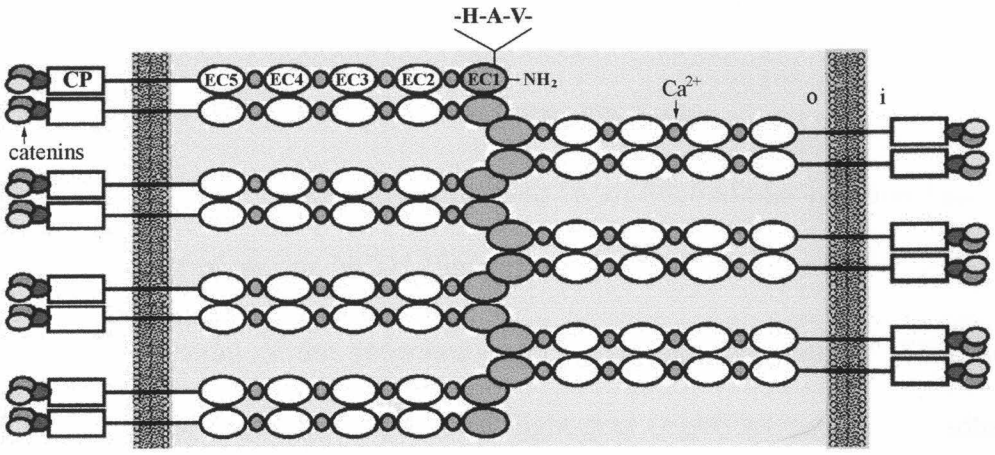
(A) Cadherins mediate Ca^{2+}-dependent homophilic cell-cell interactions. The extracellular domain is composed of five repeats (EC1-EC5) with the most distal repeat (EC1), especially a region containing the highly conserved His-Ala-Val (HAV) sequence, being critical for homophilic binding. The Ca^{2+}-binding sites are presumably between adjacent repeats. They may stabilize the interface between successive EC repeats to give a stiff, rod-like molecule. The highly conserved cytoplasmic C termini of cadherins interact with the catenins and cytoskeleton.

(B) IgSF members mediate Ca^{2+}-independent cell adhesion. The extracellular domain contains one or more copies of Ig-like domains. Some members have fibronectin type III (FNIII) repeats in the extracellular domain. IgSF members either are integral membrane proteins or associate with the membrane via a GPI anchor.

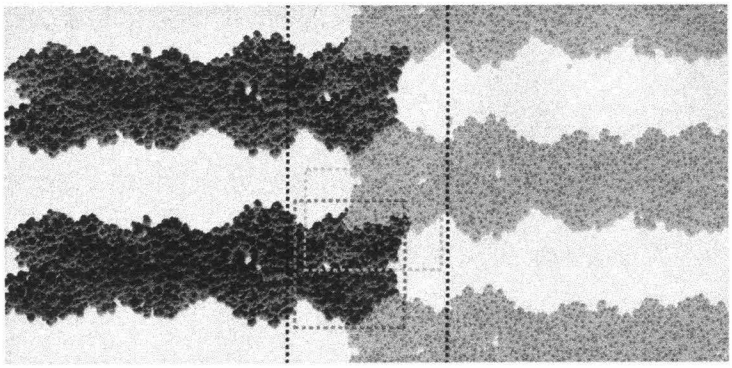
(C) Integrin family members mediate Ca^{2+}-dependent cell-cell and cell-matrix interactions. They are integral membrane glycoproteins composed of non-covalently associated α and β subunits. They are receptors of extracellular matrix molecules (ECMs).

(D) Proteoglycans are a special class of ECMs. They have a high content of highly negatively charged glycosaminoglycans (GAGs). Proteoglycans are present as ECM components, or in cell surface, membrane-spanning, soluble, and intracellular forms.

A

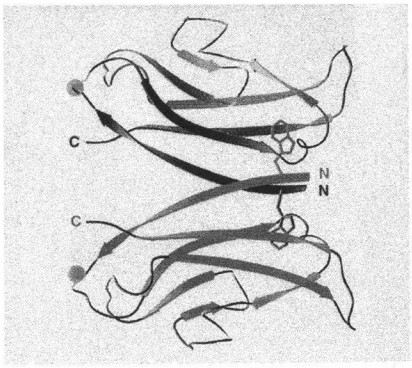


B



C

strand dimer



adhesion dimer

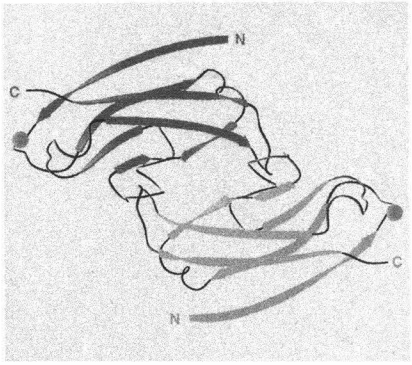


Figure 1-4. Cadherin junction complex and dimer interfaces.

(A) Schematic diagram of cadherin interactions in an intercellular junction based on the crystal structure of murine NCD1 (Shapiro et al., 1995).

(B) Space-filling model of the proposed cadherin cell-adhesion zipper (Shapiro et al., 1995). Molecules in dark gray (left) and light gray (right) correspond to cadherin extracellular segments emanating from opposing cell surfaces. The strand and adhesion dimers are enclosed in dashed boxes.

(C) Left, Ribbon drawing of the strand dimer. The termini are denoted by N and C. The side chain of Trp 2 from each protomer extends into a hydrophobic pocket of its partner in the dimer. Right, Ribbon drawing of the adhesion dimer (Shapiro et al., 1995).

Chapter 2. Expression of cadherins in young adult hippocampus

2.1 Introduction

As discussed earlier, expression of each of the identified classic cadherin subtypes is tissue- and time-dependent (see Section 1.2.3.4). The dynamic expression patterns of cadherins, especially N- and E-cadherin, and their roles in animal morphogenesis have been well studied in early developmental stages (Takeichi, 1987). In contrast to this, it is not clear about the expression of these cadherins in adult mammalian brain, especially adult hippocampus (of 5-8 weeks of age) where most studies of synaptic plasticity have been conducted.

Some controversies exist regarding the expression of N- and E-cadherin in the brain. A Northern blot analysis of rat hippocampus and forebrain of E15 to 720 days of age suggested that the 4.3-kb mRNA for N-cadherin was relatively abundant at postnatal days 1 and 21 days but down-regulated thereafter (Wagner et al., 1992). This is in contrast with the result from Linnemann et al. where they suggested that expression level of mRNAs for N-cadherin as well as N-cadherin protein only has a slight change from P1 to P730 in rat brain, liver, muscle and other tissues (Linnemann et al., 1994). A Northern blot analysis in this same study failed to reveal the mRNAs for E-cadherin in P4-10 rat brain. An *in situ* hybridization in mouse brain indicated that N-cadherin is ubiquitously expressed throughout the brain at E12-E16 but restricted to particular nuclei or laminae

that share common functional features and neuroanatomical connections at E16-P6 (Redies et al., 1992). Recent western blots of rat forebrain of 28-35 days of age suggests that N-cadherin is a major glycoprotein component of isolated rat forebrain postsynaptic densities (PSDs) and that it may play a role in stabilizing synaptic structure and early synaptogenesis (Beesley et al., 1995; Beesley et al., 1995). Due to the lack of clarity about the expression of cadherins in adult brain, especially in the hippocampus, it is thus necessary to examine closely the expression and cellular localization of cadherins in adult hippocampus before the putative role of cadherins in synaptic plasticity could be examined. The examination of cadherin expression and cellular localization in adult hippocampus thus constitutes the first part of this thesis.

2.2 Results

2.2.1 *In situ* hybridization

To examine first whether mRNAs of the two well-studied cadherins, N-cadherin and E-cadherin, are present in adult hippocampus, *in situ* hybridization of digoxigenin-labeled riboprobes for N- and E-cadherin was performed. When compared to sense controls (Figure 2-1 C, F, I), mRNAs for both N- (Figure 2-1 A-B) and E-cadherin (Figure 2-1 D-E, G-H) were found to be expressed in adult hippocampal slices. The signal for N-cadherin appears weaker than E-cadherin using these particular riboprobes in these experiments. The expression of mRNA for N-cadherin in the adult hippocampus is consistent with some earlier studies (Redies et al., 1992; Linnemann et al., 1994) whereas expression of E-cadherin mRNA is in contrast with an earlier study (Linnemann et al.,

1994). The expression of E-cadherin in adult hippocampus was surprising because this epithelial form of cadherin had not been characterized in the adult nervous system before.

2.2.2 Western blot analysis

To visualize the expression of cadherin protein in the adult hippocampus, a western blot analysis was performed on different hippocampal preparations including crude homogenate, membrane, cytosol and synaptosome fractions. A Pan-cadherin antibody, which was raised against cytoplasmic domain of N-cadherin and recognizes various classic cadherin subtypes including N-cadherin and E-cadherin, was used in the analysis (see Materials and Methods: Antibodies, also see Geiger et al., 1990).

I observed that cadherin protein is present in the hippocampal homogenate, membranous, and synaptosome fractions as 130 kDa bands, but not in the cytosolic fraction (Figure 2-2). The absence of cadherins in the cytosolic fraction is consistent with the fact that cadherins are integral membrane proteins. The presence of cadherins in the synaptosome fraction suggests that cadherins associate with synaptic structures.

2.2.3 Immunohistochemistry

In order to examine the cellular localization of cadherins, immunofluorescent labeling combined with confocal microscopy was performed to visualize the labeled sites in mouse (E-cadherin) and rat (N-, Pan-cadherin, α- and β-catenin) hippocampal slices and cultured hippocampal neurons (N-, Pan-cadherin, α- and β-catenin).

2.2.3.1 Single immunofluorescence labeling in hippocampal slice sections

At low power (10x), strong cadherin-positive staining was evident throughout the stratum radiatum and stratum oriens; four different antibodies, anti-N-cadherin, anti-E-cadherin, and anti-Pan-cadherin (anti-N-cadherin$_{cyto}$) and anti-Pan-cadherin (anti-E-cadherin$_{cyto}$), yielded similar patterns of labeling in the dendritic regions (Figure 2-3 A-C, F). Like the cadherins, immunostaining for α-, β- and γ-catenin was present throughout the synaptic neuropil in stratum radiatum and stratum oriens (Figure 2-3 G-I).

At high power (63x), immunostaining of N-cadherin revealed punctate staining throughout the synaptic neuropil, suggesting localization of N-cadherin proteins at or near synaptic sites.

Glypican, a GPI-anchored heparan sulfate proteoglycan, was also found to be expressed in hippocampal slices (Figure 2-3 E). The expression of glypican in the adult hippocampus has also been shown by others (Karthikeyan et al., 1994; Litwack et al., 1994).

2.2.3.2 Dual immunofluorescence labeling in hippocampal slice sections

To examine whether the cadherins (and catenins) are expressed at or near synaptic sites, I compared the labeling for N-, E-, and Pan-cadherin with that of the presynaptic protein synapsin I in the same hippocampal section, using either FITC-conjugated (green; cadherin) or Cy3-conjugated (red; synapsin I) secondary antibodies with minimal cross-reactions.

Synapsin I labeling was punctate and abundant throughout the slice (Figure 2-4 B) and the majority of the N-cadherin signal colocalized with the synapsin I signal (Figure

2-4 A, C). A similar pattern was observed for E-cadherin staining, although the labeling for E-cadherin was less abundant, and the synapsin I staining was more diffuse in mouse slices (Figure 2-4 D-F). There were also a number of synapsin I-positive sites that were not colabeled with a cadherin Ab, as has been observed by others (Fannon and Colman, 1996). Similar experiments conducted with synapsin I and a Pan-cadherin Ab still indicate the presence of sites that are only recognized by the synapsin I Ab (Figure 2-4 G-I) suggesting that there exists a population of synapses that contain cadherins not recognized by this Ab or, alternatively, other types of adhesion molecules.

2.2.3.3 Single immunofluorescence labeling in cultured hippocampal neurons

To better visualize the expression of cadherins and catenins in individual hippocampal neurons, immunostaining was performed in dissociated hippocampal neurons cultured *in vitro* for 5-7 weeks. At high power, staining for Pan-cadherin (Figure 2-5 A, B), N-cadherin (Figure 2-5 C, D), α-catenin (Figure 2-5 E, F) and β-catenin (Figure 2-5 G, H) all showed immunopositive puncta surrounding cell bodies and along neuronal processes, suggesting localization of these proteins at/near synaptic sites.

2.2.3.4 Dual immunofluorescence labeling in cultured hippocampal neurons

Like dual immunolabeling in hippocampal slices, dual immunolabeling in cultured hippocampal neurons indicated that the majority of the Pan-cadherin, α-catenin, and β-catenin colocalized with the synapsin I signal (Pan-cadherin + Syn I: Figure 2-6 A, D, G, and J, α-catenin + Syn I: Figure 2-6 B, E, H, and K, β-catenin + Syn I: Figure 2-6 C, F, I, and L). In these cultured neurons, there were also some synapsin-I positive but cadherin/catenin-negative sites, consistent with the staining pattern in hippocampal slices.

2.3 Discussion

My studies thus far have indicated that classic cadherins, especially N- and E-cadherins, and their intracellular partners, catenins, are not only present in adult hippocampus, but are molecular constituents of hippocampal synapses as well.

During the course of my experiments, several other groups had reported similar findings about the expression of cadherins/catenins at synaptic sites. As mentioned before, N-cadherin was found to be a constituent of a PSD fraction (Beesley et al., 1995). N-cadherin was also found at synapses between retinotectal axons and tectal neurons in the chick brain (Yamagata et al., 1995).

Recently, Fannon and Colman found discrete cadherin-positive sites at mouse cerebellar synapses (Fannon and Colman, 1996). Using immunofluorescent labeling and confocal microscopy, they found these cadherin-labeled sites ranged from small "dots" to larger disks (with a diameter of ~ 500 nm). The larger disks were associated with labeling of synaptophysin labeling (a presynaptic vesicle protein), and adjacent to or surrounded transmitter release zones. Moreover, N- and E-cadherins were found to be present on the neuropil of the CA3 region of hippocampus. The N-cadherin signals did not overlap E-cadherin signal. Some synapses were negative for either N- or E-cadherins, suggesting the presence of other adhesion molecules at these synapses (Fannon and Colman, 1996). This is consistent with our findings in the CA1 synapses. Based on the immunostaining data, Fannon et al. proposed that the cadherins function as primary adhesive moieties between pre- and postsynaptic membranes (Figure 3-14 A), and that locking in and fine-tuning of specific synaptic connections may be achieved through the use of multiple cadherins.

Uchida et al. also performed double immunolabeling of catenins and synaptophysin and found that the proteins extensively colocalized in neurons throughout the brain. By immuno-electron microscopy, catenins were found to exist in clusters distributed underneath pre- and post-synaptic plasma membranes. These clusters were often located beside transmitter-release zones and were associated with electron-dense material along apposed pre- and postsynaptic membranes. The catenin-positive regions of contact were cleanly segregated from the transmitter-release zones at asymmetrical synapses, while catenin signals tended to overlap the release zones at symmetrical synapses.

Together with my results, these studies indicate that cadherins and catenins are clearly molecular constituents of adherens-type junctions at many CNS synapses, including the CA1 synapses of adult hippocampus.

Aside from the expression of classic cadherin at synaptic sites, Kohmura et al. have demonstrated that a protein of a novel nonclassic cadherin subfamily, CNR (cadherin-related neuronal receptor), is expressed at synaptic sites in postnatal neocortex and interacts with Fyn, a non-receptor type tyrosine protein kinase of the Src family (Kohmura et al., 1998). As data on *in situ* hybridization showed that each neuron expresses a distinct set of CNR genes, proteins of multiple subclasses seem to coexist in a single pre- or postsynaptic cell. Therefore, proteins of multiple cadherin subtypes (classic, non-classic) seem to coexist in the brain, or in the same cell. Morphologically, however, the subcellular localization of CNRs appears different from that of classical cadherins: the CNRs are found within the active zones whereas catenins/cadherins are in the area bordering the active zones (Uchida et al., 1996).

Besides a critical role in animal morphogenesis, cadherins localized at synaptic sites also play a role in synaptic plasticity (see later part of my thesis). CNR expressed at synapses has also been speculated to play a role in conveying diverse extracellular signals and modulating the activity of Fyn and thus regulating NMDA receptor function, or in building specific synaptic connections (Miyakawa et al., 1997).

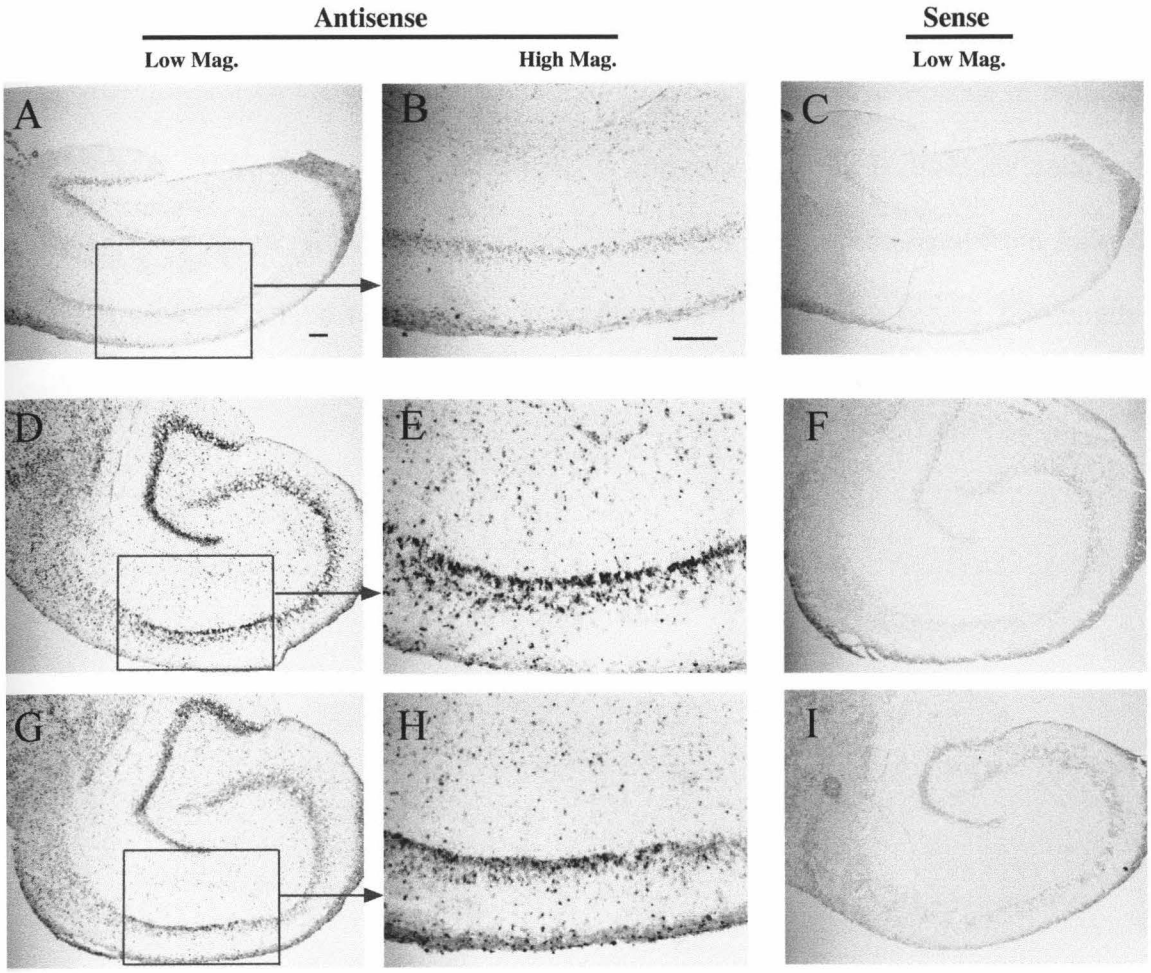

Antisense
Sense
Low Mag.
High Mag.
Low Mag.
A
B
C
D
E
F
G
H
I

Figure 2-1. *In situ* hybridization for N- and E-cadherin in hippocampal sections.

Digoxigenin-labeled cRNA probes were hybridized to 16 μm cryosections of hippocampal slices at 70°C. Sense probes were used as controls (C, F, I). B, E, H are enlarged as indicated regions in A, D, G, respectively. Riboprobes were synthesized from following cDNAs: A-C: a 2.4 kb rat N-cadherin fragment, D-F: a 4.3 kb mouse full-length E-cadherin, G-I: a 0.5 kb mouse E-cadherin fragment. Scale bars: 200 μm.

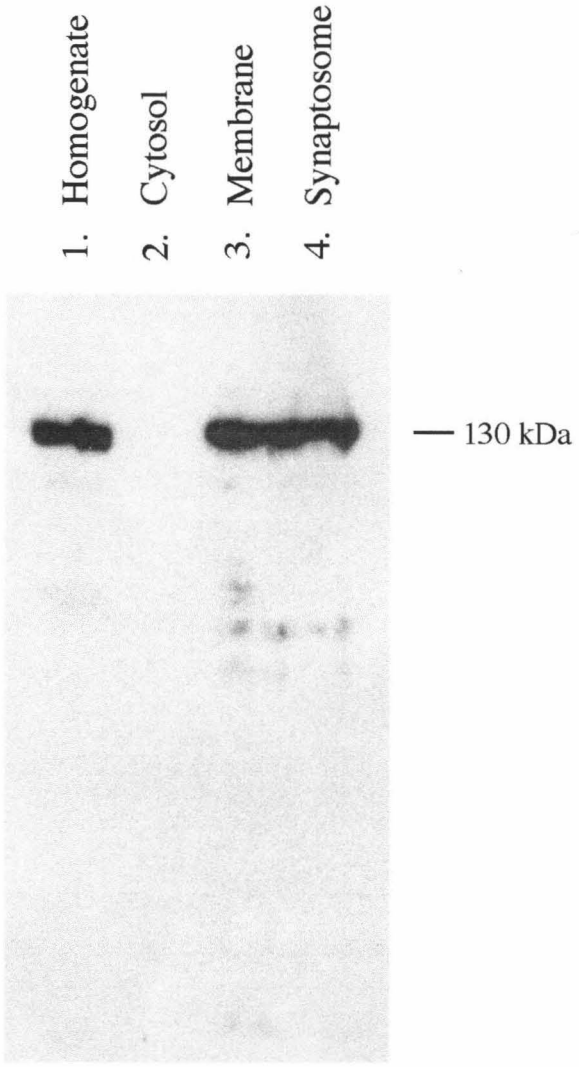

1. Homogenate
2. Cytosol
3. Membrane
4. Synaptosome
130 kDa

Figure 2-2. Immunoblot analysis indicates the presence of cadherins in adult hippocampus.

An anti-Pan-cadherin antibody was used for immunoblot analysis on hippocampal homogenate (lane 1), cytosolic fraction (lane 2), membranous fraction (lane 3) and synaptosome (lane 4).

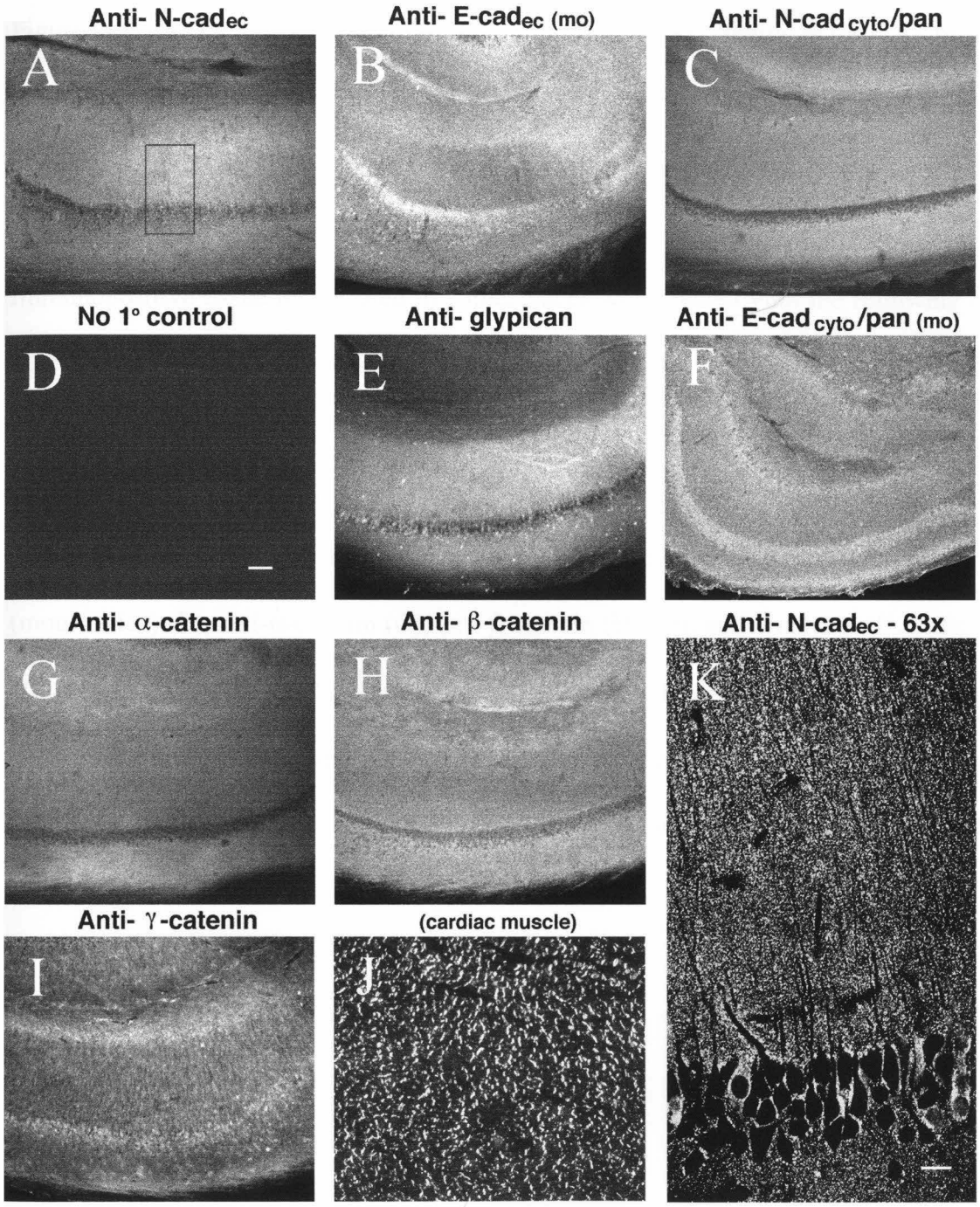

Anti- N-cadec
Anti- E-cadec (mo)
Anti- N-cadcyto/pan
A
B
C
No 1° control
Anti- glypican
Anti- E-cadcyto/pan (mo)
D
E
F
Anti- α-catenin
Anti- β-catenin
Anti- N-cadec - 63x
G
H
K
Anti- γ-catenin
(cardiac muscle)
I
J

Figure 2-3. Immunohistochemical labeling of cadherins and catenins in the CA1 region of the adult hippocampus.

(A-I) Low power (10x) confocal images of the CA1 region of a hippocampal slice, showing portions of stratum oriens (bottom), pyramidale (middle) and radiatum (top). Immunopositive tissue is indicated in white. Slices were treated with the following antibodies: anti-N-cadherin to the extracellular region of N-cadherin (A), anti-E-cadherin to the extracellular region of E-cadherin (mouse slice) (B), anti-N-cadherin to the cytoplasmic region of N-cadherin (i.e., anti-Pan-cadherin) (C), no primary antibody (control) (D), anti-glypican (E), anti-E-cadherin to a recombinant E-cadherin cytoplasmic domain (mouse slice) (F), anti-α-catenin (G), anti-β-catenin (H), and anti-γ-catenin (I). Scale bar: 100 μm. (K) High power (63x) confocal images of an indicated region in (A), indicating puncta surrounding pyramidal cell bodies and along neuronal processes. (J) High power (63x) confocal images of staining of anti-N-cadherin in cardiac muscle (positive control). Scale bars: 20 μm.

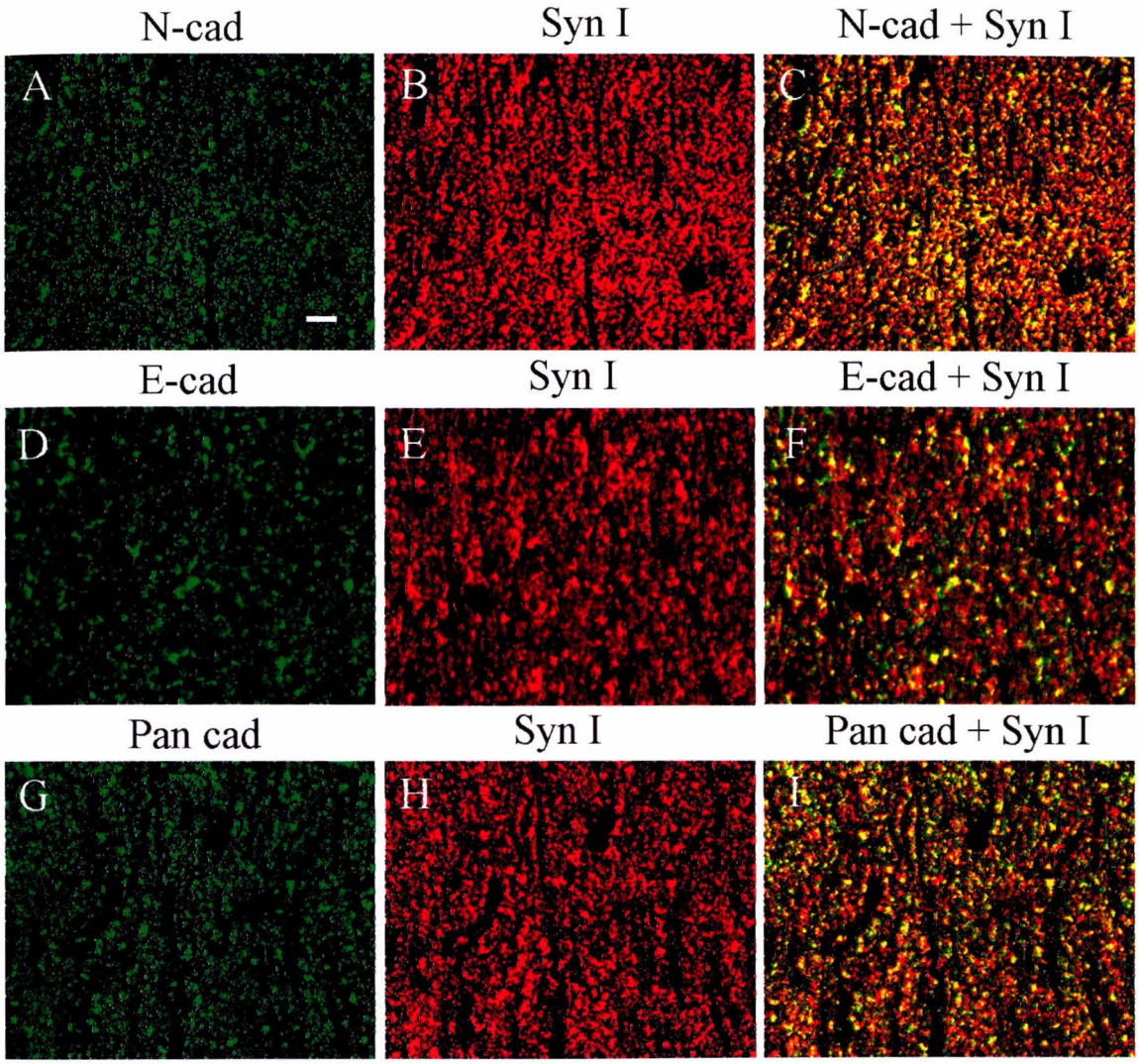

N-cad
Syn I
N-cad + Syn I
A
B
C
E-cad
Syn I
E-cad + Syn I
D
E
F
Pan cad
Syn I
Pan cad + Syn I
G
H
I

Figure 2-4. Dual immunolabeling suggests synaptic localization of cadherins in adult hippocampus.

High power (63x) confocal images of dual immunolabeled slice sections from stratum radiatum in CA1 region of the adult hippocampus. Immunopositive tissue is indicated in either green (FITC) for cadherins (leftmost column) or red (Cy3) for synapsin I (middle column). Immunolabeling was done with the following antibodies: anti-N-cadherin (A, green), anti-E-cadherin (D, green), anti-Pan-cadherin (G, green), anti-synapsin I (B, E, H, red). (C), (F), and (I) are overlay images of (A) + (B), (D) + (E), (G) + (H), respectively, showing coincidence of immunolabeling of the two antigens in yellow. Scale bar: 10 μm.

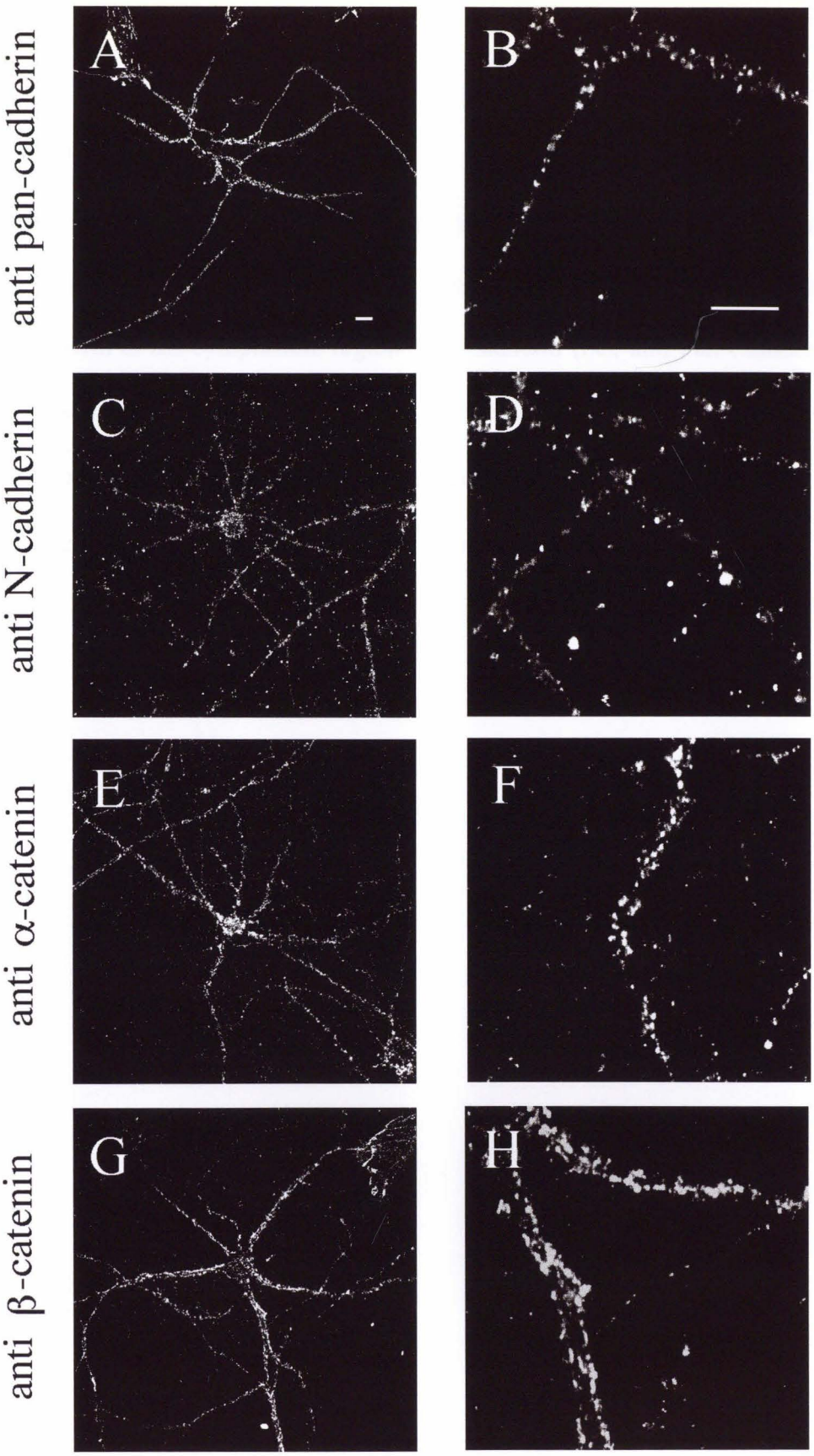

anti pan-cadherin
anti N-cadherin
anti α-catenin
anti β-catenin
A
B
C
D
E
F
G
H

Figure 2-5. Immunohistochemical labeling of cadherins and catenins in cultured hippocampal neurons.

High power (63x) confocal images of immunolabeled hippocampal neurons. Immunopositive is indicated in white. Cultured cells were treated with the following antibodies: anti-N-cadherin to the cytoplasmic region of N-cadherin (i.e., anti-Pan-cadherin) (A, B), anti-N-cadherin to the extracellular region of N-cadherin (C, D), anti-α-catenin (E, F), and anti-β-catenin (G, H). B, D, F, H are enlarged images (x4) of certain regions in A, C, E, G, respectively, showing puncta along neuronal processes. Scale bar: 10 μm.

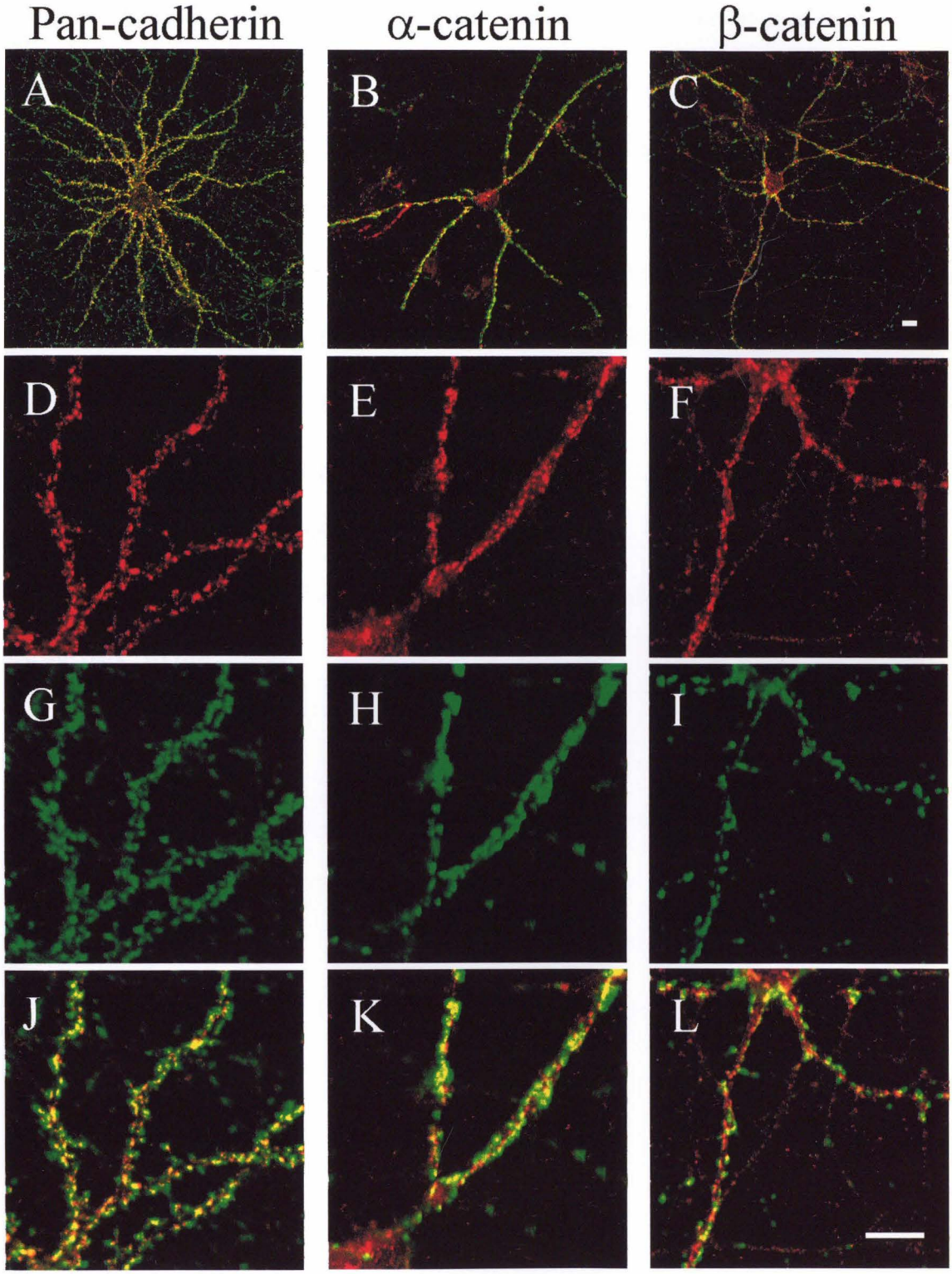

Pan-cadherin
α-catenin
β-catenin
A
B
C
D
E
F
G
H
I
J
K
L

Figure 2-6. Dual immunolabeling suggests synaptic localization of cadherins and catenins in hippocampal neurons.

High power (63x) confocal images of dual immunolabeled dissociated hippocampal neurons cultured *in vitro* for 5-7 weeks. Immunopositive is indicated in either green (FITC) for synapsin I or red (Cy3) for Pan-cadherin (leftmost column), α-catenin (middle column), or β-catenin (rightmost column). Immunolabeling was done for the following antibodies: anti-Pan-cadherin (anti-N-cadherin$_{cyto}$) (D, red), anti-α-catenin (E, red), anti-β-catenin (F, red), anti-synapsin I (G-I, green). J, K, and L are overlay images of D + G, E + H, F + I, respectively, showing coincidence of immunolabeling of the two antigens in yellow. J, K, and L are enlarged images (x4) of certain regions in A, B, C, respectively. Scale bars: 10 μm.

Chapter 3. Roles of cadherins in hippocampal synaptic plasticity

3.1 Introduction

The localization of cadherins and their signal transduction partners catenins at synaptic sites suggests that they may play a role in synaptic function. Several recent developmental studies have provided evidence for cadherin involvement in neurite outgrowth and the formation and/or maintenance of synapses (Yamagata et al., 1995; Riehl et al., 1996; Inoue and Sanes, 1997; also see Section 1.2.3.4). This raises the possibility that cadherins may play an analogous role in the activity-dependent rearrangement of synaptic structures in the adult CNS.

The established signaling capabilities of the cadherins coupled with their synaptic localization suggest that they may regulate synaptic transmission and synaptic plasticity. In the remainder of this thesis, I asked whether or not cadherins participate in synaptic transmission and plasticity. Specifically, I examined whether disrupting cadherin function, using a number of different approaches, affects synaptic transmission and long-term potentiation (LTP) in adult hippocampal slices.

3.2 Results

3.2.1 Function-blocking cadherin antibodies inhibit LTP

To perturb cadherin function, I used adhesion-blocking antibodies (Abs) raised against the extracellular region of two different cadherin family members, N- or E-cadherin. These monoclonal Abs were also used in the preceding chapter describing immunohistochemical studies of N- and E-cadherin localization.

The function-blocking anti-N-cadherin (also called anti-ACAM) Ab reacts with the N-terminal half of the extracellular domain of N-cadherin (Volk and Geiger, 1986). It had been used by others for immunostaining (Salomon et al., 1992; Schulze and Firth, 1993; Gilbertson-beadling and Fisher, 1993; Fannon et al., 1995; Hertig et al., 1996) and to interfere with cadherin-mediated cell interactions (Volk et al., 1990), cell adhesion between rat smooth muscle cells and endothelial cells (Gilbertson-beadling and Fisher, 1993), and other cadherin-dependent processes such as gap junction assembly in a rat liver tumor cell line (Meyer et al., 1992).

The function-blocking anti-E-cadherin (also called anti-Uvomorulin) Ab has been used for immunostaining (Fannon et al., 1995; Mbalaviele et al., 1995), and to block the aggregation of mouse embryonal carcinoma cells and the compaction of pre-implantation embryos (Vestweber and Kemler, 1985).

3.2.1.1 Ab penetration and persistence in slices

To examine the effect of the function-blocking cadherin Abs on hippocampal LTP, an acute hippocampal slice was incubated in a well containing either N- or E-cadherin Ab diluted in normal ACSF for 2-3 hours. The slice was then transferred to a recording

chamber and perfused with normal artificial cerebrospinal fluid (ACSF, i.e., slice medium) for 15-30 minutes before LTP-inducing stimulation was delivered. As a control, adjacent slices were incubated in a control solution containing one of the following: normal ACSF, preimmune rat IgG, a non-function-blocking E-cadherin Ab, or an Ab to glypican. Glypican is a cell-surface GPI-anchored heparan sulfate proteoglycan (see Section 1.2.2.). Its expression in adult hippocampus has been shown by my work and the work of others (see Section 2.4.1).

To confirm that the Ab incubations resulted in successful penetration into the depth of the slice, the extent of Ab labeling in incubated slices was analyzed. As shown in Figure 3-1 for the anti-N-cadherin Ab, it was found that 2-3 hr Ab incubations were sufficient to penetrate the most interior region of the slice, and that Abs remained in the slice following perfusion with ACSF for at least 1 hour. The full penetration and persistence of Ab following perfusion with ACSF (containing no Ab) during recordings was seen for anti-N-cadherin Ab (Figure 3-2 A-D), as well as the other Abs such as anti-E-cadherin Ab (Figure 3-2 C-H) and anti-glypican Ab (Figure 3-2 I-L) as well.

3.2.1.2 Function-blocking cadherin antibodies do not affect basal synaptic parameters

One major concern about using Abs that disrupt cell adhesion molecules is a disturbance of the structural integrity of the hippocampal slice, which could influence synaptic plasticity. I therefore determined whether the cadherin Abs affected basal synaptic transmission by examining the relationship between stimulus strength and the size of the postsynaptic response (I/O), paired-pulse facilitation (PPF), and posttetanic potentiation (PTP). It is known that these parameters are sensitive to the structural integrity and the

health status of slices, and have been used to examine basal synaptic function (e.g., Kantor et al., 1996). Cadherin Abs had no effect on the I/O relation (Figure 3-3 A, B), PPF (Figure 3-3 C, D), or PTP (Figure 3-4).

Taken together, these observations indicate that the function-blocking cadherin Abs do not affect basal synaptic transmission, and that it is less likely that the Abs disturb the structural integrity of the hippocampal slice.

3.2.1.3 Function-blocking cadherin antibodies inhibit LTP

Adjacent slices from the same hippocampus were treated with either a function-blocking cadherin Ab or a control Ab (or solution) for 2-3 hours prior to electrophysiological recordings and LTP induction by high frequency stimulation (HFS) of the Schaffer collateral axons. Incubation of slices in an N-cadherin function-blocking Ab significantly attenuated the magnitude of LTP relative to control slices treated with an equal dilution of a non-function-blocking cadherin Ab (Figure 3-4 A, C) (mean percent of baseline 1 hr after LTP induction: N-cadherin, 109.1% ± 7.1%; control, 147.6% ± 9.5% [n = 8]). Similarly, slices treated with a function-blocking E-cadherin Ab also exhibited significantly less LTP than adjacent control slices incubated in ACSF (Figure 3-4 B, C) (mean percent of baseline 1 hr after LTP induction: E-cadherin, 122.9% ± 7.9%; control, 150.7% ± 11.5% [n = 9]).

In contrast, in paired experiments examining LTP in slices exposed to normal ACSF versus slices exposed to non-function-blocking Abs raised against the cytoplasmic domain of N- or E-cadherin, these non-function-blocking Abs did not reduce LTP (Figure 3-4 C) (mean percent of baseline 1 hr after LTP induction: $\text{cadherin}_{\text{cyto}}$, 148.7% ± 6.7%;

control, 148.6% ± 4.9% [n = 16]). In addition, rat hippocampal slices pretreated with an additional control Ab to glypican exhibited robust and significant potentiation of synaptic transmission that did not differ significantly from that observed in control ACSF slices (Figure 3-4 C) (mean percent of baseline 1 hr after LTP induction: glypican, 146.2% ± 7.5%; control, 152.8% ± 5.5% [n = 12]).

3.2.2 HAV peptides inhibit LTP

3.2.2.1 Introduction

As a second approach to disturb cadherin function and ask whether synaptic plasticity is affected, I used synthetic peptides that were derived from the amino acid sequences of EC1 region of N- or E-cadherin and contain the highly conserved His-Ala-Val (HAV) sequence (HAV peptides) (Figure 3-5).

It is known that the homophilic binding specificity of cadherins is determined by the EC1 domain (Nose et al., 1990). Site directed mutations in the His-Ala-Val (HAV) sequence and its flanking amino acids of the EC1 repeat (Figure 1-3 A) result in loss of cadherin binding specificity (Nose et al., 1990). The HAV sequence in the EC1 repeat is highly conserved among the classic cadherins (Blaschuk et al., 1990; Blaschuk et al., 1990), and has been shown by crystallographic studies to exist in the adhesion dimer interface of N-cadherin EC1 domain (Shapiro et al., 1995) and in a similar conformation in E-cadherin EC1 domain (Overduin et al., 1995; Tomschy et al., 1996).

Synthetic peptides containing HAV have been used to inhibit cadherin-mediated cell adhesion and cadherin-dependent processes. Earlier studies showed that an HAV 10-mer peptide derived from chicken N-cadherin sequence (LRAHAVDVNG, 1 mM) completely

blocked E-cadherin-dependent mouse embryo compaction and partially inhibited N-cadherin-mediated neurite extension from rat DRG neurons or olfactory epithelial neurons growing on astrocyte monolayer (Blaschuk et al., 1990; Chuah et al., 1991). An HAV 6-mer peptide (AHAVSE, a composite of N- and E-cadherin sequences, 1 mM) also inhibited these cadherin-dependent processes by 50-80%. Doherty et al. (1991) found that a 16-mer HAV peptide (0.06-0.25 mM) derived from human N-cadherin sequence (HLGAHAVDINGNQVET) or a 10-mer peptide (0.4 mM) derived from mouse N-cadherin sequence (LRAHAVDING) blocked N-cadherin-dependent neurite outgrowth from the E6 rat RGCs or P7 rat cerebellar neurons grown on mouse 3T3 fibroblasts expressing exogenous chicken N-cadherin. Moreover, a 17-mer, 10-mer or 8-mer HAV peptide derived from mouse E-cadherin sequence (AKYILYSHAV SSNGNAV, LYSHAVSSNG, and YSHAVSSN) were found to block E-cadherin-mediated mouse osteoclast formation with the 17-mer HAV peptide (AKYILYSHAVSSNGNAV, 0.55 mM) being most effective (Mbalaviele et al., 1995). Similarly, a 10-mer HAV peptide (0.2-2 mM) derived from mouse N-cadherin sequence (LRAHAVDING) blocked N-cadherin-dependent mouse myoblast fusion in a concentration-dependent manner whereas a scrambled sequence (DANGALHIVR) was without effect (Mege et al., 1992).

Direct *in vitro* cell adhesion assays indicated that a 10-mer HAV peptide (0.2 mM) derived either from human N-cadherin (LRAHAVDING), or E-cadherin (LFSHAVSS NG), or P-cadherin sequence (LFHHAVSENG), or rat extracellular super-oxide dismutase (EC-SOD, REMHAVSRVQ) was found to inhibit cadherin-mediated adhesion of chicken or human E-cadherin, or mouse P-cadherin expressing cells by 50-80% (Willems et al., 1995).

In this thesis, I used HAV-containing peptides derived from mouse N- or E-cadherin sequences as inhibitors to disrupt cadherin dimerization and examine whether LTP is subsequently affected. The HAV peptides I used were of two different lengths: a 17-mer and a 5-mer. Scrambled versions of these HAV peptides were used as controls.

3.2.2.2 HAV peptides inhibit LTP without disrupting normal synaptic function

In the first set of experiments, slices were incubated in ACSF containing long (17-mer) HAV peptides, attempting to maximize the inhibition and specificity of the peptide interaction with endogenous cadherins (see Section 3.3.1). The incubation time and recording configurations were the same as in the function-blocking Ab experiments.

As was the case for the function-blocking Ab, incubation with the 17-mer peptides had no apparent effect on either the input-output relations of synaptic transmission (Figure 3-6 A-C) or PPF (Figure 3-6 D-F). In addition, slices incubated with either an N-cadherin-derived or an E-cadherin-derived 17-mer peptide (1 mM) exhibited significantly less LTP than adjacent controls slices incubated with a scrambled version of the peptide (Figure 3-7 A, C) (mean percent of baseline: N-cadherin 17-mer, 109.7% ± 9.0%; scrambled 17-mer, 144.3% ± 9.6% [n = 6]; E-cadherin 17-mer, 119.7% ± 8.3%; scrambled 17-mer, 177.7% ± 20.2% [n = 7]).

The combined preincubation of slices with both N- and E-cadherin-derived peptides (1 mM for each peptide) did not result in greater inhibition than that observed with a single peptide (Figure 3-7 B, C) (mean percent of baseline: N-cadherin [1 mM] + E-cadherin [1 mM] 17-mer, 113.3% ± 5.2% [n = 6]; scrambled 17-mer [2 mM], 146.0% ±

11.2% [n = 6]), suggesting that each peptide may be capable of inhibiting both N- and E-cadherin function.

3.2.2.3 Inhibition of LTP induction by HAV peptides

Because relatively short peptides can be introduced into slices in the perfusion media, I was able to address specifically whether peptides containing the HAV alter the initiation and/or maintenance of LTP. The experimental design of two independent pathways is shown in Figure 3-8. Two stimulating electrodes and one recording electrode (in the middle of stimulating electrodes) were placed in a single slice. The stimulating electrode activated two independent sets of Schaffer collateral axons making synapses to the same set of CA1 postsynaptic cells, whose EPSPs were recorded from the recording electrode (Figure 3-8 A). The experimental design was as follows: LTP was induced in one pathway. Thirty minutes later the peptide (0.2 mM) was introduced. After another 30 minutes, LTP-inducing stimulus was delivered to the second pathway in the continued presence of the peptide. Thus the effects of the peptide on LTP maintenance (pathway 1) and initiation (pathway 2) were studied.

The introduction of two different HAV peptides, in which the flanking amino acids were derived from either N- (AHAVD) or E-cadherin (SHAVS) sequences, attenuated LTP induction in the second pathway (Figure 3-9 A, C) (mean percent of baseline: AHAVD, 114.8% ± 4.3% [n = 6]; control pathway, 143.2% ± 8.7% [n = 6]). The same peptide applied 30 minutes after LTP induction, however, had no effect on previously established LTP (Figure 3-9 A). The combined application of both the N- and E-cadherin-derived peptides (AHAVD and SHAVS; 0.2 mM each) did not produce greater inhibition of LTP than either peptide alone (Figure 3-9 C) (mean percent of baseline:

AHAVD + SHAVS, 132.7% ± 6.9% [n = 6]; control pathway, 175.2% ± 13.0% [n = 6]), suggesting that there may be a cadherin-independent aspect to potentiation that cannot be blocked by the peptides.

In several sets of control experiments, introduction of either a scrambled (AADHV; VSHSA) or a single amino acid mutation (AEAVD) peptide had no apparent effect on either LTP induction (Figure 3-9 B, C) or maintenance in the second pathway (Figure 3-9 B).

These results indicate that LTP induction requires normal cadherin function since the HAV-containing peptides can block LTP when present at the time of induction. The lack of effect of the HAV peptides on previously established LTP reflects a time window of vulnerability of LTP to HAV peptides: the peptides are only effective inhibitors of LTP when applied around the time of LTP induction (but see Section 3.2.3).

3.2.2.4 Dose-dependent inhibition of LTP by HAV peptides

Previous experiments have shown that HAV peptides (10-mer) block N-cadherin-dependent processes such as myoblast fusion in a concentration-dependent manner (Mege et al., 1992). The inhibitory effect on LTP by HAV peptides at several different concentrations (200 μM, 50 μM, and 10 μM) was also studied in the present experiments.

As shown in Figure 3-10, the inhibition of LTP by 5-mer HAV peptides is dose-dependent: lower concentration of AHAVD produces less inhibition of LTP (mean percent inhibition of LTP: 200 μM AHAVD, 65.6% ± 10.0% [n = 6], 50 μM AHAVD, 43.8% ± 10.7% [n = 5], 10 μM AHAVD, 6.8% ± 20.0% [n = 5]).

3.2.2.5 HAV peptides also block LTP induced by other protocols

Several studies have shown that different LTP induction protocols can utilize distinct biochemical signaling pathways (e.g., Kang et al., 1997). Therefore, the sensitivity of LTP induced by several additional protocols to the HAV peptides was examined in this study. These additional LTP inducing protocols include theta burst stimulation (TBS) (Bliss and Lomo, 1973; Gustafsson et al., 1987) and pairing postsynaptic depolarization with low frequency stimulation (Larson and Lynch, 1986).

In two-pathway experiments, it was found that pathways exposed to the HAV peptide (200 μM) exhibited significantly reduced TBS-induced LTP relative to the control pathway (Figure 3-11 A) (mean percent of baseline: AHAVD pathway, 112.4% ± 8.7%; control pathway, 139.0% ± 10.8% [n = 5]). In addition, slices treated with HAV peptide (200 μM) exhibited significantly less pairing-induced LTP than control slices treated with a scrambled peptide (Figure 3-11 B) (mean percent of baseline: AHAVD slices, 111.4% ± 11.9%; AADHV slices, 183.6% ± 23.9% [n = 5]).

Taken together, these data indicate that the HAV peptides are capable of inhibiting LTP induced by several different protocols, suggesting a fundamental role of cadherins in synaptic plasticity.

3.2.2.6 HAV peptides do not affect NMDA receptor-mediated responses

One potential mechanism for the inhibitory effect on LTP by the HAV peptides could be that the HAV peptides blocks LTP by interfering with NMDA receptor activation, rather than disturbing cadherin function *per se*. I therefore examined the pharmacologically isolated NMDA receptor-mediated responses in the presence or absence of the HAV

peptides. The peptides were found to have no effect on NMDA receptor-mediated field EPSPs (Figure 3-12 A) (mean percent of baseline value after 30 minutes: fEPSP, 111.6% ± 11.5% [n = 5]) or the magnitude of excitatory postsynaptic currents (EPSCs) recorded at a range of holding potentials (Figure 3-12 B) (mean percent of control value: at –60 mV, 102.0% ± 12.0%; -50 mV, 105.0% ± 5.0%; 40 mV, 108.0% ± 2.0%; -30 mV, 94.0% ± 16.0%; -20 mV, 94.0% ± 10.0% [n = 2]).

3.2.2.7 HAV peptides do not affect the postsynaptic neuron's ability to depolarize

Like the cadherin Abs and the long HAV peptides, the 5-mer HAV-containing peptides had no detectable effect on basal synaptic responses, PTP (Figure 3-10), or PPF (Figure 3-13 B). Other possible effects of the HAV peptides, such as alteration of inhibitory responses and the ability of the postsynaptic neuron to depolarize during high frequency stimulation, were also examined.

Application of AHAVD had no effect on the magnitude of the inhibitory postsynaptic potential (IPSP) recorded intracellularly in pyramidal neurons (mean percent of baseline after 30 minutes peptide exposure: 97.4% ± 15.4% [n = 2]). In addition, the application of an HAV-containing peptide did not significantly alter the postsynaptic neuron's ability to depolarize during high frequency stimulation (Figure 3-13 A, C, D).

Taken together, these results rule out any obvious effect of the HAV peptide on the first few events (e.g., postsynaptic depolarization and NMDA receptor activation) associated with LTP induction, and argue for a fundamental role of cadherins in synaptic plasticity.

3.2.3 The inhibition of LTP by cadherin inhibitory reagents: Dependence on $[Ca^{2+}]_o$

3.2.3.1 Introduction

The two-pathway experiments (Figure 3-9 and Figure 3-11 A) indicate that HAV peptides are effective inhibitors of LTP when applied around the time of LTP induction. There are two general possibilities to account for the exclusive inhibitory effect of cadherin manipulations on the induction rather than the maintenance of plasticity. (1) LTP induction might render the adhesive junctions formed by existing cadherins susceptible to inhibition by the peptide inhibitors. For example, reductions in extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) the synaptic cleft during high frequency stimulation (Nicholson et al., 1978; Krnjevic et al., 1982; Smith, 1992) might transiently destabilize the Ca^{2+}-dependent cadherin-cadherin interaction (Hyafil et al., 1981) (Figure 3-14 B). More recently, a computational modeling study by Egelman and Montague shows that a presynaptic action potential (AP) invasion (1 msec) may cause an extracellular calcium fluctuation of about 2.0 mM (personal communication, also see Egelman and Montague, 1998). According to this study, a resting Ca^{2+} concentration of 2.5 mM (in normal ACSF) drops transiently to ~ 0.5 mM by a presynaptic AP invasion, and a resting concentration of 5.0 mM drops to ~ 3.0 mM. It is likely the drop of $[Ca^{2+}]_o$ in synaptic cleft by LTP-inducing stimulus destabilizes cadherin bonds (Takeichi, 1977; Hyafil et al., 1981; Ozawa et al., 1990) and increases their vulnerability to the peptide inhibitors. (2) New cadherin bonds may be formed during LTP induction (Figure 3-14 C); as such, the peptide could block the formation of these new junctions. Inhibition of existing and/or new cadherin bonds would thus eventually lead to decreased LTP via cadherin-dependent mechanisms. The former possibility has been test in this thesis.

3.2.3.2 High Ca^{2+} protects LTP from inhibition by cadherin inhibitory reagents

We reasoned that if the former possibility were true, the inhibition of LTP by HAV peptides could be prevented if the extracellular Ca^{2+} concentration was raised. I thus elevated the Ca^{2+} concentration from 2.5 mM to 5.0 mM and reexamined the inhibitory efficacy of the HAV peptides in two-pathway experiments. As would be expected, the increased concentration of Ca^{2+} in the extracellular solution had a modest (15.5%) enhancing effect on basal synaptic transmission. The ability of the HAV peptide to inhibit LTP, however, was completely prevented by the elevated Ca^{2+} in the ACSF (Figure 3-15 A) (mean percent of baseline: AHAVD, 138.3% ± 7.4%; control pathway, 142.4% ± 9.2% [n = 9]). Similarly, when slices were pretreated with N-cadherin Ab in the presence of elevated Ca^{2+}, the Ab no longer attenuated LTP (Figure 3-15 B) (mean percent of baseline: N-cadherin Ab/high Ca^{2+}, 141.7% ± 13.5% [n = 6]; control Ab/high Ca^{2+}, 138.8% ± 4.6% [n = 6]).

These results suggest that elevated extracellular Ca^{2+} can protect synaptic cadherins from inhibition by the HAV peptide and function-blocking Ab.

3.2.3.3 Low Ca^{2+} manipulation reveals cadherin-dependency during LTP maintenance

The protection of LTP from inhibition by elevating extracellular Ca^{2+} is consistent with the hypothesis that a transient drop in extracellular Ca^{2+} engendered during LTP induction destabilizes cadherin bonds. My earlier two-pathway results demonstrated that HAV peptides do not affect established LTP. The lack of effect during LTP maintenance may be due to a protection of cadherin bonds by existing Ca^{2+} levels. I therefore exam-

ined whether LTP maintenance could be rendered susceptible to inhibition by the peptides by a transient reduction in extracellular Ca^{2+}.

In two-pathway experiments, the slice was exposed for 30 minutes to low Ca^{2+} ACSF (0.5 mM Ca^{2+}) containing HAV peptides 30 minutes after LTP induction in pathway 1. As would be expected, low Ca^{2+} concentration caused progressively decreased basal synaptic transmission that recovered after normal Ca^{2+} ACSF was re-introduced (Figure 3-16 A, B). The potentiation in the LTP pathway, however, did not recover following exposure to HAV peptides in a low Ca^{2+} ACSF (Figure 3-16). Recovery of LTP was observed when a scrambled peptide, rather than HAV, was introduced (normalized potentiation, AADHV + low Ca^{2+} before treatment, 143.8% ± 3.7%, after, 145.2% ± 9.1% [n = 7], AHAVD + low Ca2+ before treatment, 147.1% ± 6.7%, after, 120.1% ± 9.2% [n = 7]).

These results suggest that maintenance of LTP also requires cadherin function that may be disturbed by HAV peptides combined with a low concentration of Ca^{2+}.

3.3 Discussion

As shown above, hippocampal slices pretreated with the function-blocking cadherin Abs exhibited decreased LTP without apparent disruption of structural integrity of slices and basal synaptic properties. This suggests that these Abs inhibit LTP by perturbing mechanisms involved in synaptic plasticity rather than a general anti-adhesive effect on the slices. This segregates the disruptive effect on synaptic structure and the cadherin-dependent synaptic plasticity.

Preincubation of hippocampal slices with function-blocking cadherin antibodies or antagonistic peptides containing HAV sequence greatly reduced hippocampal long-term potentiation (LTP) whereas basal synaptic properties including input-output relations, and paired-pulse facilitation (PPF), were normal. The inhibition of LTP by peptide inhibitors exhibited a number of properties including the following: (1) concentration-dependence: lower concentrations of HAV peptides inhibit LTP to a lesser degree, (2) stimulation protocol-independence: HAV peptides inhibit LTP induced by either theta burst stimulation, intracellular pairing, or tetanus. The HAV peptides do not affect NMDA receptor-mediated responses, nor do they affect the ability of postsynaptic neurons to depolarize during the LTP inducing stimulus.

Previous studies have shown that HAV peptides can interfere with a number of cadherin-dependent processes, such as neurite outgrowth (Chuah et al., 1991; Doherty et al., 1991), osteoclast formation (Mbalaviele et al., 1995), and myoblast fusion (Mege et al., 1992). I have shown that hippocampal slices treated with HAV peptides exhibited significantly reduced LTP. The HAV motif may also mediate heterophilic interactions of cadherins with another HAV-containing molecule, the fibroblast growth factor (FGF) receptor (Williams et al., 1994). Given that FGF does not appear to modulate LTP (Hisajima et al., 1992), perturbations of an FGFR-cadherin interaction is an unlikely explanation for our results. Since the HAV motif is present in the adhesion interface for cadherin-cadherin interactions (Shapiro et al., 1995; Vaughn and Bjorkman, 1996), the inhibition of LTP by the HAV peptides thus suggests that intercellular interactions between cadherins are essential for LTP formation. Cadherin-mediated interactions could be required for intracellular signaling on either side of the synapse, or to increase the area

of contact between pre- and postsynaptic elements, or even to transmit mechanical signals across the synaptic cleft.

Furthermore, my results indicated that blockade of LTP by the HAV peptides and the N-cadherin Ab is Ca^{2+} concentration-dependent. These reagents do not inhibit LTP when extracellular Ca^{2+} is elevated from 2.5 mM to 5 mM. Studies using ion-sensitive microelectrodes have shown that repetitive stimulation in the hippocampus (Krnjevic et al., 1982) and cerebellum (Nicholson et al., 1978) can cause dramatic reductions in extracellular Ca^{2+}. Moreover, models of Ca^{2+} dynamics in the synaptic cleft and extrasynaptic space predict that action potential activity can transiently decrease or even deplete Ca^{2+} in the synaptic cleft due to flux through voltage-gated Ca^{2+} channels and NMDA channels (Smith, 1992; Egelman and Montague, 1998). Cadherin-cadherin interactions exhibit a strong Ca^{2+} dependence: removal of Ca^{2+} from the medium results in a loss of adhesion (Hyafil et al., 1981) and a change in the structure of the cadherin extracellular domains from their native rod-like structure to a globular structure (Pokutta et al., 1994; Koch et al., 1997). Thus bouts of intense synaptic activity associated with LTP induction may transiently destabilize existing cadherin homophilic bonds (Takeichi, 1977; Hyafil et al., 1981; Ozawa et al., 1990), rendering them susceptible to inhibition by the HAV peptides. Nascent cadherin-cadherin bonds might also be vulnerable at this time, due to reduced extracellular Ca^{2+}. The inability of the HAV peptides to block at elevated Ca^{2+} concentrations may thus reflect a relative protection of the cadherin bonds owing to a less dramatic reduction in cleft Ca^{2+} during stimulation. In the case of the N-cadherin Ab, the results do not distinguish between a protective effect of elevated Ca^{2+} associated with

LTP induction versus protection associated with the Ab preincubation period (2-3 hr), during which time the Ab may gain access to synaptic sites.

The combined treatment of the HAV peptides with low Ca^{2+} (0.5 mM) ACSF during the LTP maintenance phase reversed LTP in the potentiated pathway, suggesting that expression of LTP also requires cadherin function for at least 30 minutes following the induction of plasticity. It is an open question as to how long the expression of LTP is vulnerable to the treatment of the HAV peptide in low Ca^{2+} ACSF. Application of the HAV peptide in low Ca^{2+} at different time points would be useful to address this question. In my earlier 2-pathway experiments, however, application of HAV peptides in a normal ACSF had no effect on the established LTP (see Section 3.2.2.2). The lack of effect of HAV peptides during LTP maintenance is likely due to a protection of cadherin homophilic bonds by Ca^{2+} ions in the normal ACSF.

The basal synaptic transmission in the control pathway recovered to the original level following replacement of the low Ca^{2+} ACSF and HAV peptides (Section 3.2.3.3). Moreover, HAV peptides delivered in a normal ACSF were found not to alter the basal synaptic transmission in the control pathway (Section 3.2.2.2). Therefore, the basal synaptic transmission of naïve synapses are not affected by bath application of HAV peptides alone or combined with low Ca^{2+}, whereas the potentiated synapses are sensitive to the combined treatment of HAV peptides with low Ca^{2+}. These results suggest that normal cadherin function is required for both the induction and maintenance of hippocampal LTP. This further supports the notion that these cadherin inhibitory reagents inhibit LTP by perturbing mechanisms involved in synaptic plasticity rather than a general disruptive effect on synaptic structure of the slices.

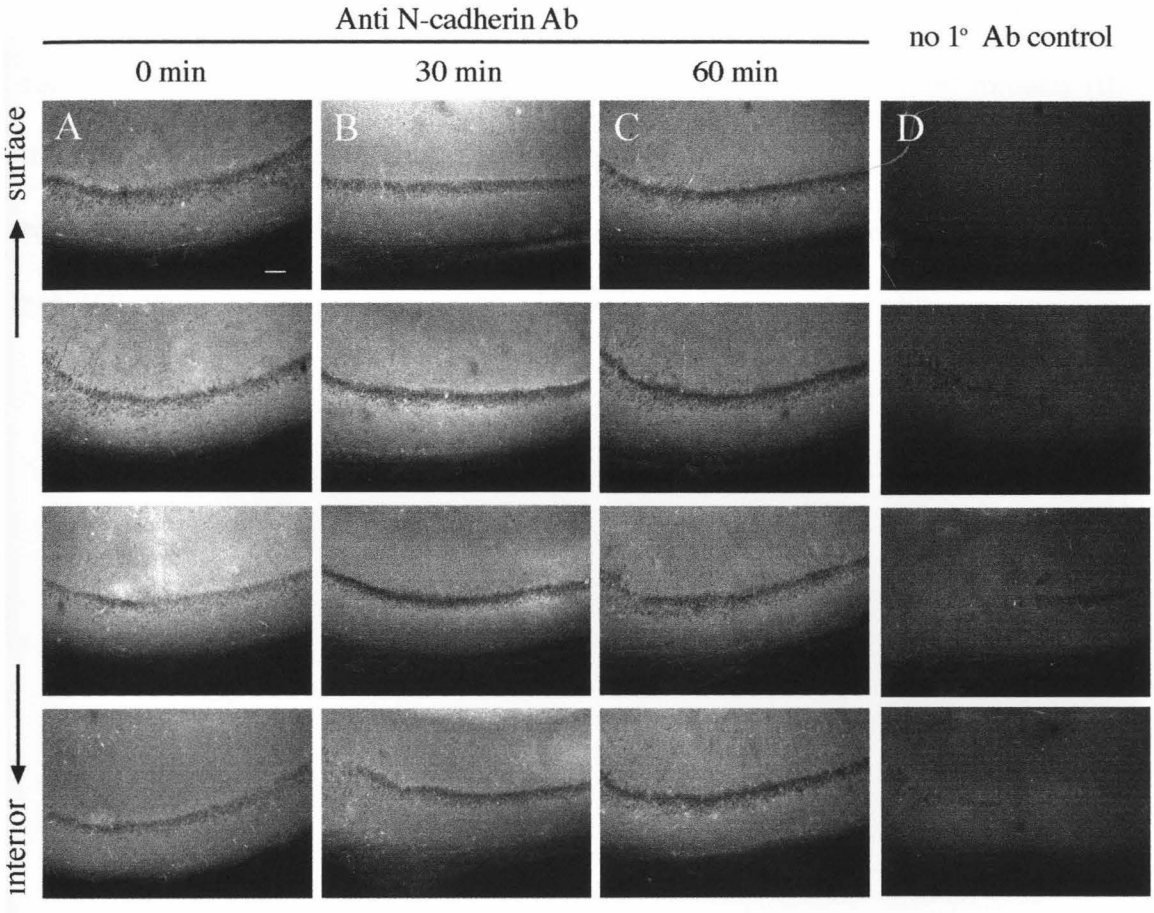

Anti N-cadherin Ab
no 1° Ab control
0 min
30 min
60 min
A
B
C
D
surface
interior

Figure 3-1. Full penetration and persistence of N-cadherin antibodies in hippocampal slices.

Each column shows 50 μm thick sections taken from a slice incubated in the anti-N-cadherin Ab for 2-3 hrs and perfused with ACSF for 0 minute (A), 30 minutes (B), 60 minutes (C), or a slice incubated with no primary Ab (control, D). The most exterior sections are shown in the top row; the most interior sections are in the bottom row. Scale bar, 100 μm.

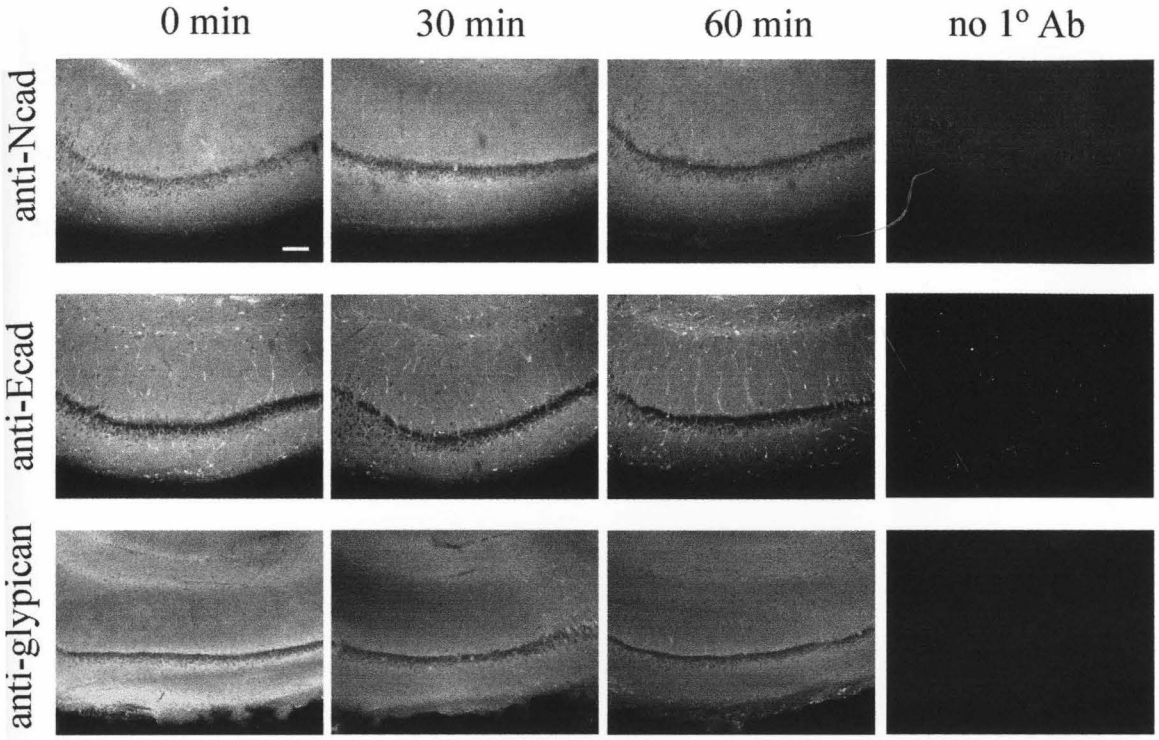

0 min
30 min
60 min
no 1° Ab
anti-Ncad
anti-Ecad
anti-glypican

Figure 3-2. Persistence of various antibodies in hippocampal slices for at least 60 minutes following perfusion with normal ACSF.

Each row shows sections taken from the middle 50 μm of Ab-treated slices perfused with normal ACSF for 0, 30, or 60 minutes as well as a no primary Ab control. Abs are anti-N-cadherin, anti-E-cadherin, anti-glypican. Scale bar, 100 μm.

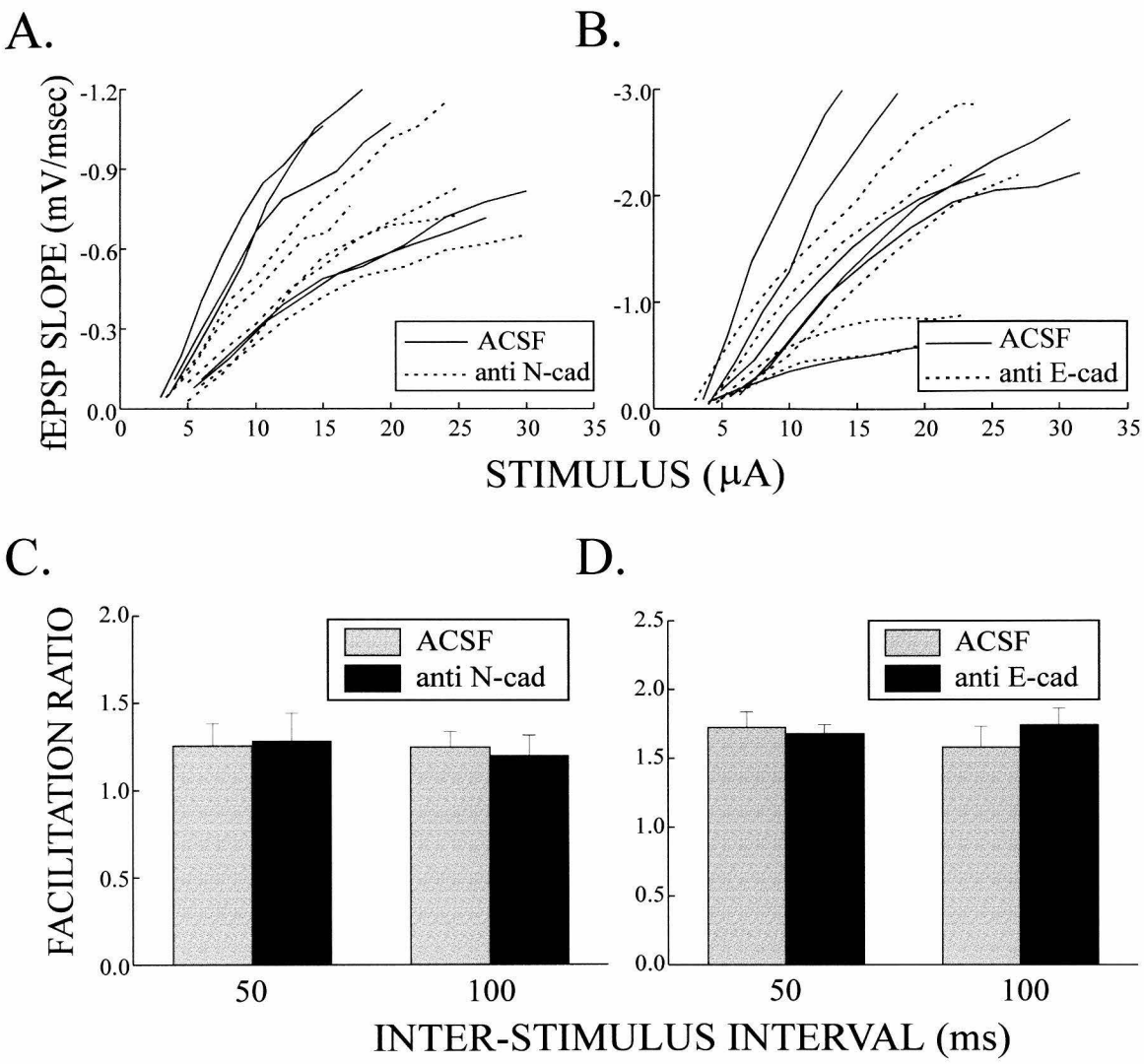

A.
B.
fEPSP SLOPE (mV/msec)
-1.2
-0.9
-0.6
-0.3
0.0
-3.0
-2.0
-1.0
0.0
0
5
10
15
20
25
30
35
ACSF
anti N-cad
ACSF
anti E-cad
STIMULUS (μA)
C.
D.
FACILITATION RATIO
2.0
1.5
1.0
0.5
0.0
2.5
50
100
ACSF
anti N-cad
ACSF
anti E-cad
INTER-STIMULUS INTERVAL (ms)

Figure 3-3. Cadherin antibodies do not alter basal properties of synaptic transmission in hippocampal slices.

(A and B) Input-output curves depicting the relationship between stimulus current intensity and the size of the field EPSP slope. Slices treated with either an N-cadherin (A) or an E-cadherin (B) Ab were not significantly different from ACSF controls in their input-output curves.

(C and D) Facilitation ratio for anti-N-cadherin-treated and anti-E-cadherin-treated slices and controls. The facilitation ratio represents the slope of the second field EPSP divided by the slope of the first field EPSP for the interstimulus intervals shown.

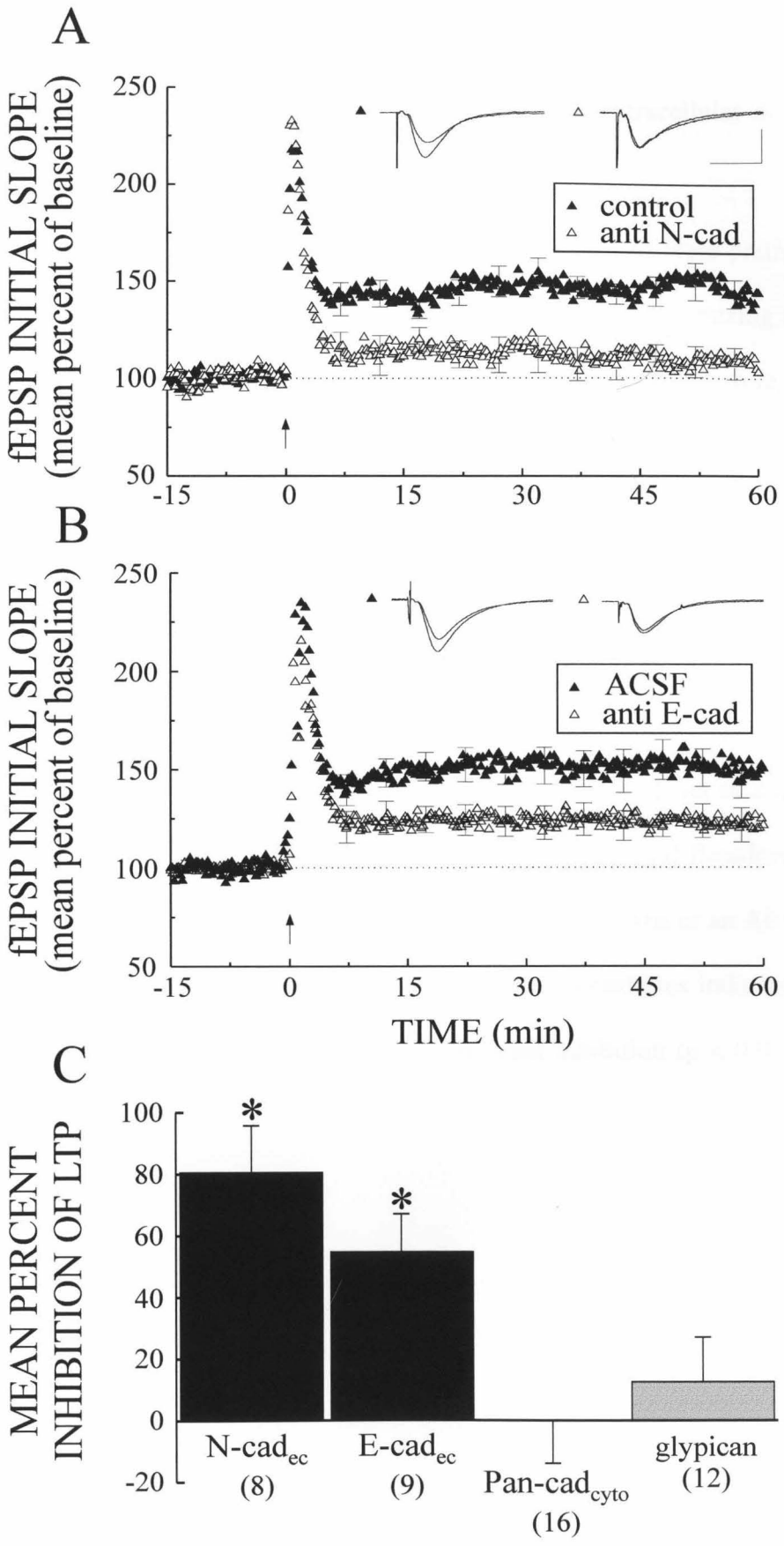

A
fEPSP INITIAL SLOPE
(mean percent of baseline)
250
200
150
100
50
-15
0
15
30
45
60
control
anti N-cad
B
fEPSP INITIAL SLOPE
(mean percent of baseline)
ACSF
anti E-cad
TIME (min)
C
MEAN PERCENT
INHIBITION OF LTP
100
80
60
40
20
0
-20
*
*
N-cad$_{ec}$
(8)
E-cad$_{ec}$
(9)
Pan-cad$_{cyto}$
(16)
glypican
(12)

Figure 3-4. Adhesion-blocking antibodies raised against the extracellular domains of either N- or E-cadherin attenuate LTP.

(A) Ensemble average of all experiments (n = 8) in which rat slices were pretreated with an N-cadherin Ab or a non-function-blocking E-cadherin Ab (recognizing mouse but not rat E-cadherin). Superimposed representative field EPSPs taken before and 50-60 minutes after tetanus for each group are shown in (A) and (B).

(B) Ensemble average of all experiments (n = 9) in which mouse slices were pretreated with a function-blocking E-cadherin Ab or normal ACSF. Scale bar, 0.5 mV and 20 ms.

(C) Summary of all Ab experiments. Mean percent inhibition of LTP is expressed relative to the amount of LTP observed in the control pathway for each set of experiments. Shown are the summary data for the N-cadherin-treated (A) and E-cadherin-treated (B) slices as well as slices treated with cytoplasmic cadherin Abs or an Ab to the cell surface GPI-anchored molecule glypican. Numbers in parentheses indicate the n for each set of experiments. Asterisks indicate significant inhibition ($p < 0.05$, paired t-test) relative to control.

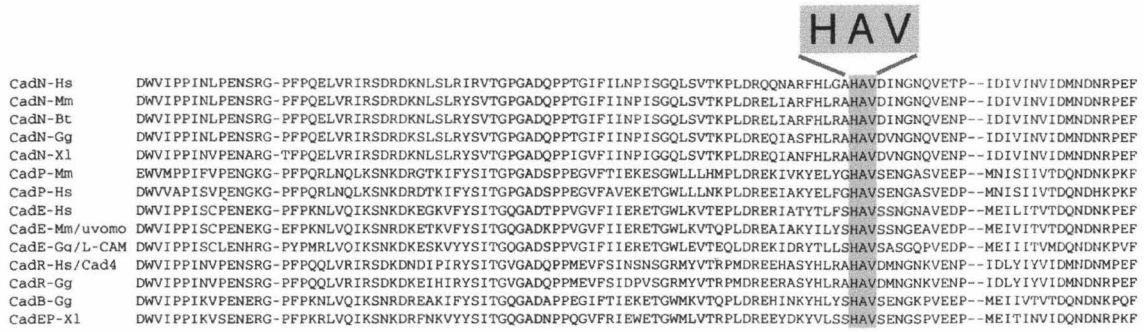

HAV
CadN-Hs DWVIPPINLPENSRG-PFPQELVRIRSDRDKNLSLRIRVTGPGADQPPTGIFILNPISGQLSVTKPLDRQQNARFHLGAHAVDINGNQVETP--IDIVINVIDMNDNRPEF
CadN-Mm DWVIPPINLPENSRG-PFPQELVRIRSDRDKNLSLRYSVTGPGADQPPTGIFIINPISGQLSVTKPLDRELIARFHLRAHAVDINGNQVENP--IDIVINVIDMNDNRPEF
CadN-Bt DWVIPPINLPENSRG-PFPQELVRIRSDRDKNLSLRYSVTGPGADQPPTGIFIINPISGQLSVTKPLDRELIARFHLRAHAVDINGNQVENP--IDIVINVIDMNDNRPEF
CadN-Gg DWVIPPINLPENSRG-PFPQELVRIRSDRDKSLSLRYSVTGPGADQPPTGIFIINPISGQLSVTKPLDREQIASFHLRAHAVDVNGNQVENP--IDIVINVIDMNDNRPEF
CadN-Xl DWVIPPINVPENARG-TFPQELVRIRSDRDKNLSLRYSVTGPGADQPPIGVFIINPIGGQLSVTKPLDREQIANFHLRAHAVDVNGNQVENP--IDIVINVIDMNDNRPEF
CadP-Mm EWVMPPIFVPENGKG-PFPQRLNQLKSNKDRGTKIFYSITGPGADSPPEGVFTIEKESGWLLLHMPLDREKIVKYELYGHAVSENGASVEEP--MNISIIVTDQNDNKPKF
CadP-Hs DWVVAPISVPENGKG-PFPQRLNQLKSNKDRDTKIFYSITGPGADSPPEGVFAVEKETGWLLLNKPLDREEIAKYELFGHAVSENGASVEDP--MNISIIVTDQNDHKPKF
CadE-Hs DWVIPPISCPENEKG-PFPKNLVQIKSNKDKEGKVFYSITGQGADTPPVGVFIIERETGWLKVTEPLDRERIATYTLFSHAVSSNGNAVEDP--MEILITVTDQNDNKPEF
CadE-Mm/uvomo DWVIPPISCPENEKG-EFPKNLVQIKSNRDKETKVFYSITGQGADKPPVGVFIIERETGWLKVTQPLDREAIAKYILYSHAVSSNGEAVEDP--MEIVITVTDQNDNRPEF
CadE-Gg/L-CAM DWVIPPISCLENHRG-PYPMRLVQIKSNKDKESKVYYSITGQGADSPPVGIFIIERETGWLEVTEQLDREKIDRYTLLSHAVSASGQPVEDP--MEIIITVMDQNDNKPVF
CadR-Hs/Cad4 DWVIPPINVPENSRG-PFPQQLVRIRSDKDNDIPIRYSITGVGADQPPMEVFSINSNSGRMYVTRPMDREEHASYHLRAHAVDMNGNKVENP--IDLYIYVIDMNDNMPEF
CadR-Gg DWVIPPINVPENSRG-PFPQQLVRIRSDKDKEIHIRYSITGVGADQPPMEVFSIDPVSGRMYVTRPMDREERASYHLRAHAVDMNGNKVENP--IDLYIYVIDMNDNRPEF
CadB-Gg DWVIPPIKVPENERG-PFPKNLVQIKSNRDREAKIFYSITGQGADAPPEGIFTIEKETGWMKVTQPLDREHINKYHLYSHAVSENGKPVEEP--MEIIVTVTDQNDNKPQF
CadEP-Xl DWVIPPIKVSENERG-PFPKRLVQIKSNKDRFNKVYYSITGQGADNPPQGVFRIEWETGWMLVTRPLDREEYDKYVLSSHAVSENGSPVEEP--MEITINVIDQNDNRPKF

Figure 3-5. Sequence alignment of the EC1 domain of cadherins showing the highly conserved HAV motifs.

The alignment was generated manually and is centered around well-conserved sites such as the HAV and LDRE motifs (Pouliot, 1992). Hs: *Homo sapiens*; Mm: *Mus musculus*; Gg: *Gallus gallus*; Bt: *Bos taurus*; Xl: *Xenopus laevis*.

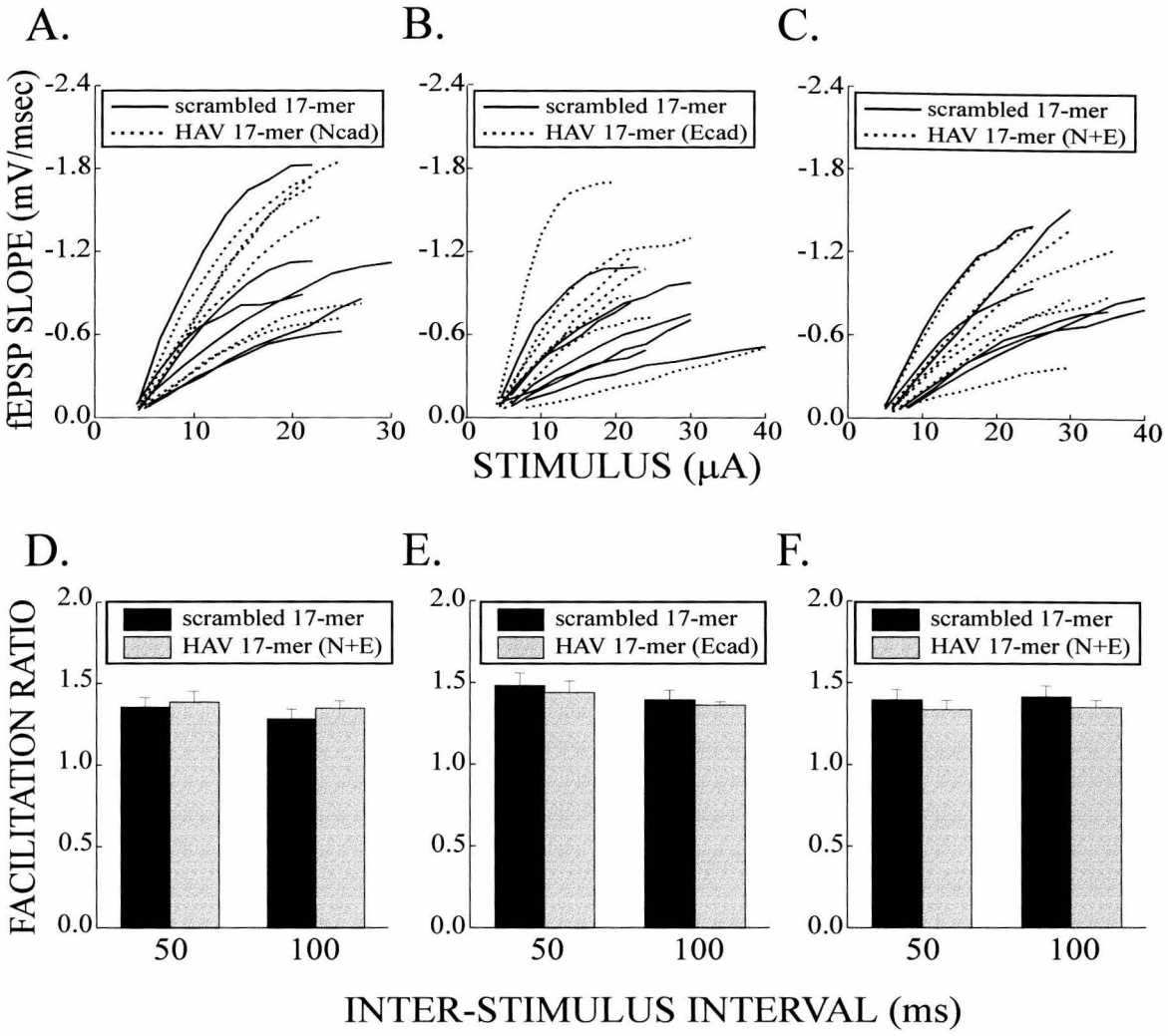

A.
B.
C.
fEPSP SLOPE (mV/msec)
-2.4
-1.8
-1.2
-0.6
0.0
0
10
20
30
40
scrambled 17-mer
HAV 17-mer (Ncad)
HAV 17-mer (Ecad)
HAV 17-mer (N+E)
STIMULUS (μA)
D.
E.
F.
FACILITATION RATIO
2.0
1.5
1.0
0.5
0.0
50
100
INTER-STIMULUS INTERVAL (ms)

Figure 3-6. HAV peptides do not alter basal properties of synaptic transmission in hippocampal slices.

(A-C) Input-output curves depicting the relationship between stimulus current intensity and the size of the field EPSP slope. Slices were treated with either an N-cadherin-derived HAV peptide (17-mer) (A), an E-cadherin-derived HAV peptide (17-mer) (B), or a combination of the two (C). These slices did not differ significantly from scrambled HAV peptide in their input-output curves.

(D-F) Facilitation ratios for HAV peptide-treated slices and controls. The facilitation ratio represents the slope of the second field EPSP divided by the slope of the first field EPSP for the interstimulus intervals shown.

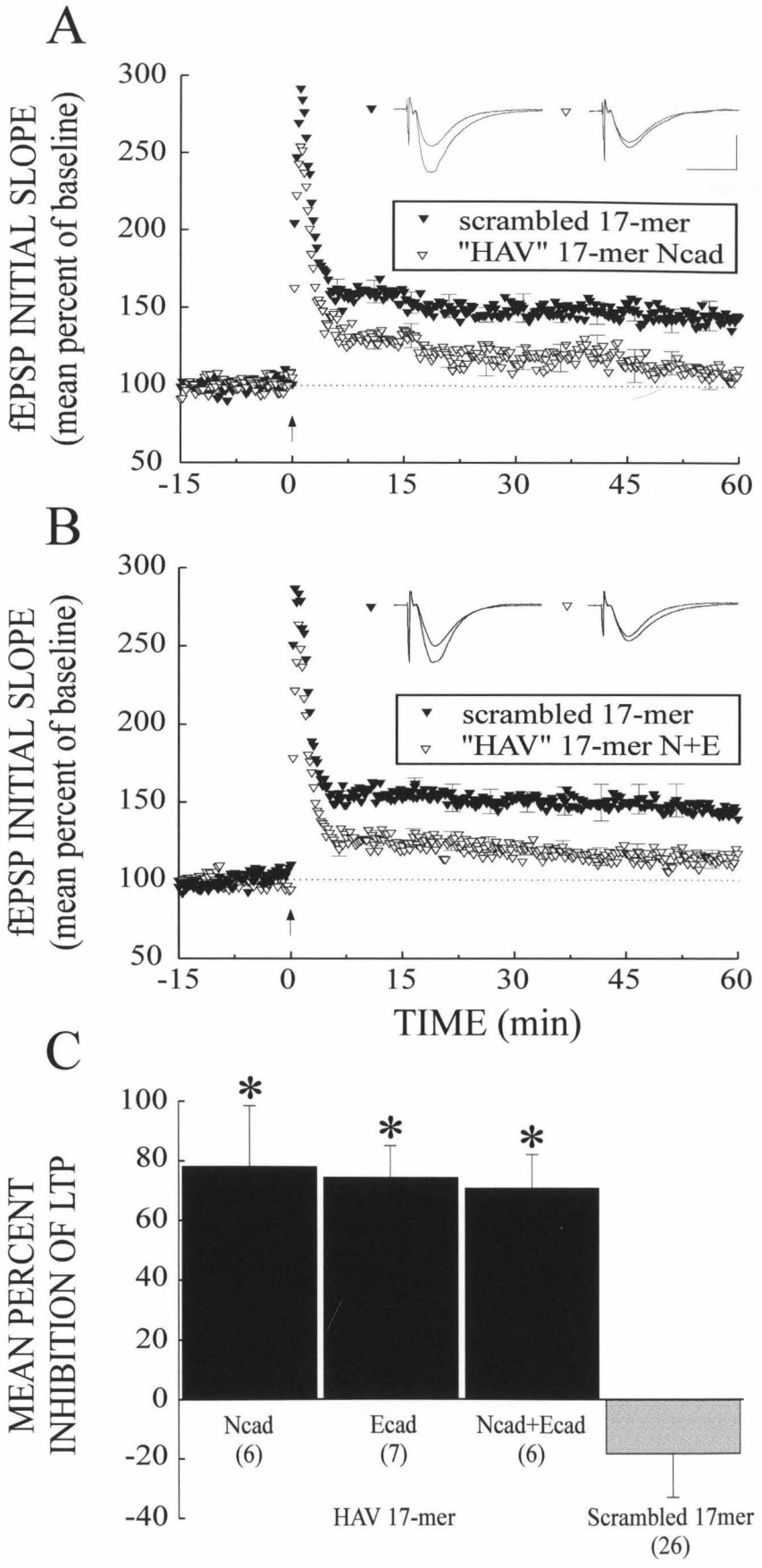

A
fEPSP INITIAL SLOPE
(mean percent of baseline)
300
250
200
150
100
50
-15
0
15
30
45
60
scrambled 17-mer
"HAV" 17-mer Ncad
B
fEPSP INITIAL SLOPE
(mean percent of baseline)
scrambled 17-mer
"HAV" 17-mer N+E
TIME (min)
C
MEAN PERCENT
INHIBITION OF LTP
100
80
60
40
20
0
-20
-40
*
Ncad
(6)
Ecad
(7)
Ncad+Ecad
(6)
HAV 17-mer
Scrambled 17mer
(26)

Figure 3-7. Slices incubated in HAV peptides exhibit diminished LTP.

Superimposed representative field EPSPs taken before and 50-60 minutes after tetanus for each group are shown in (A) and (B). Scale bar, 0.5 mV and 20 ms.

(A) Slices pretreated with an N-cadherin-derived HAV 17-mer showed significantly reduced LTP relative to adjacent slices pretreated with a scrambled 17-mer.

(B) Slices pretreated with a combination of N-cadherin-derived (1 mM) and E-cadherin-derived (1 mM) HAV 17-mer showed significantly reduced LTP relative to slices pretreated with a scrambled 17-mer (2 mM).

(C) Summary graph for all 17-mer HAV peptide experiments. Mean percent inhibition of LTP is expressed relative to the amount of LTP observed in the control pathway for each set of experiments. Numbers in parentheses indicate the "n" for each set of experiments. Asterisks indicate significant inhibition ($p < 0.05$, paired t-test) relative to control slice LTP. The controls for the scrambled peptide experiments were adjacent to ACSF-treated slices.

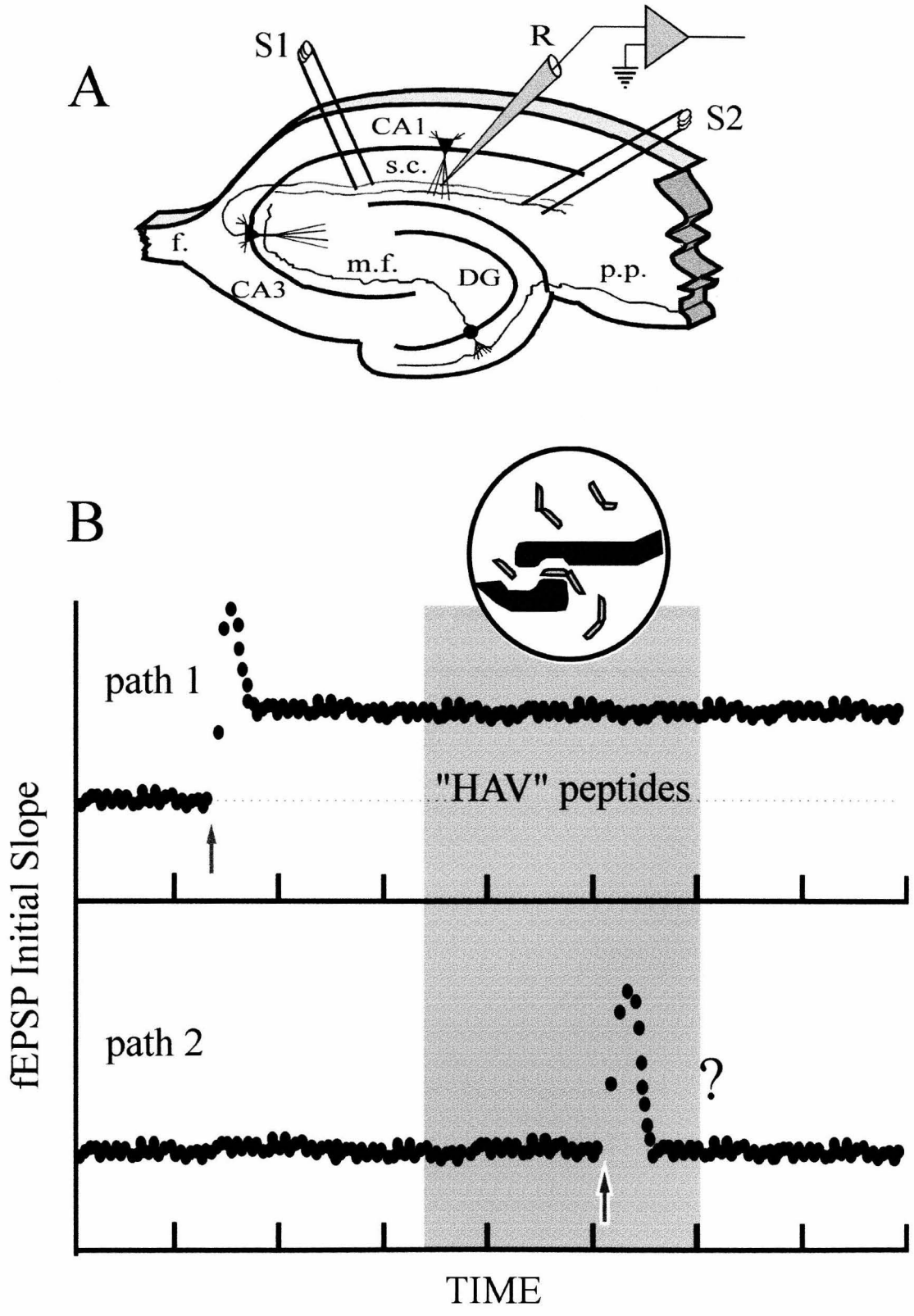

A
S1
R
S2
CA1
s.c.
f.
m.f.
DG
p.p.
CA3
B
path 1
"HAV" peptides
path 2
?
fEPSP Initial Slope
TIME

Figure 3-8. Two-pathway experimental design with HAV peptides.

(A) The approximate position of the recording electrode (R) and two stimulating electrodes (S1 and S2) in a single slice is shown. The stimulating electrode activated two independent sets of Schaffer collateral axons making synapses onto CA1 postsynaptic cells whose EPSPs were recorded from the recording electrode.

(B) A schematic diagram of experimental design to examine the effect of HAV peptides on synaptic transmission and LTP. Thirty minutes following LTP induction (arrow) in the first pathway, the HAV peptide (0.2 mM) was introduced into the ACSF to disrupt cadherin dimerization (shown schematically in the large circle). High frequency stimulation (arrow) was then delivered to the second pathway in the continued presence of the peptide. The amount of potentiation (question mark) in the second pathway was measured and compared to that in the first pathway.

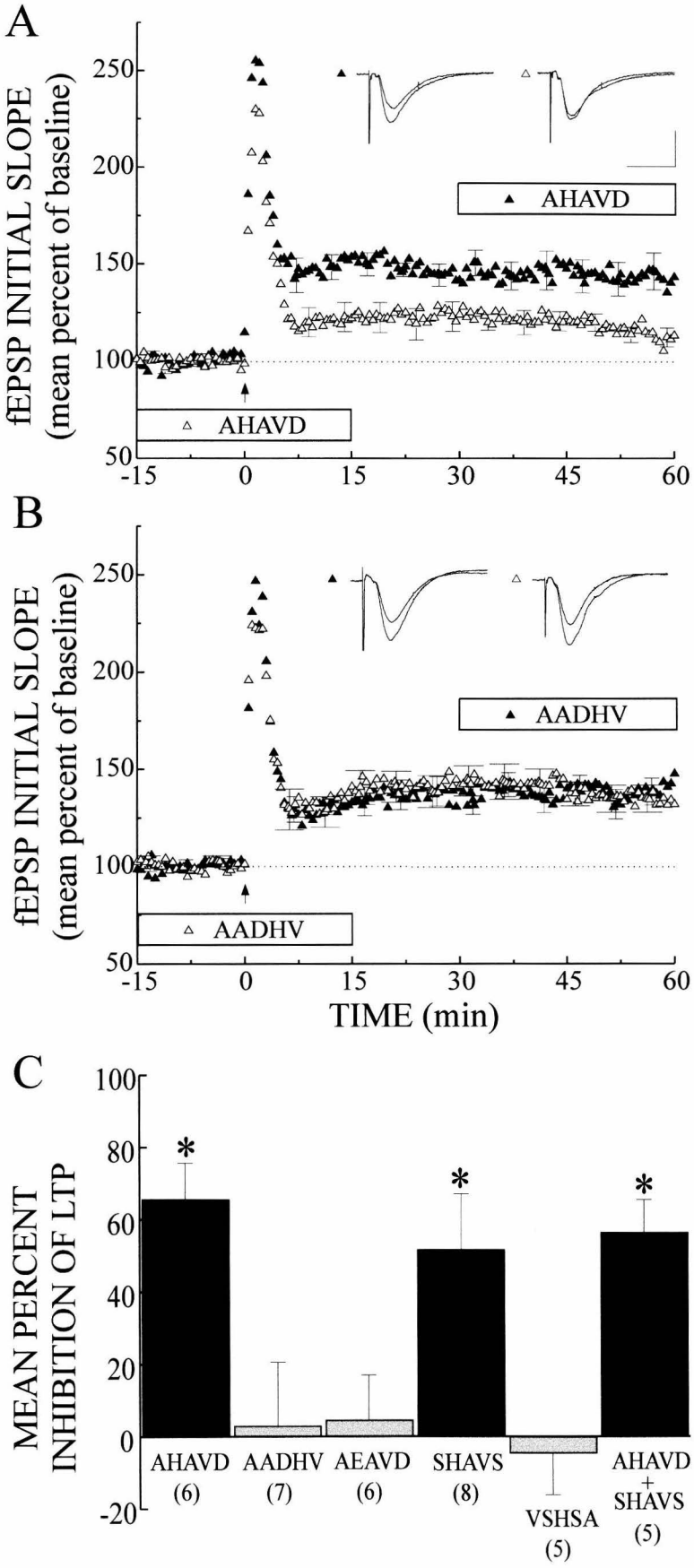

A
fEPSP INITIAL SLOPE
(mean percent of baseline)
250
200
150
100
50
AHAVD
AHAVD
-15
0
15
30
45
60
B
fEPSP INITIAL SLOPE
(mean percent of baseline)
250
200
150
100
50
AADHV
AADHV
-15
0
15
30
45
60
TIME (min)
C
MEAN PERCENT
INHIBITION OF LTP
100
80
60
40
20
0
-20
*
*
*
AHAVD
(6)
AADHV
(7)
AEAVD
(6)
SHAVS
(8)
VSHSA
(5)
AHAVD
+
SHAVS
(5)

Figure 3-9. HAV peptides perturb the induction of LTP.

Superimposed ensemble averages from two-pathway experiments conducted in the same slices. Individual superimposed representative electrophysiological traces are shown for each experimental group 10 minutes before and 50-60 minutes after LTP induction. Boxes indicate application of the peptide.

(A) Bath application of an AHAVD peptide for 30 minutes prior to tetanus significantly reduced LTP in pathway 2 (open triangles), whereas application of the same peptide 30 minutes after LTP induction in pathway 1 had no significant effect on established potentiation (closed triangles). Scale bar, 0.5 mV and 20 ms.

(B) Bath application of a control AADHV peptide for either 30 minutes prior to tetanus (open triangles) or 30 minutes after tetanus (closed triangles) had no significant effect on the initiation or maintenance of LTP.

(C) Summary of all two-pathway experiments conducted with 5-mer HAV peptides; only peptides containing the HAV motif in the correct orientation showed inhibitory activity. Mean percent inhibition of LTP is expressed relative to the amount of LTP observed in the control pathway for each set of experiments. The numbers in parentheses indicate the "n" for each experimental group. Asterisks indicate significant inhibition ($p < 0.05$, paired t-test) relative to LTP of control pathways.

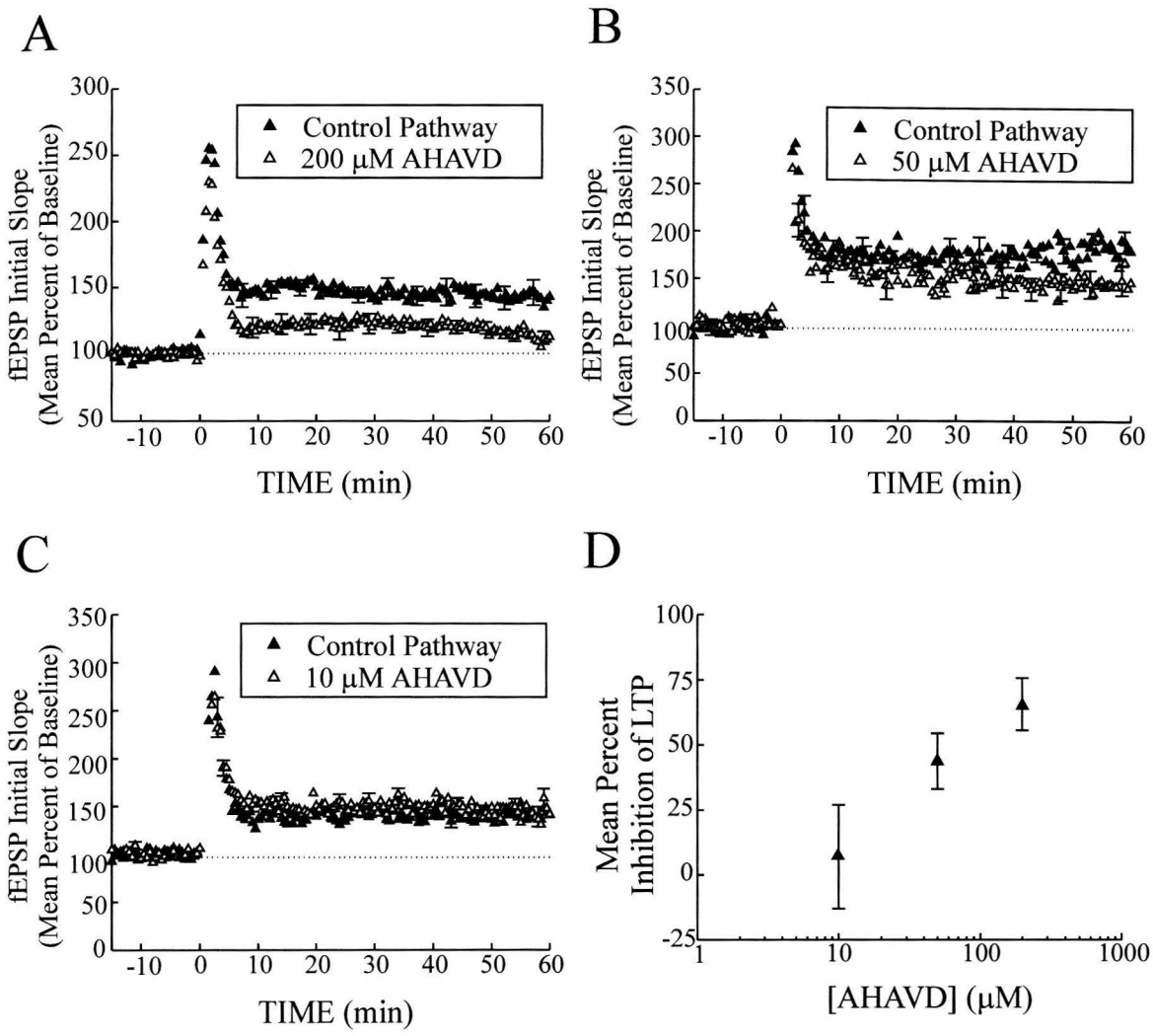

A
B
C
D
Control Pathway
200 μM AHAVD
50 μM AHAVD
10 μM AHAVD
fEPSP Initial Slope
(Mean Percent of Baseline)
TIME (min)
Mean Percent
Inhibition of LTP
[AHAVD] (μM)

Figure 3-10. Concentration-dependent inhibition of LTP by HAV peptides.

(A) Ensemble average of two-pathway experiments (n = 6) in which the effect of bath application of 200 μM AHAVD for 30 minutes prior to (open symbols) or 30 minutes after tetanus (closed symbols) is examined. LTP was significantly reduced by 200 μM AHAVD ($p < 0.05$, paired t-test).

(B) Ensemble average of two-pathway experiments (n = 5) in which the effect of bath application of 50 μM AHAVD for 30 minutes prior to (open symbols) or 30 minutes after tetanus (closed symbols) is examined. LTP was significantly reduced by 50 μM AHAVD ($p < 0.05$, paired t-test).

(C) Ensemble average of two-pathway experiments (n = 5) in which the effect of bath application of 10 μM AHAVD for 30 minutes prior to (open symbols) or 30 minutes after tetanus (closed symbols) is examined. LTP was not significantly affected by 10 μM AHAVD.

(D) Summary graph depicting the relations between the concentration of HAV peptides and the mean percent inhibition of LTP shown in (A), (B) and (C). Mean percent inhibition of LTP is expressed relative to the amount of LTP observed in the control pathway for each set of experiments.

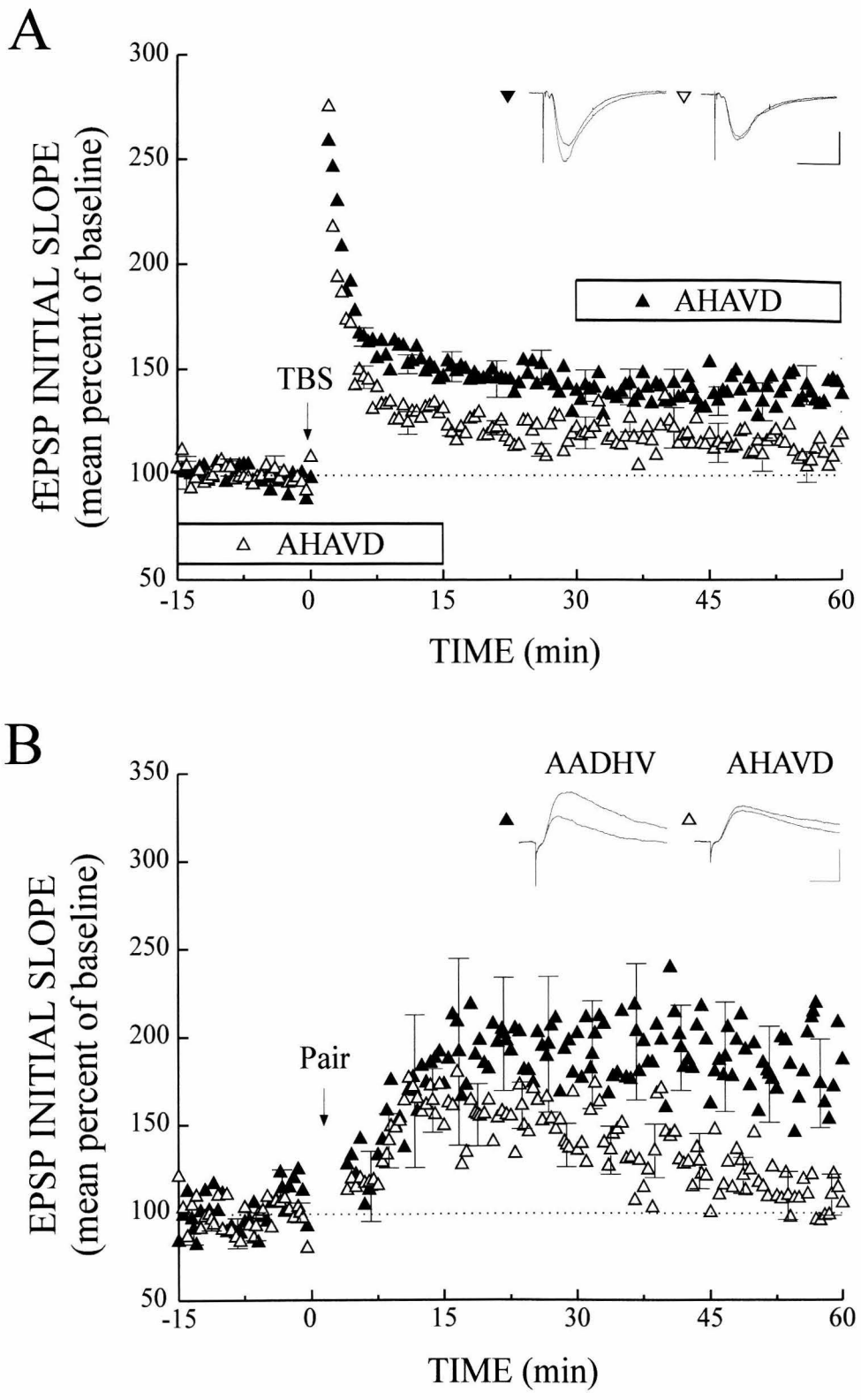

A
fEPSP INITIAL SLOPE
(mean percent of baseline)
300
250
200
150
100
50
-15
0
15
30
45
60
TIME (min)
TBS
AHAVD
AHAVD
B
EPSP INITIAL SLOPE
(mean percent of baseline)
350
300
250
200
150
100
50
-15
0
15
30
45
60
TIME (min)
Pair
AADHV
AHAVD

Figure 3-11. HAV peptides also inhibit LTP induced by other protocols.

Individual superimposed representative electrophysiological traces are shown for each experimental group 10 minutes before and 50-60 minutes after LTP induction.

(A) Ensemble average for a series of two-pathway experiments in which the HAV peptide was applied either 30 minutes before (open triangles) or after (closed triangles) LTP induction by TBS. Scale bar, 0.5 mV and 20 ms. Boxes indicate application of the peptide.

(B) Ensemble average for experiments in which LTP was induced by pairing postsynaptic depolarization with low frequency stimulation (1 Hz, 1 minute) in the intracellular recording configuration in the presence of the correct orientation HAV peptide (open triangles) or the scrambled (closed triangles) peptide. Scale bar, 5.0 mV and 20 ms.

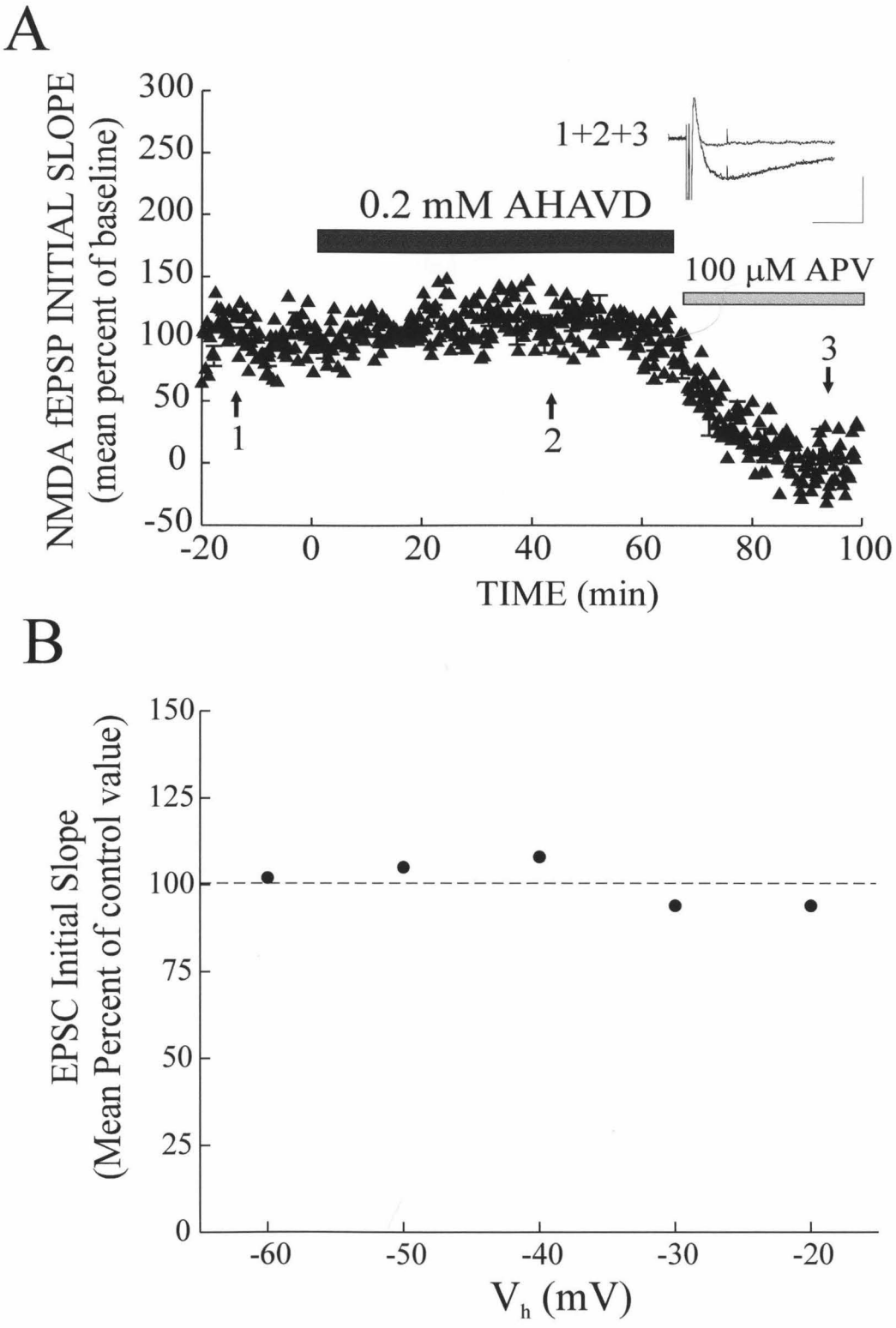

A
NMDA fEPSP INITIAL SLOPE
(mean percent of baseline)
300
250
200
150
100
50
0
-50
1+2+3
0.2 mM AHAVD
100 μM APV
1
2
3
-20
0
20
40
60
80
100
TIME (min)
B
EPSC Initial Slope
(Mean Percent of control value)
150
125
100
75
50
25
0
-60
-50
-40
-30
-20
V_h (mV)

Figure 3-12. HAV peptides do not affect NMDA receptor-mediated responses.

(A) Ensemble average of experiments (n = 5) examining field EPSPs mediated by NMDA receptors before and after the addition of the AHAVD peptide.

(B) Summary of NMDA receptor-mediated EPSCs at a range of membrane holding potentials (n = 2). Mean percent of control value is expressed as EPSC initial slope after addition of 200 μM AHAVD relative to that before addition of the peptide.

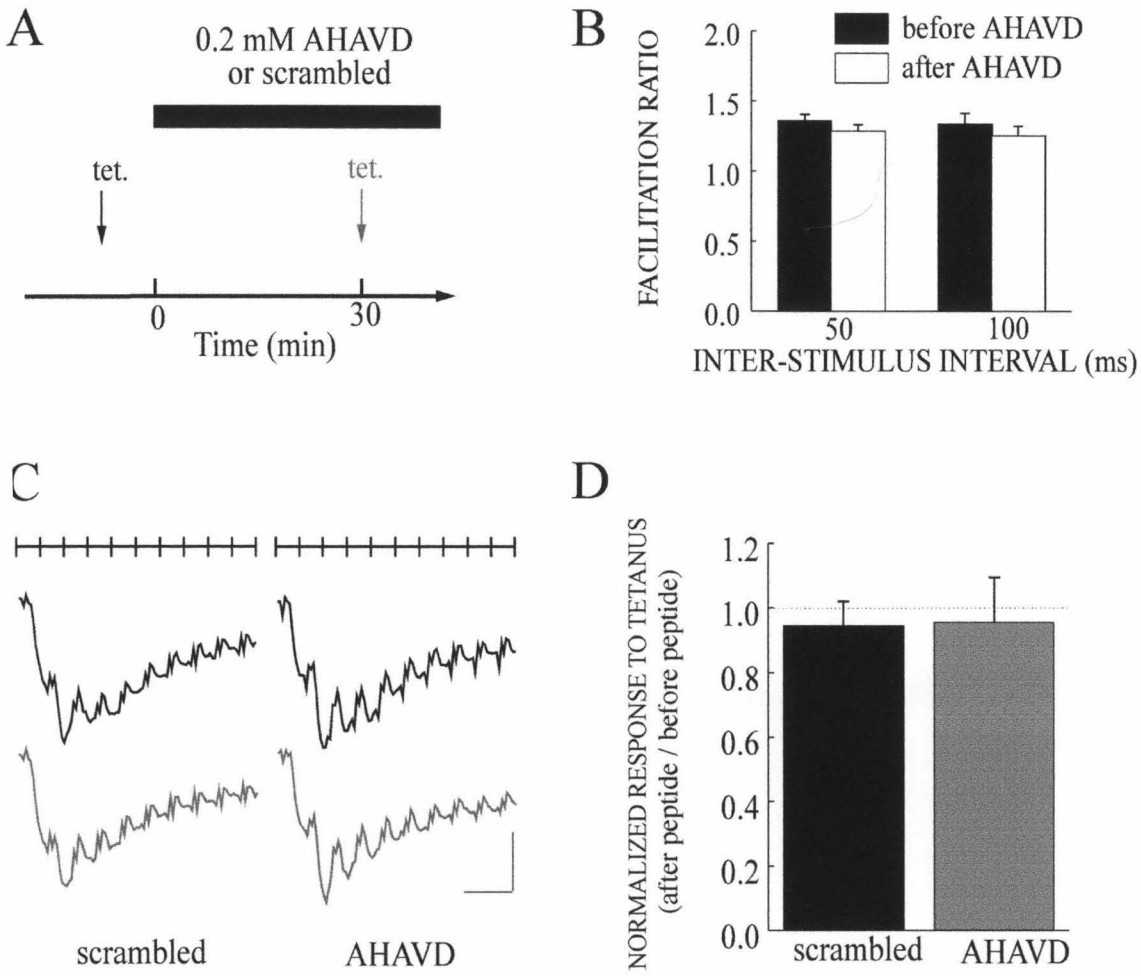

A
0.2 mM AHAVD
or scrambled
tet.
tet.
0
30
Time (min)
B
before AHAVD
after AHAVD
FACILITATION RATIO
2.0
1.5
1.0
0.5
0.0
50
100
INTER-STIMULUS INTERVAL (ms)
C
scrambled
AHAVD
D
NORMALIZED RESPONSE TO TETANUS
(after peptide / before peptide)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
scrambled
AHAVD

Figure 3-13. HAV peptides do not inhibit PPF, or postsynaptic depolarization mechanisms.

(A) Experiment design to examine the postsynaptic neuron's ability to depolarize before and after addition of 0.2 mM AHAVD.

(B) Facilitation ratios for HAV peptide-treated slices. The facilitation ratio represents the slopes of the second field EPSP divided by the slope of the first field EPSP for the interstimulus intervals shown.

(C) Representative field potential recordings obtained during a tetanus before (top) and after (bottom) addition of either a scrambled (left) or correct (right) orientation HAV peptide. Scale bar, 0.5 mV and 20 ms.

(D) Summary of the normalized response to tetanus for either the scrambled or the correct orientation HAV peptide.

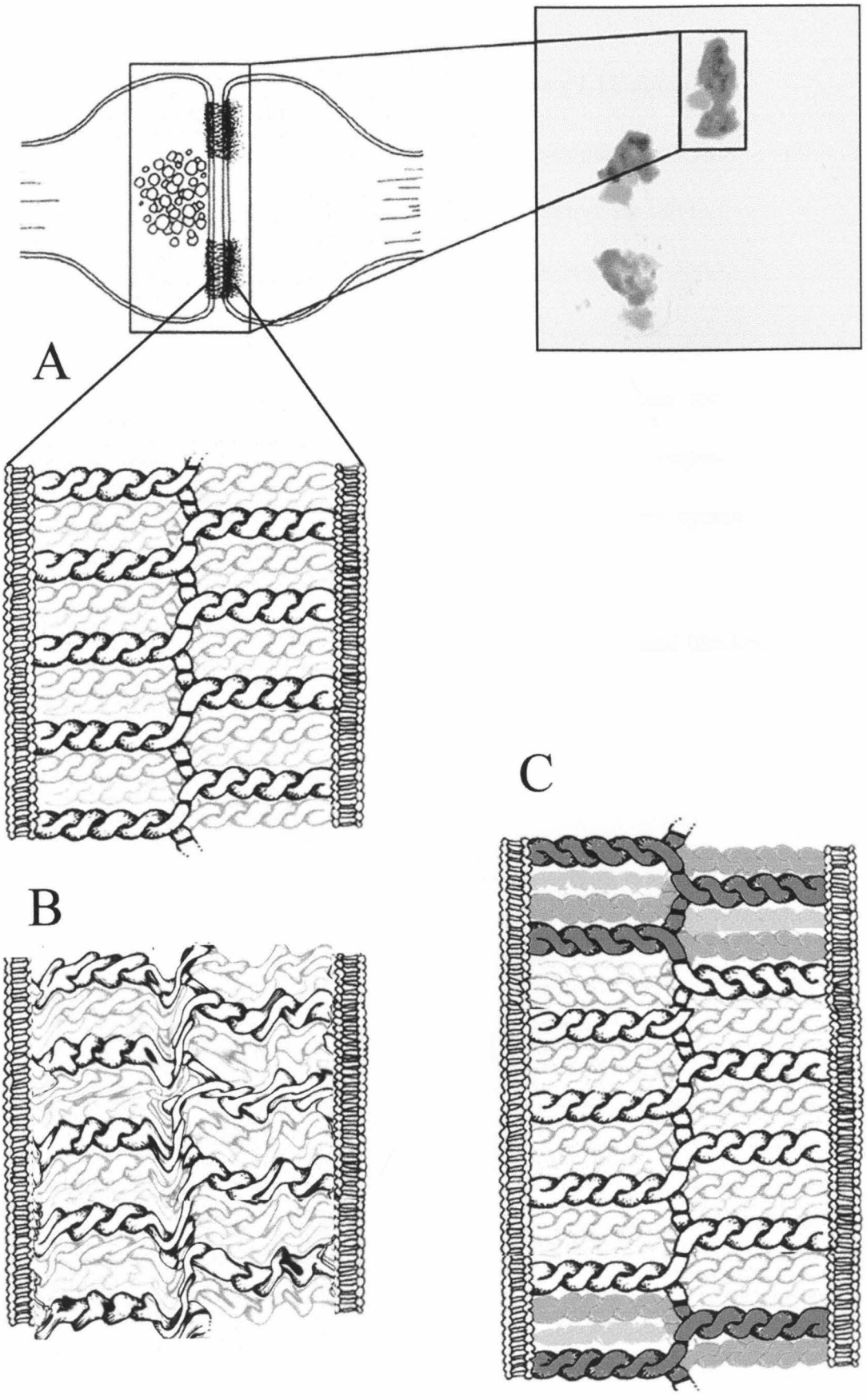
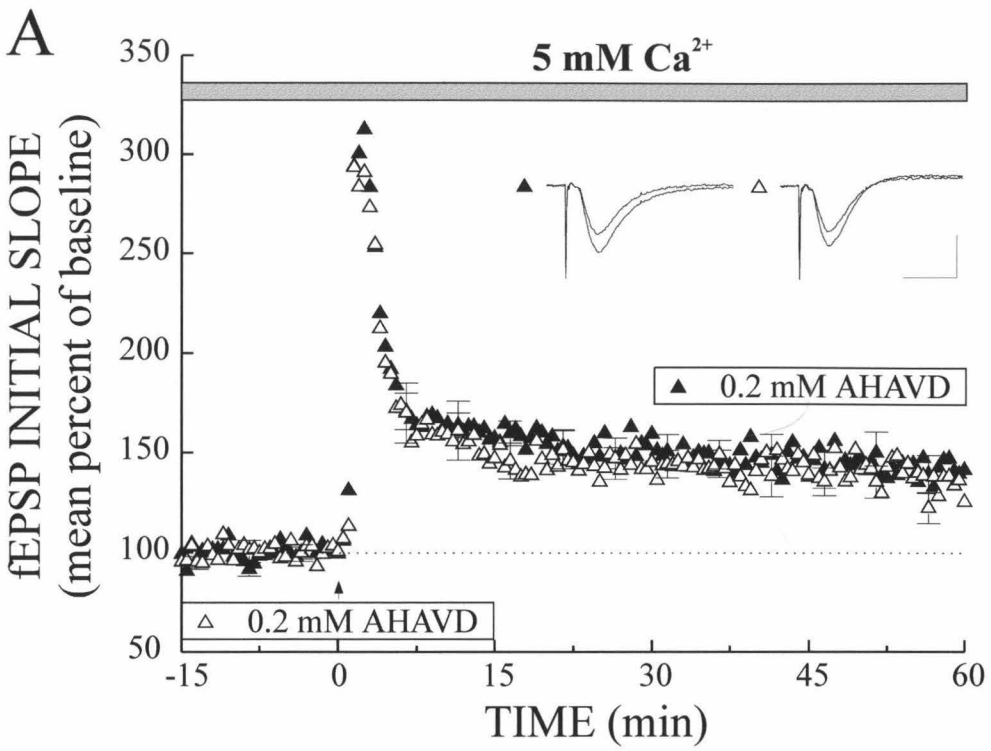

A
B
C

Figure 3-14. Hypothetical model of cadherin action during LTP induction.

(A) Modified from a model of cadherin-based central synapse proposed by Fannon et al. (1996). This model is based on the dual immunofluorescent labeling in mouse cerebellar synapses in which annular cadherin signal surrounding central synaptophysin (a presynaptic vesicle protein) signal was observed (Fannon and Colman, 1996).

(B) Intensive synaptic activity associated with LTP induction may render the cadherin junctions susceptible to inhibition by the cadherin inhibitory reagents. This could be achieved by transient reductions in Ca^{2+} concentration in the synaptic cleft during LTP induction.

(C) New cadherin bonds may be formed during LTP induction and blocked by the cadherin inhibitory reagents.


A
5 mM Ca2+
fEPSP INITIAL SLOPE
(mean percent of baseline)
350
300
250
200
150
100
50
0.2 mM AHAVD
0.2 mM AHAVD
-15
0
15
30
45
60
TIME (min)

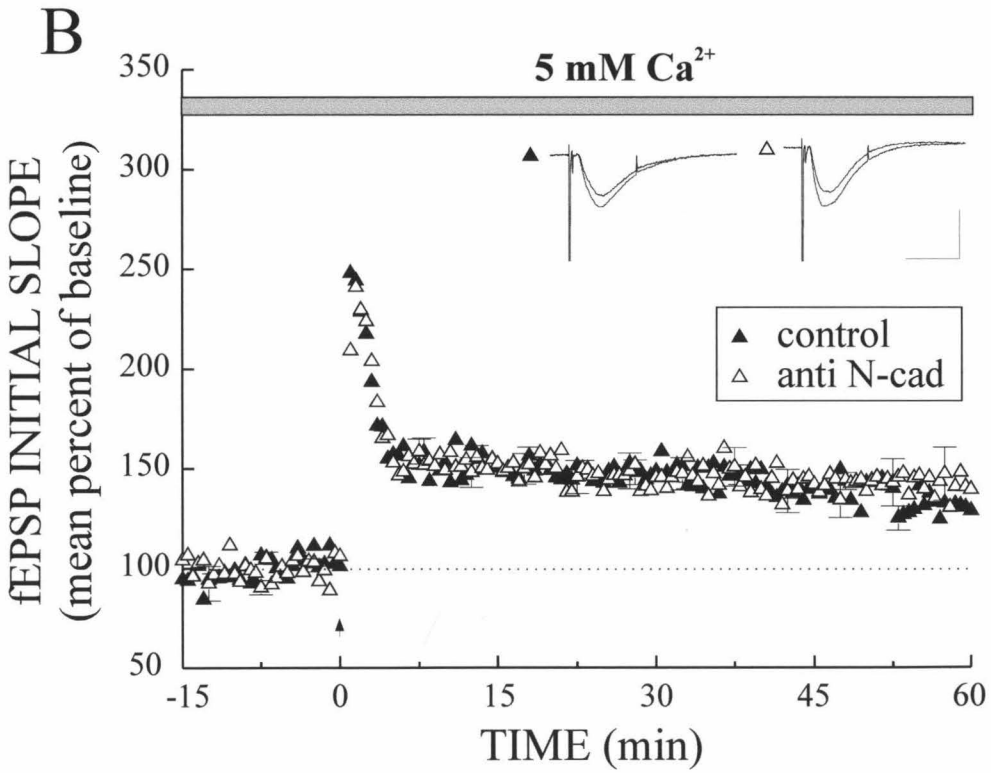

B
5 mM Ca2+
fEPSP INITIAL SLOPE
(mean percent of baseline)
350
300
250
200
150
100
50
control
anti N-cad
-15
0
15
30
45
60
TIME (min)

Figure 3-15. Elevated extracellular Ca^{2+} prevents the block of LTP by cadherin inhibitory reagents.

Individual superimposed representative electrophysiological traces are shown for each experimental group 10 minutes before and 50-60 minutes after LTP induction. Scale bar, 0.5 mV and 20 ms.

(A) Ensemble average for two-pathway experiments (n = 9) in which the HAV peptide was applied either 30 minutes before (open symbols) or after (closed symbols) LTP induction by tetanus in an altered ACSF containing 5.0 mM Ca^{2+}. Boxes indicate application of the peptide.

(B) Ensemble average of all experiments (n = 6) in which slices were pretreated with either a function-blocking N-cadherin Ab or a non-function-blocking E-cadherin Ab in the presence of an ACSF containing 5.0 mM Ca^{2+}.

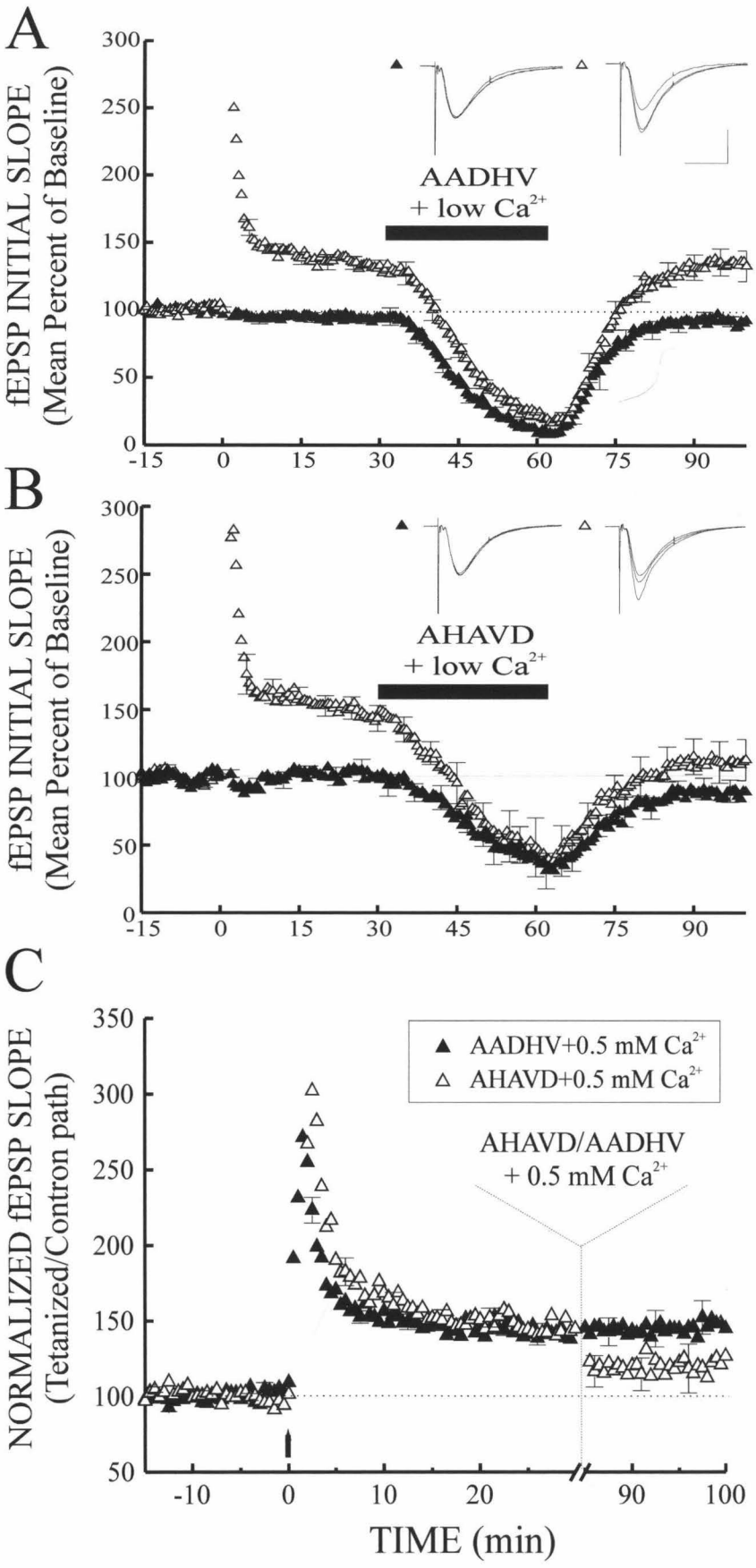

A
fEPSP INITIAL SLOPE
(Mean Percent of Baseline)
AADHV
+ low Ca2+
B
fEPSP INITIAL SLOPE
(Mean Percent of Baseline)
AHAVD
+ low Ca2+
C
NORMALIZED fEPSP SLOPE
(Tetanized/Contron path)
AADHV+0.5 mM Ca2+
AHAVD+0.5 mM Ca2+
AHAVD/AADHV
+ 0.5 mM Ca2+
TIME (min)

Figure 3-16. HAV peptides delivered in low Ca^{2+} ACSF reverse LTP.

Individual superimposed representative electrophysiological traces are shown for each experimental group 10 minutes before, 20-30 minutes, and 90-100 minutes after LTP induction. Scale bar, 0.5 mV and 20 ms.

(A) Ensemble average for two-pathway experiment (n = 7) in which the scrambled version of peptide (AADHV, 0.2 mM) in an altered ACSF containing 0.5 mM Ca^{2+} was applied 30 minutes after LTP induction and washed out 60 minutes after LTP induction.

(B) Ensemble average for two-pathway experiment (n = 7) in which the AHAVD (0.2 mM) in an altered ACSF containing 0.5 mM Ca^{2+} was applied 30 minutes after LTP induction and washed out 60 minutes after LTP induction.

(C) Normalized field EPSP from (A) and (B) showing LTP is reduced after treatment with HAV peptides in 0.5 mM Ca^{2+} ACSF ($p < 0.05$, paired t-test). Normalized field EPSP initial slope is expressed as a ratio between field EPSP initial of the LTP pathway and that of the control pathway. Significance is calculated between the amount of potentiation at 90-100 minutes and that at 20-30 minutes after LTP induction.

Chapter 4. Recombinant Ad-virus containing mutant cadherin DNA

4.1 Introduction

In recent years there has been a rapid development in gene transfer techniques based on the properties of DNA viruses such as adenovirus and recombinant DNA technology (Becker et al., 1994). Adenoviruses engineered to carry a foreign DNA has been a useful tool to deliver foreign genes into host cells (Berkner, 1988) and study the expression and/or function of the protein of interest in the host cells (for example in synaptic plasticity, Kantor et al., 1996).

In this thesis, an engineered adenovirus containing a mutant (dominant-negative) cadherin cDNA was constructed and used to assess the role of cadherins in synaptic plasticity in adult hippocampus. Takeichi and his colleagues showed that when cDNA of cN390Δ was introduced and expressed in PAM212 cells (mouse keratinocyte cell line), cadherin-dependent adhesion mediated by endogenous E- and P-cadherins in the transfected PAM 212 cells was inhibited, resulting in the dispersion of cell colonies (Fujimori and Takeichi, 1993). Immunolabeling of transfected cells indicated that as a result of cN390Δ expression, the nonfunctional cadherins occupied the sites where endogenous

cadherins should localize through interactions with the cytoskeleton (Fujimori and Takeichi, 1993).

4.2 Construction of Ad-cN390Δ virus

The mutant cadherin DNA (cN390Δ) used in these experiments encodes a mutant chicken N-cadherin in which a large portion of the extracellular domain containing 390 amino acids was deleted using EcoRI and Nsp V (Figure 4-1) (Fujimori and Takeichi, 1993). cN390Δ was subcloned into pAC vector, which was subsequently cotransfected with adenovirus into HEK 293 cells for making recombinant, replication-deficient adenovirus.

As a control, an adenovirus containing the lacZ gene was used. The Ad-lacZ virus has been used by others as a control virus to study synaptic plasticity (Kantor et al., 1996).

4.3 Infection of slices by Ad-cN390Δ virus

To address the role of cadherins in LTP, hippocampal slices were incubated for 24 hours with Ad-cN390Δ virus diluted in the slice medium (see Appendices C-7); adjacent slices were incubated with Ad-lacZ virus diluted in the slice medium. To confirm the expression of mutant chicken N-cadherin in hippocampal slices and β-galactosidase in control slices, immunofluorescent labeling was conducted on infected slices using antibodies to either the mutant N-cadherin or β-galactosidase. Expression of these proteins after 24-30 hours infection was observed in the dendritic region of the infected hippocampal slices (Figure 4-2 A-C).

4.4 LTP is reduced in Ad-cN390Δ virus infected slices

Like function-blocking cadherin antibodies and HAV peptides, infection with the mutant N-cadherin adenovirus had no apparent effect on the basal synaptic properties such as basal synaptic transmission (Figure 4-2), posttetanic potentiation (PTP, Figure 4-2), the relationship between stimulus strength and the size of the postsynaptic response (Figure 4-3 A), and paired-pulse facilitation (PPF, Figure 4-3 B) of hippocampal slices. When LTP was examined in slices infected with Ad-cN390Δ virus or Ad-lacZ virus, however, it was found that LTP in Ad-cN390Δ virus infected slices was significantly less than that in adjacent, Ad-lacZ virus infected slices (Figure 4-2 D) (mean percent of base-line: Ad-lacZ virus, 161.2% ± 10.2% [n = 9]; Ad-cN390Δ virus, 128.7% ± 3.8% [n = 9]). These data further support that cadherins play a functional role in synaptic plasticity in the adult hippocampus.

4.5 Discussion

Interactions of the cadherin cytoplasmic domain with catenins and cytoskeletonal proteins are important for cadherin function (see Section 1.2.3.1.2); expression of a mutant cadherin lacking a cytoplasmic domain may lead to developmental defects and tumorigenesis. Injection of mRNA encoding the N-cadherin cytoplasmic domain (dominant-negative) into *Xenopus* embryos caused a dramatic inhibition of cell adhesion and cell segregation in the ectodermal cell layer (Kintner, 1992). Expression of this mutant N-cadherin also inhibited the binding of α-catenin to endogenous E-cadherin, presumably by competition for the supply of catenins (Kintner, 1992). Transfection of embryonic stem cells with a dominant negative *Xenopus* N-cadherin mutant cDNA (lacking the

extracellular region) led to an inflammatory bowel disease in the crypt and villus epithelium of adult chimeric mice (Hermiston and Gordon, 1995). Expression of the same mutant cadherin in embryonic *Xenopus* eyes impaired axon and dendrite outgrowth, particularly in retinal ganglion cells (Riehl et al., 1996). Expression of mutant chicken N-cadherin cDNA (cN390Δ) disrupts endogenous cadherin-mediated cell adhesion presumably due to occupation of expressed nonfunctional cadherins at the sites where endogenous cadherins should localize through interactions with the cytoskeleton (Fujimori and Takeichi, 1993).

As an alternative approach to access the role of cadherins in synaptic function, I used a recombinant adenovirus containing the cN390Δ cDNA to infect acute hippocampal slices and examined LTP in the infected slices. Immunofluorescent staining confirmed the expression of mutant cadherin protein or β-galactosidase in the dendritic region of Ad-cN390Δ virus or a control Ad-lacZ virus infected hippocampal slices. Ad-cN390Δ virus infected slices exhibited normal basal synaptic properties but significantly less LTP than Ad-lacZ virus infected adjacent slices.

These results, together with my earlier studies using cadherin function-blocking Abs and HAV-containing peptides, increase the evidence that cadherins play a functional role in synaptic plasticity. These data further suggest that disrupting cadherin-mediated cell adhesion affects synaptic plasticity in the adult hippocampus without compromising basal synaptic properties or the integrity of synaptic structures.

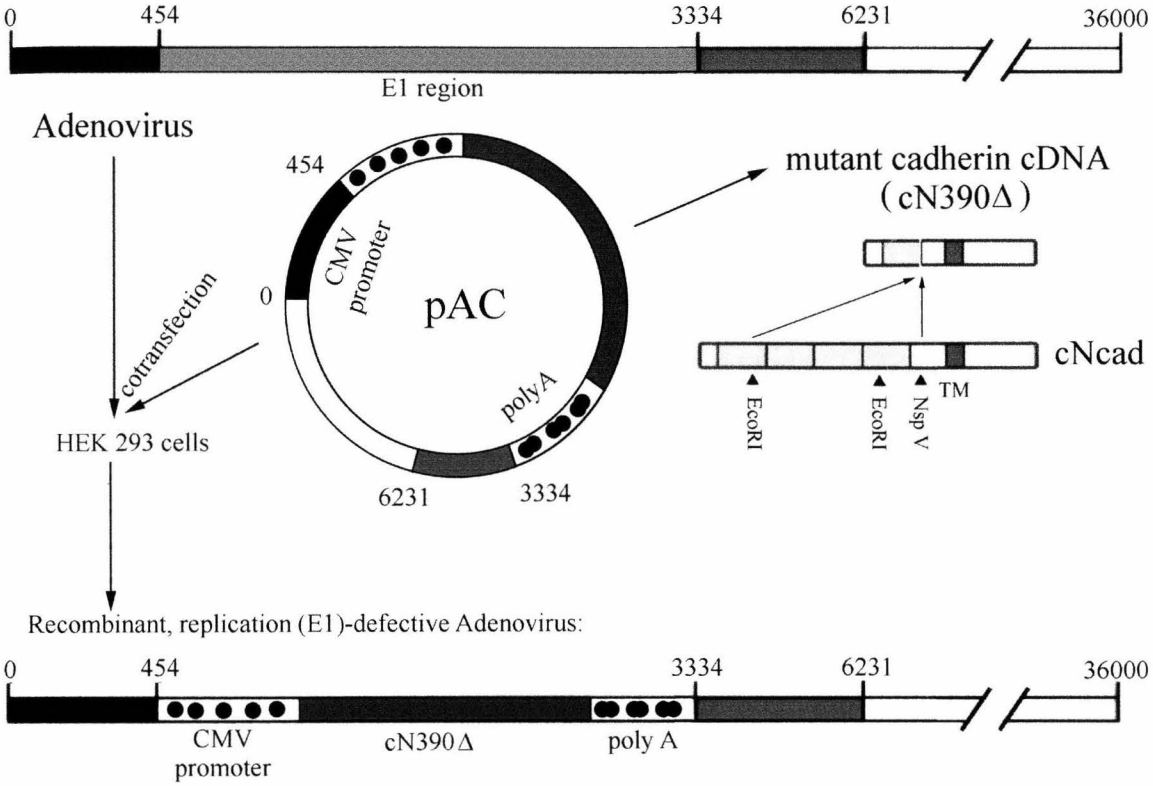
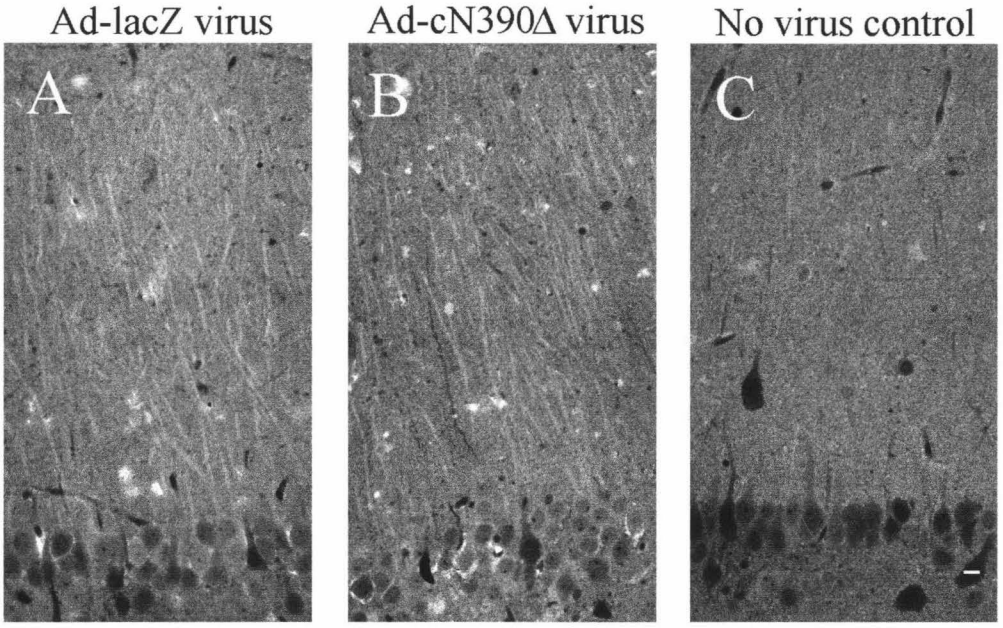

0
454
3334
6231
36000
E1 region
Adenovirus
cotransfection
HEK 293 cells
454
0
CMV promoter
pAC
polyA
6231
3334
mutant cadherin cDNA
(cN390Δ)
cNcad
EcoRI
EcoRI
Nsp V
TM
Recombinant, replication (E1)-defective Adenovirus:
0
454
3334
6231
36000
CMV promoter
cN390Δ
poly A

Figure 4-1. Construction of Ad-cN390Δ virus containing dominant-negative mutant N-cadherin cDNA.

cN390Δ was a gift from Takeichi with a large deletion of extracellular sequence encoding 390 amino acids using *EcoRI* and *Nsp V* (Fujimori and Takeichi, 1993). cN390Δ was subcloned into pAC vector, which was subsequently cotransfected with adenovirus into HEK 293 cells for making recombinant, replication-deficient adenovirus.


Ad-lacZ virus
Ad-cN390Δ virus
No virus control
A
B
C

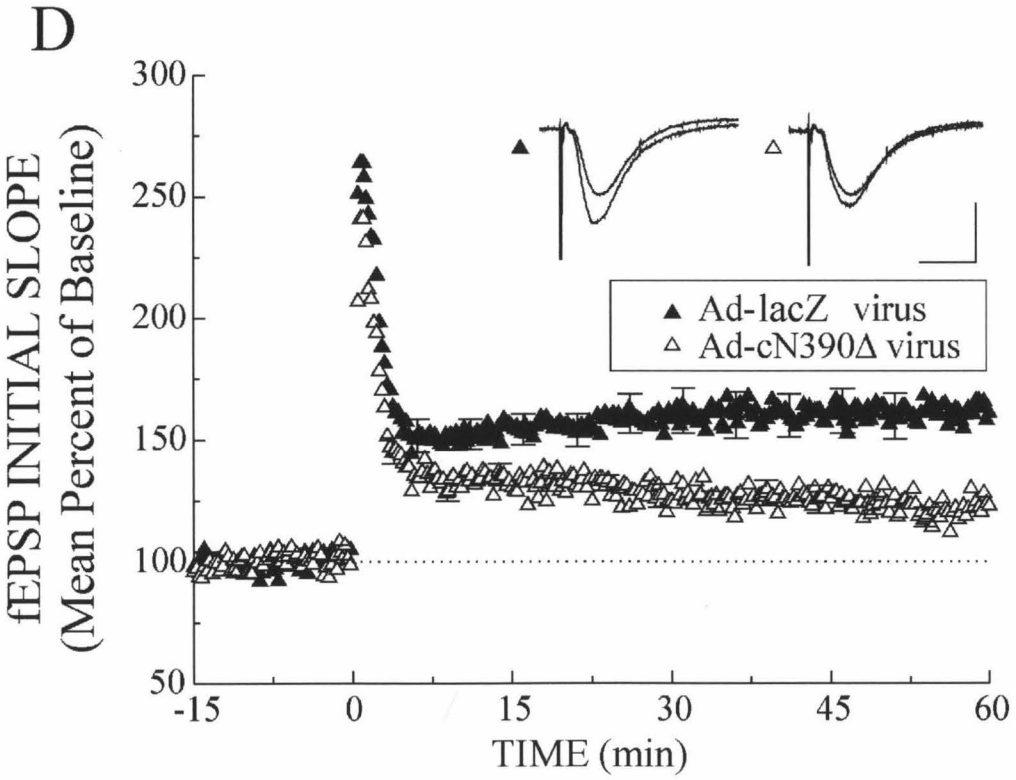

D
fEPSP INITIAL SLOPE
(Mean Percent of Baseline)
300
250
200
150
100
50
-15
0
15
30
45
60
TIME (min)
Ad-lacZ virus
Ad-cN390Δ virus

Figure 4-2. Slices infected with Ad-cN390Δ virus exhibit attenuated LTP.

(A-C) High power (63x) confocal images of the CA1 region of hippocampal slices infected with Ad-lacZ virus (A), Ad-cN390Δ virus (B), or slice medium only (C) for 24 hours, showing portions of pyramidale (bottom) and radiatum (top) layers. Immunopositive tissue is white. Slices were treated with the following primary antibodies: anti-β-galactosidase (A), NCD-2 (B), and NCD-2 (C). Scale bar: 100 μm. NCD-2: a monoclonal rat anti-N-cadherin antibody reacting with chicken N-cadherin and cN390Δ (also see Appendix C-1).

(D) Ensemble average of LTP in Ad-cN390Δ virus-infected slices (open triangles, n = 9) and in Ad-lacZ virus infected adjacent slices (closed triangles, n = 9). Individual superimposed representative electrophysiological traces are shown for each experimental group 10 minutes before and 50-60 minutes after LTP induction. Scale bar, 0.5 mV and 20 ms.

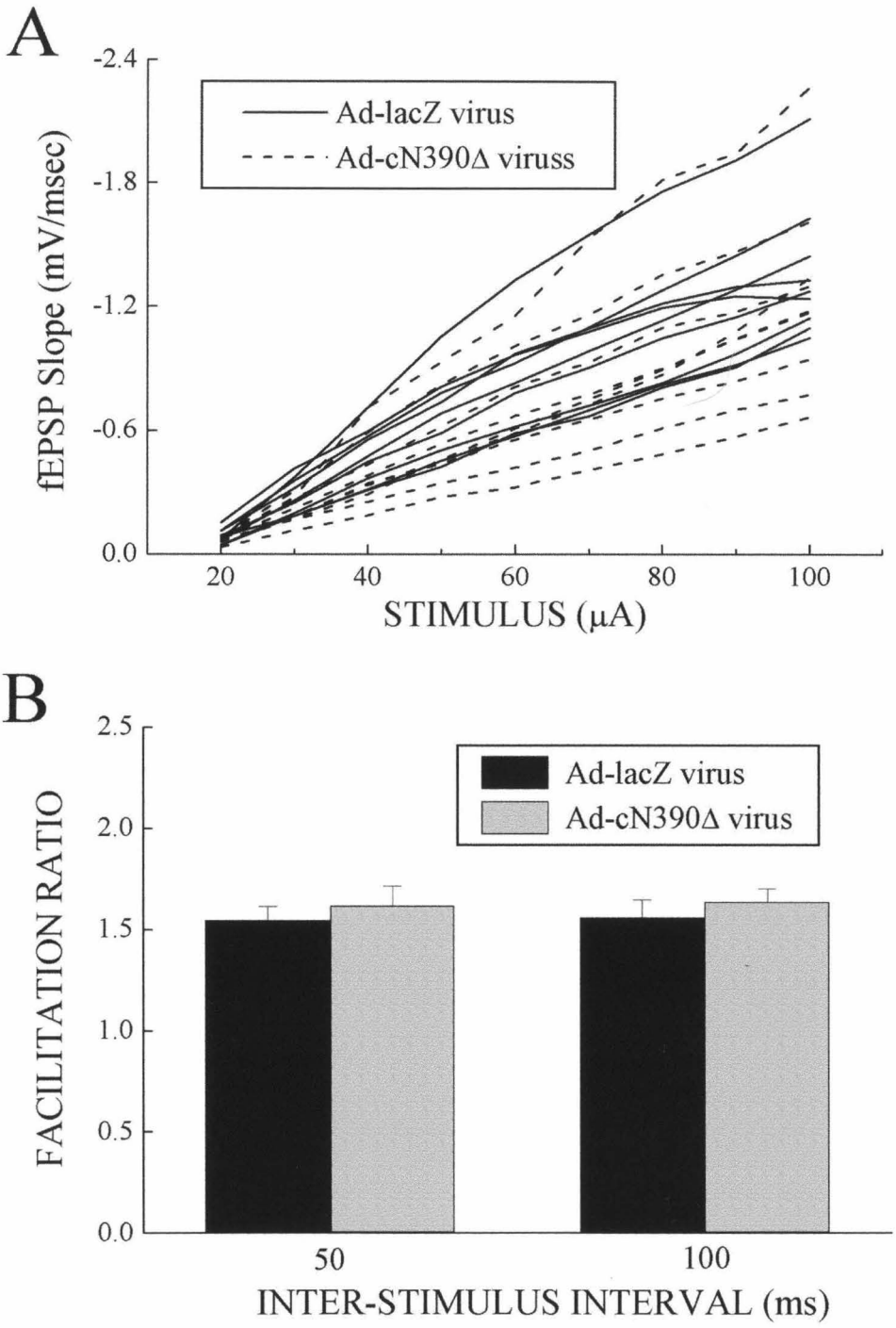

A
-2.4
-1.8
-1.2
-0.6
0.0
fEPSP Slope (mV/msec)
Ad-lacZ virus
Ad-cN390Δ viruss
20
40
60
80
100
STIMULUS (μA)
B
2.5
2.0
1.5
1.0
0.5
0.0
FACILITATION RATIO
Ad-lacZ virus
Ad-cN390Δ virus
50
100
INTER-STIMULUS INTERVAL (ms)

Figure 4-3. Basal synaptic properties in slices infected with Ad-cN390Δ virus or control Ad-lacZ virus.

(A) Input-output curves depicting the relationship between stimulus current intensity and the size of the field EPSP slope. Slices were infected with either Ad-cN390Δ virus (dotted lines), or Ad-lacZ virus (solid lines). Slices infected with Ad-cN390Δ virus did not differ significantly from Ad-lacZ virus in their input-output curves.

(B) Facilitation ratios for slices infected with Ad-cN390Δ virus (shaded) or Ad-lacZ virus (black). The facilitation ratio represents the slope of the second field EPSP divided by the slope of the first field EPSP for the interstimulus intervals shown. Slices infected with Ad-cN390Δ virus did not differ significantly from Ad-lacZ virus in the facilitation ratio.

Chapter 5. Presynaptic vesicle exocytosis

5.1 Introduction

Presynaptic neurotransmitter release is a critical component of synaptic transmission. It occurs by a process of vesicle exocytosis (Kelly, 1993). Modulation of presynaptic exocytosis is one way to change synaptic strength and function.

It is well established that vesicles that mediate fast synaptic transmission are recycled locally for repeated release (Betz et al., 1992; Ceccarelli et al., 1973; Heuser and Reese, 1973). It has been difficult to study presynaptic recycling, especially in the CNS. However, Betz and colleagues recently introduced an amphipathic fluorescent membrane probe FM 1-43 to track the recycling of cholinergic vesicles at motor nerve terminals (Betz and Bewick, 1992; Betz et al., 1992). This dye is water-soluble, which makes it virtually non-fluorescent in water-based medium. FM 1-43 is internalized within recycled vesicular membranes via endocytosis following stimulation, and becomes fluorescent (this process is called dye staining or loading). The positive charge (+2) of FM 1-43 prevents this styryl dye from crossing membranes, while the relatively short hydrocarbon chain of the dye facilitates its dissociation from the outer membrane leaflet, allowing it to be easily washed away from the outside of cells after it is released from the labeled vesicles following stimulation (this process is called dye destaining or unloading). This probe has been applied successfully to synapses in hippocampal cultures to visualize active synapses. Cultured neurons in a bath containing FM 1-43 show fluorescent punctae cor-

responding to active presynaptic boutons after stimulation by high potassium depolarization or by electrical action potentials (Ryan et al., 1993; Ryan and Smith, 1995).

In previous sections of this thesis, function-blocking cadherin antibodies or HAV-containing peptides were found to inhibit hippocampal LTP in a $[Ca^{2+}]_o$-dependent manner. It is interesting to ask whether the HAV peptides have any effect on the process of presynaptic transmitter release and what the possible cellular mechanism of HAV peptides on synaptic function would be. To begin to address this question, I used the fluorescent probe FM1-43 to examine if acute application of HAV peptides interferes with the presynaptic activity of cultured hippocampal neurons.

5.2 FM 1-43 staining and destaining

Figure 5-1 (A) shows scanning differential interference contrast (DIC) images of a representative cell culture used for the study. The morphology of cell bodies and processes is typical of pyramidal neurons from hippocampus. Figure 5-1 (B) shows a scanning fluorescence image of the same neuron after staining with 10 μM FM 1-43 in a depolarizing solution (60 mM KCl). The FM 1-43 labeled neuron show fluorescent punctae along neuronal processes, as illustrated clearly in an enlarged view in Figure 5-1 (E).

Destaining was performed by stimulating the neurons again with a KCl-containing solution without FM 1-43. Figure 5-1 (C) and (F) show the decrease of fluorescence at labeled terminals of the same neuron following the destaining of FM 1-43.

5.3 Effect of HAV peptides on the destaining of FM 1-43

To examine whether or not HAV peptides have any effect on the presynaptic vesicle exocytosis, FM 1-43 labeled cultured hippocampal neurons were incubated with either a scrambled peptide (1 mM, Figure 5-2 A) or an HAV peptide (E-cadherin-derived HAV 17-mer, 1 mM, Figure 5-2 D) in normal ACSF for 15-60 minutes. Scanning fluorescence images were taken in the continued presence of the peptides (Figure 5-2 B and E). De-staining fluorescence images of the same field were obtained 3 minutes after a puff of KCl solution was added to the neurons (Figure 5-2 C and F). The percent change in fluorescence in each labeled synaptic terminal was measured. The average percent change in fluorescence of the HAV peptide or scrambled peptide-treated neurons were compared.

As shown in Figure 5-3 (A), the HAV 17-mer peptide (1 mM) do not appear to have a significant effect on the destaining of FM 1-43 in the pretreated neuronal culture when compared to the scrambled peptide (mean percent of fluorescence change: E-cadherin 17-mer, 21.8% ± 3.1%, [n = 5]; scrambled 17-mer, 23.4% ± 3.4% [n = 5], N.S.).

My previous experiments suggested that lower extracellular Ca^{2+} may render cadherin-cadherin bonds more susceptible to the blockade of HAV peptides. In consideration of this, the above experiments were repeated with a revised experimental design. The peptide incubation was performed in a modified ACSF containing 0 Ca^{2+}. Since the destaining of presynaptic boutons is dependent upon extracellular Ca^{2+}, the incubation solution was changed into peptides in normal ACSF before destaining was performed. Under these conditions, HAV peptides (1 mM) of two different lengths (17-mer, 5-mer) did not affect the release of FM 1-43 when compared to the scrambled peptide controls

(mean percent of fluorescence change: E-cadherin 17-mer, 22.6% ± 1.9%, [n = 5]; scrambled 17-mer, 20.8% ± 2.3% [n = 9], N. S.; AHAVD, 22.7% ± 2.2%, [n = 5]; scrambled 17-mer, 24.4% ± 2.8% [n = 9], N. S.).

Since FM 1-43 labels recycling synaptic vesicles and the destaining represents vesicle turnover of labeled terminals (Ryan et al., 1993), these data suggest that acute application of HAV peptides has no significant effect on exocytosis of neurotransmitters.

5.4 Discussion

Using the fluorescent membrane label FM 1-43, I have measured the destaining of the dye in active synapses of cultured hippocampal neurons pretreated with either scrambled or HAV peptides. There is no significant difference in the destaining of synaptic boutons between HAV peptide and scrambled peptide-treated neuronal cultures. As discussed in Section 3.3, it was suggested that decreasing extracellular Ca^{2+} concentration either by modifying ACSF or by LTP induction may render a more effective blockade of cadherin homophilic bonds by HAV peptides. Therefore, further experiments to incubate neurons with the peptides in a modified ACSF containing 0 mM Ca^{2+} have also performed. Under these conditions, however, HAV peptides were not found to have significant effect on the change in fluorescence intensity following destaining of synaptic boutons by KCl stimulation. Taken together, HAV-containing peptides do not affect the exocytotic activity of synaptic vesicles of presynaptic boutons.

The lack of effect of HAV peptides on the presynaptic vesicle exocytosis seems consistent with my earlier data that HAV peptides have no appreciable effect on basal synaptic transmission in acute hippocampal slices. In 2-pathway experiments, HAV

peptides were also found not to alter the synaptic transmission in the control or potentiated pathway (Section 3.2.2.3). In these earlier experiments, HAV peptides were introduced in a normal ACSF. In later 2-pathway experiments, HAV peptides combined with a low concentration (0.5 mM) of Ca^{2+} in the ACSF were found to reverse LTP. In contrast, basal synaptic transmission in the control pathway recovered to the original level following replacement of the low Ca^{2+} ACSF (Section 3.2.3.3). Therefore, for naïve synapses, bath application of HAV peptides alone or combined with low Ca^{2+} do not affect synaptic transmission in acute hippocampal slices. Here the data of FM 1-43 experiments suggest that acute application of HAV peptides alone or in a Ca^{2+}-free ACSF does not affect the presynaptic exocytotic activity in dissociated hippocampal cultures. For synapses that have undergone LTP in hippocampal slices, cadherin function was found to be required for the maintenance of LTP since treatment with HAV peptides in 0.5 mM Ca^{2+} ACSF reversed the potentiation. The "plasticity state" of the FM 1-43 labeled synapses in the cultured hippocampal neurons before the incubation with HAV peptides in the Ca^{2+}-free ACSF is not known. It is possible that some of those synapses were potentiated, for example, by the KCl stimulation used to load FM 1-43. If that is true, one might speculate that some effect by HAV peptides in the Ca^{2+}-free ACSF should have been observed in the FM 1-43 experiments. However, the lack of effect on presynaptic exocytosis by HAV peptides, even in the Ca^{2+}-free ACSF, could be explained by the following: (1) hippocampal slices and cultures are different preparations. Intrinsic differences between them may exist. (2) Even if there is an effect on potentiated synapses by HAV peptides in the Ca^{2+}-free ACSF in these cultured neurons, modulation of presyn-

aptic exocytosis may not be the mechanism. Other presynaptic and/or postsynaptic mechanisms may operate.

Taken together, the data suggest that HAV peptides do not alter presynaptic exocytotic activity.

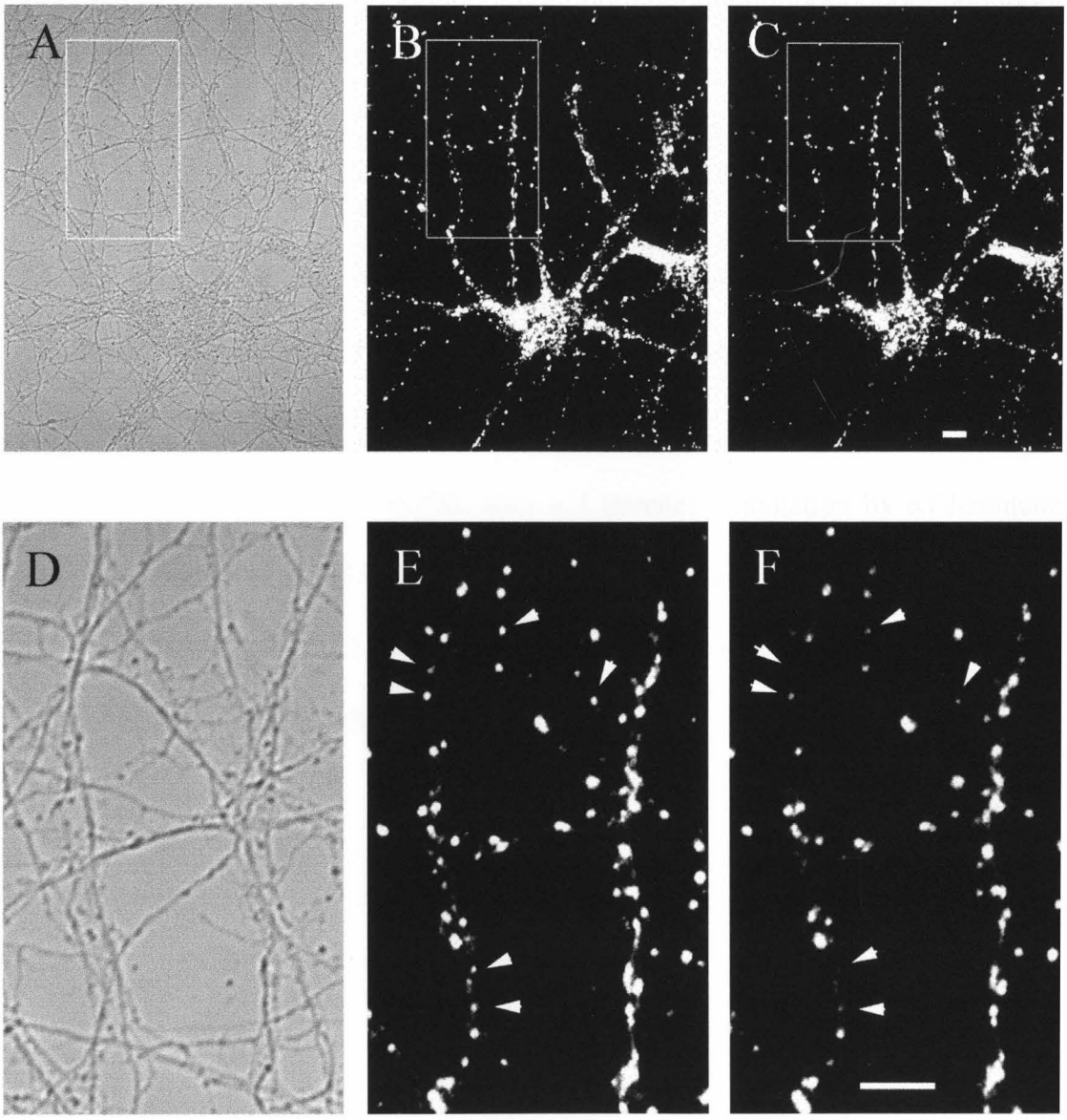

A
B
C
D
E
F

Figure 5-1. Staining and destaining of FM 1-43 labeled synaptic terminals in cultured hippocampal neurons.

(A) A scanning DIC/Normarski image of a cultured hippocampal neuron. An enlarged view of the labeled rectangle area is shown in (D).

(B) A scanning fluorescence image of the same neuron following staining with FM 1-43 following stimulation with high potassium depolarization. An enlarged view of the labeled rectangle area is shown in (E).

(C) The same field of view as in (B), after a 3 minutes stimulation by KCl-containing solutions without FM 1-43. An enlarged view of the labeled rectangle area is shown in (F). Arrowheads in (E) and (F) indicate labeled boutons undergoing apparent de-staining. Scale bars, 10 μm.

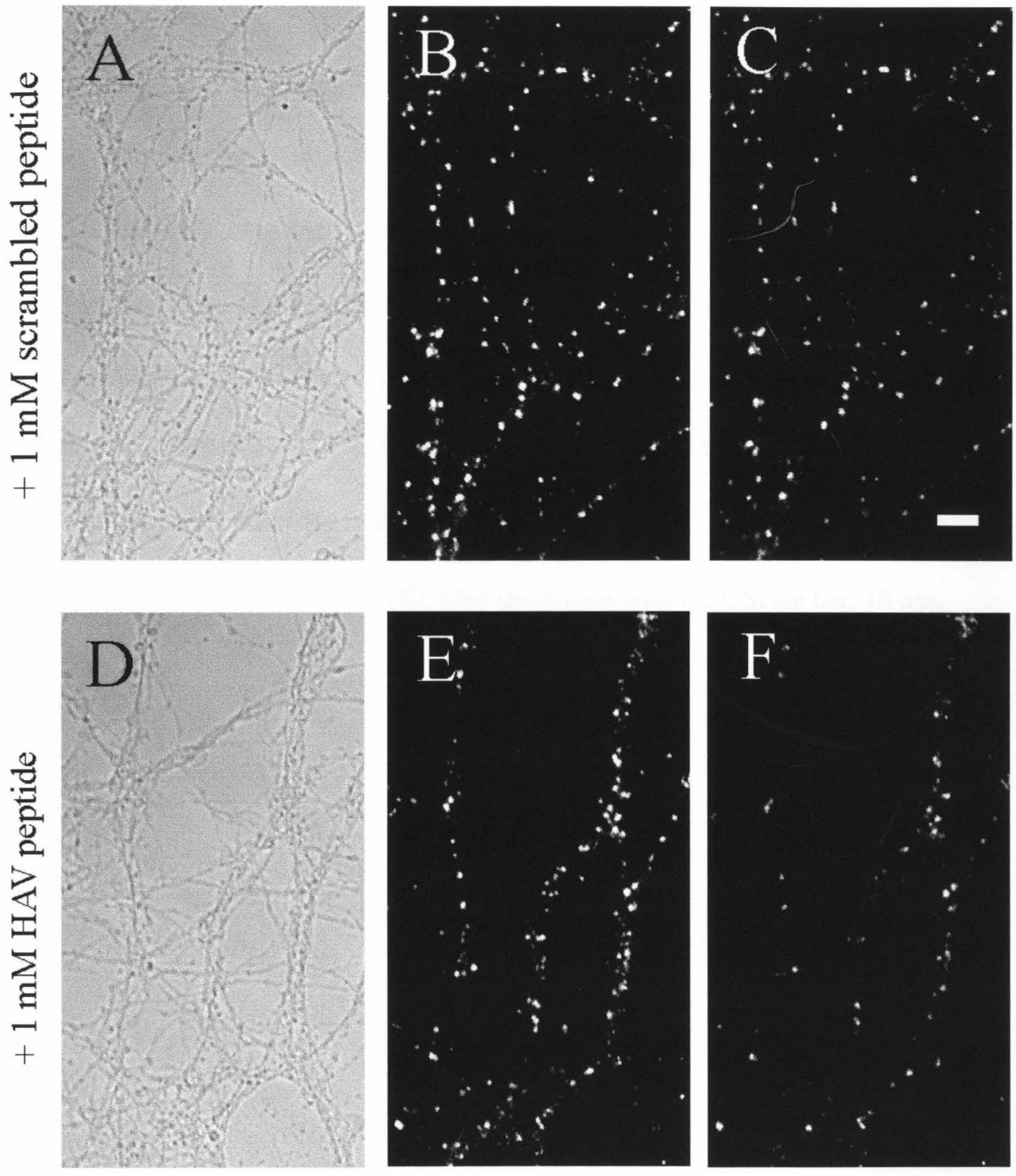

+ 1 mM scrambled peptide
A
B
C
+ 1 mM HAV peptide
D
E
F

Figure 5-2. Destaining of synaptic terminals in HAV peptide-treated hippocampal neurons.

(A) A scanning DIC/Normarski image of a cultured hippocampal neuron (processes shown) pretreated with scrambled peptides (1 mM).

(B) A scanning fluorescence image of the same neuron.

(C) The same field of view as in (B), after destaining with KCl.

(D) A scanning DIC/Normarski image of a cultured hippocampal neuron (processes shown) pretreated with E-cadherin-derived HAV-containing peptides (1 mM).

(E) A scanning fluorescence image of the same neuron.

(F) The same field of view as in (E), after destaining with KCl. Scale bar, 10 μm.

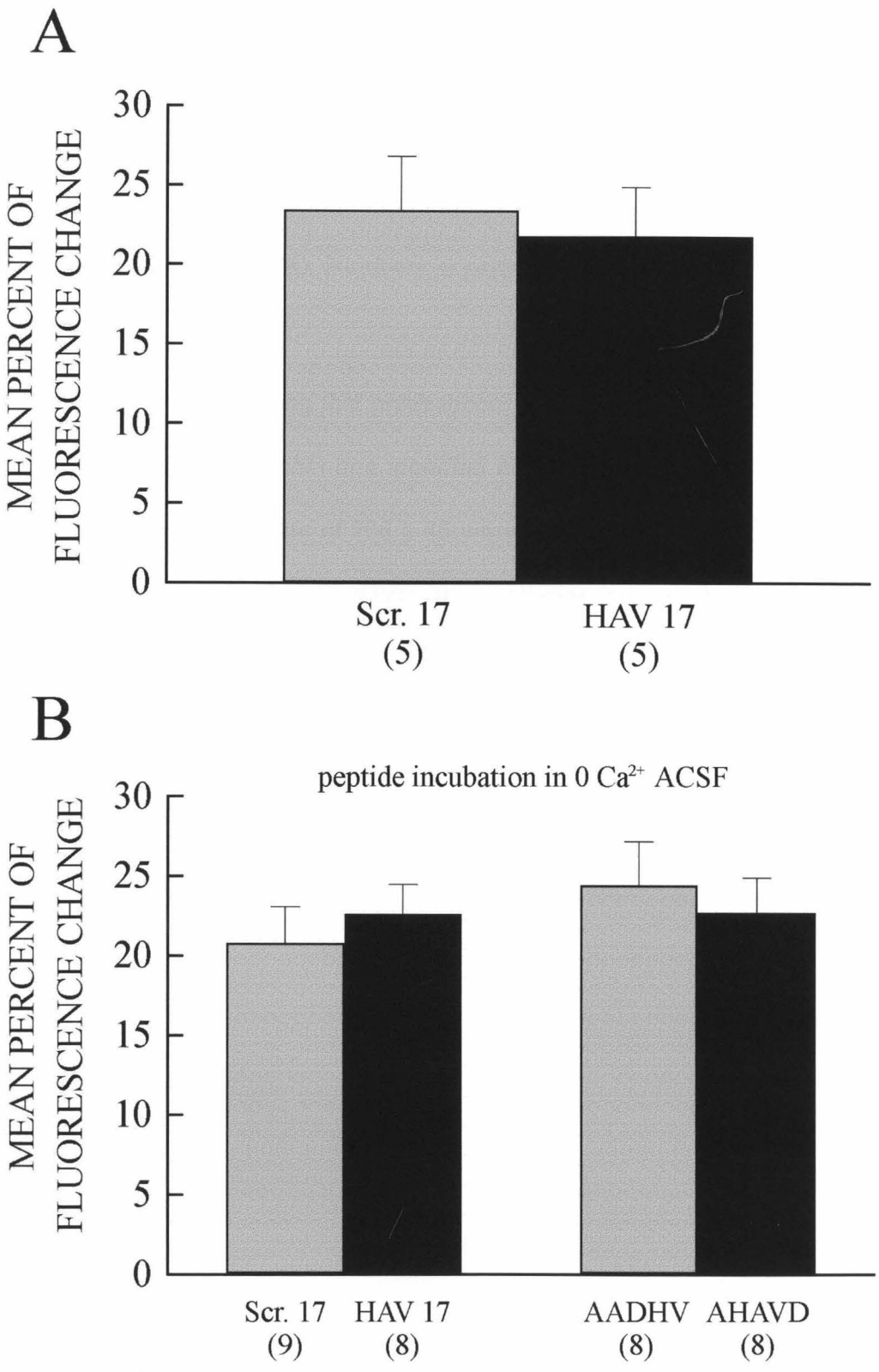

A
MEAN PERCENT OF FLUORESCENCE CHANGE
30
25
20
15
10
5
0
Scr. 17
(5)
HAV 17
(5)
B
peptide incubation in 0 Ca2+ ACSF
MEAN PERCENT OF FLUORESCENCE CHANGE
30
25
20
15
10
5
0
Scr. 17
(9)
HAV 17
(8)
AADHV
(8)
AHAVD
(8)

Figure 5-3. HAV peptides do not appear to affect the release of FM 1-43.

(A) A summary graph showing that pretreatment with E-cadherin-derived HAV 17-mer (1 mM) does not significantly affect the release of FM 1-43 when compared to the scrambled 17-mer (1 mM). Numbers in parentheses indicate the "n" for each set of experiments.

(B) A summary graph showing that pretreatment with E-cadherin-derived (1 mM) HAV 17-mer or AHAVD (1 mM) in a modified ACSF containing no Ca2+ does not significantly affect the release of FM 1-43 when compared to the scrambled 17-mer (1 mM) or AADHV (1 mM). Numbers in parentheses indicate the "n" for each set of experiments.

Chapter 6. General Discussion: cell adhesion molecules in the modulation of synaptic transmission and plasticity

6.1 Role of other cell adhesion molecules in synaptic plasticity

During the course of my thesis study on the role of cadherins in synaptic function, a number of studies on the role of other cell adhesion molecules in modulating synaptic transmission and plasticity were reported. These other cell adhesion types include Ig family members and integrins. I would like to summarize these studies before discussing the potential function of synaptic cadherins in early development (synaptogenesis) and in the adult brain (this thesis).

As stated above, many different adhesion molecules appear to participate in activity-dependent synaptic plasticity. What is the relative contribution of each of these adhesion types in synaptic plasticity? Do they play redundant or independent roles in the modulation of synaptic transmission?

6.1.1 Ig family members in synaptic plasticity

Besides LTP in adult hippocampus, another well-characterized model of synaptic plasticity is long-term facilitation (LTF) of the sensory-motor synaptic connections in the mollusk *Aplysia* california. Long-term sensitization (LTS) of the gill/siphon-withdrawal

reflex parallels LTF and a long-lasting increase in the number of the sensory-motor synaptic connections. Serotonin (5-HT) is believed to be released by interneurons onto sensory neurons during sensitization training and to play an important role in plasticity of sensory-motor synapses (Montarolo et al., 1986; Glanzman et al., 1989). Both the electrophysiological and the structural changes that underlie LTF can be mimicked by repeated or prolonged 5-HT application to sensory-motor co-cultures (Glanzman et al., 1990). A single 5-HT application causes short-term facilitation that lasts < 2 hours, whereas repeated (5 times) 5-HT application causes LTF that lasts more than 1 day.

Mayford et al. found that 5-HT application produced an actinomycin D-sensitive decrease in 4 cell surface proteins (Mayford et al., 1992). Through cloning and characterization, it was determined that these proteins (called apCAMs) are homologous to mammalian N-CAM and *Drosophila* Fasciclin II. Immunolabeling revealed that 5-HT produced a down-regulation of apCAM in the presynaptic sensory neuron. Immuogold-EM studies demonstrated that apCAM was actually decreased in the surface membrane. There was a protein-synthesis dependent internalization of apCAM via coated pits and a degradation of the protein via the endosomal pathway (Bailey et al., 1992). Further studies using epitope-tagged apCAMs indicated that only the transmembrane (not GPI-linked) apCAM was internalized (Bailey et al., 1997). Both LTF and apCAM internalization were dependent upon the MAP kinase pathway and the apCAM cytoplasmic domain containing a PEST sequence. It was thus hypothesized that repeated or prolonged application of 5-HT activates the MAPK pathway leading to the phosphorylation of the cytoplasmic tail of apCAM. This phosphorylation may alter the conformation of the intracellular domain of apCAM or its interactions with cytoskeletal elements. This conforma-

tional change may localize apCAM to coated pits where it undergoes MAPK or proteolysis-dependent internalization and degradation. These processes ultimately lead to the disruption of homophilic interactions between presynaptic axons, facilitating their outgrowth and the subsequent formation of new synapses with postsynaptic neurons.

It is known that N-CAM and L1 are expressed in the rodent hippocampus (for example, Miller et al., 1993). The role of these IgSF members in hippocampal LTP and spatial learning has also been studied. Doyle et al. (1992) found that infusion of an anti-NCAM Ab into rat lateral ventricles, when administered in 6-8 hour posttraining, inhibited the consolidation of a passive avoidance response, without affecting the acquisition processes associated with learning. NCAM knockout mice exhibited apparently normal hippocampal structure and normal motor activity but deficits in performance in the Morris water maze task (Cremer et al., 1994).

The direct examination of the involvement of NCAM and L1 in hippocampal LTP comes from the study by Luthi et al. (1994) where a microinjection pipette was used to deliver a number of inhibitory reagents onto hippocampal slices. LTP was blocked by an anti-L1 Ab in a dose-dependent manner while basal synaptic transmission and NMDA receptor-mediated responses were not affected. LTP was also blocked by an anti-NCAM Ab as well as sugar/peptide inhibitors that affect the NCAM-L1 complex. Interestingly, the blockade of LTP by these reagents had a restricted time window: they were effective in blocking when applied 10 minutes before, but not after, LTP induction.

One interesting feature of NCAM is the attachment of the negatively charged homopolymer – polysialic acid (PSA) during development. This has led to the hypothesis that cells expressing PSA-NCAM have an increased capacity for structural plasticity. Using

hippocampal organotypic slice cultures, Muller et al. found that both pre- and postsynaptic neurons expressed PSA-NCAM (Muller et al., 1996). LTP was reversibly blocked by Endo-N, an enzyme that removes PSA moieties from NCAM and does not affect NMDA receptor-dependent responses. This blockade of LTP was paralleled by a decrease in PSA immunolabeling. Furthermore, they found that NCAM knockout mice exhibited decaying LTP that was unaffected by further treatment with Endo-N, consistent with the role of PSA-NCAM in hippocampal LTP. Interestingly, an earlier behavioral study indicated that the NCAM-deficient mice showed deficits in spatial learning when tested in the Morris water maze despite normal motor abilities (Cremer et al., 1994).

In parallel with the studies of mammalian NCAM, the role of the *Drosophila* homolog Fasciclin II (Fas II) in synaptic plasticity has also been systematically studied. Goodman and his colleagues took advantage of the powerful genetics, cell biology, biophysical and behavioral approaches available in *Drosophila* to study the role of FasII in synaptic plasticity (Davis et al., 1996; Schuster et al., 1996; Schuster et al., 1996). Immuno-EM showed that FasII is expressed in both presynaptic nerve and postsynaptic muscle cells. Simultaneous pre- and postsynaptic expression of FasII is required for the synapse to survive and stabilize. The expression level of FasII influences synaptic structure since decreased level of FasII in mutants leads to presynaptic sprouting similar to *eag Shaker* and *dunce* mutants. Driving the expression of FasII in *eag Shaker* and *dunce* mutants causes suppression of sprouting. However, unlike *eag Shaker* and *dunce* mutants that exhibit increased synaptic strength, mutants that have decreased FasII expression exhibit normal synaptic strength. Therefore, FasII is necessary but not sufficient for an increase in synaptic strength/function. It was found that CREB acts in parallel with FasII

to cause an increase in synaptic strength (Davis et al., 1996). Only in FasII mutants having structural sprouting does expression of a CREB activator increase synaptic strength. It is interesting to note that increased NCAM expression (FasII homolog) is correlated with synaptic plasticity in vertebrates; whereas in invertebrates decreased FasII expression is correlated with synaptic plasticity. It is likely that different mechanisms operate in different species to control synaptic plasticity. Other factors that may contribute to this difference are (1) developmental stages of the tissue studied, (2) alternatively spliced forms of NCAM, and (3) post-translational modifications to NCAM.

6.1.2 Integrins in synaptic plasticity

Integrins, another class of cell adhesion molecule, are also implicated in modulating synaptic transmission. Using a frog sartorius nerve-muscle preparation, Chen et al. studied the role of integrins in the stretch-induced enhancement of transmitter release. At this synapse, increased stretch of muscle leads to an increase in both spontaneous and evoked transmitter release. It was found that integrin Abs or a synthetic peptide GRGDSP (mimicking the main integrin binding site in several ECM molecules) inhibited the stretch-induced enhancement of transmitter release (Chen and Grinnell, 1994; Chen and Grinnell, 1995).

An integrin-like receptor has also been shown to participate in hippocampal LTP (Staubli et al., 1990; Peng et al., 1991; Bahr et al., 1997; Staubli et al., 1998). Lynch and his colleagues found that preincubation of hippocampal slices with a GRGDSP peptide caused an inhibition of LTP in a dose-dependent manner. This peptide did not affect basal synaptic transmission, NMDA receptor-mediated responses or other synaptic parameters. It was also found that application of the peptide before, or 10 minutes after,

theta burst stimulation inhibited LTP. By affinity purification, they found a 55 kDa integrin-like protein named synaptegrin-1 that binds to GRGDSP.

Integrins have also been shown to be involved in *Drosophila* short-term memory. Using enhancer detector screening for mushroom body expression, Groteweil et al. discovered a *Drosophila* gene named *Volado*. This gene codes for two forms of α-integrins, Vol-l and Vol-s, preferentially expressed in the mushroom bodies (Grotewiel et al., 1998). Vol mutants exhibit memory deficits in odor avoidance conditioning without neuroanatomical defects or sensorimotor defects. The memory deficits of Vol mutant were rescued by conditional expression of Vol-s. The memory rescue was found to parallel Vol protein expression (Grotewiel et al., 1998). In a parallel study, an integrin-associated protein (IAP) was found to participate in memory formation in rats (Huang et al., 1998). By PCR differential display following a one-way inhibitory avoidance learning task, Huang et al. discovered three unique cDNA bands in "good-memory" rats that performed well in a memory-retention test in choosing the safe- vs. dangerous cells during an inhibitory avoidance task. By further cloning and characterization, it was found that one cDNA encodes a protein homologous to mouse or human IAP. *In situ* hybridization analysis showed that good memory rats had higher IAP mRNA expression in the hippocampus than "poor-memory" rats. Further, injection of antisense oligonucleotides for IAP into the dentate gyrus of rats inhibited both LTP and memory retention *in vivo*.

Taken together, these data suggest that integrins and their related protein(s) may play a role in hippocampal synaptic plasticity and memory formation.

6.1.3 Neuronal activity regulates the expression of CAMs

Since many CAMs are required for activity-dependent synaptic plasticity, it has been postulated that the expression of CAMs at the cell surface could also be regulated by neuronal activity.

For example, the effect of synaptic activity on the reexpression of PSA (following treatment with Endo N) was examined in hippocampal organotypic slice culture (Muller et al., 1996). The reexpression of PSA was completely blocked by TTX, a sodium channel blocker, or low Ca^{2+} medium, but enhanced by bicuculline, an antagonist of GABAergic inhibition. Facilitation of AMPA receptor-mediated synaptic transmission by allosteric modulation of AMPA receptor was found to activate the NCAM promoter (Holst et al., 1998), suggesting that NCAM synthesis is regulated in part by synaptic activity. Furthermore, expression of an ECM glycoprotein, tenascin-C, was up-regulated in the hippocampus after injection of the excitotoxin kainic acid and the occurrence of seizures (Nakic et al., 1996).

Fields and his colleagues studied whether different patterns of neuronal activity regulate the expression of cell adhesion molecules using mouse DRG neurons (~E14) in a multicompartment cell culture preparation. They found that application of 0.1 Hz electrical stimulation for 5 days to these cells caused decreased expression of L1 mRNA and protein, and decreased cell adhesion and DRG neurite fasciculation, without causing cell death (Itoh et al., 1995; Fields and Itoh, 1996). Expression of different CAMs (NCAM, L1, N-cadherin) was found to be regulated by distinct patterns of neural impulses (Itoh et al., 1997). For example, N-cadherin was down-regulated by 0.1 or 1 Hz stimulation, but NCAM mRNA and protein levels were not altered by stimulation. L1 was down-

regulated by 0.1 Hz stimulation, but not by 0.3 Hz, 1 Hz, or pulsed stimulation. Expression of N-cadherin and L1 was lowered with different kinetics. These changes correlated with firing patterns of DRG neurons and certain developmental events such as fasciculation, synaptogenesis, and synapse stabilization. Therefore, the regulated expression of different CAMs may control intercellular interactions and coordinate the structure and function of nervous system during development and regeneration.

6.2 Role of cadherins in synaptogenesis

The role of cadherins in synaptogenesis is one of the actively researched areas in developmental neurobiology. Synaptic specificity could be achieved through cadherin homophilic binding specificity; diversity could be generated by the expression of different cadherin subtypes. Currently, there are about 40 cadherin and cadherin-related proteins that have been reported in the brain, supporting their potential for adhesive specificity. For example, Sanes and his colleagues studied synapse formation in the developing chick optic tectum and found that N-cadherin and certain cell surface glycoconjugates are selectively associated with different "retinorecipient" laminae as synapses form (Yamagata et al., 1995). Moreover, A function-blocking antibody to N-cadherin perturbed laminar selectivity (Inoue and Sanes, 1997). More recently, Benson and Tanaka (1998) used cultured hippocampal neurons to study the role of N-cadherin in synapse formation. They found that N-cadherin and β-catenin are present in axons and dendrites before synapse formation and then cluster at developing synapses between hippocampal neurons. N-cadherin is expressed initially at all synaptic sites but rapidly becomes restricted to excitatory synapses whereas inhibitory synapses may now express a different

cadherin. This differential cadherin expression may orchestrate the point-to-point specificity of synapses (Benson and Tanaka, 1998).

6.3 Role of cadherins in adult synapses

6.3.1 Thesis summary

The functional role of cadherins in adult hippocampal synaptic plasticity has been studied in this thesis research. In summary, my data suggest that the classic cadherins (especially N- and E-cadherin) and the cytoplasmic catenins are expressed in the adult hippocampus. These proteins are largely localized to synaptic sites. Cadherin function is required for initial triggering of synaptic enhancement: LTP was reduced by cadherin function-blocking Abs or HAV-containing synthetic peptides. This blockade was independent of the particular stimulus protocol used to induce LTP. LTP induced by theta burst stimulation, intracellular pairing or high-frequency stimulation was inhibited by HAV peptides. The inhibition of LTP by cadherin inhibitory reagents is concentration-dependent: lower concentrations of HAV peptides inhibit LTP to a lesser degree. The inhibition of LTP by cadherin inhibitory reagents is also contingent upon a change in extracellular Ca^{2+} concentration. This change may be intrinsic to the synaptic activity associated with high frequency stimulation. Raising extracellular Ca^{2+} from 2.5 mM to 5.0 mM completely prevented inhibition of LTP by a cadherin antibody or HAV peptides. Moreover, cadherin function is also required during the maintenance phase of LTP. A combined treatment of HAV peptides and low Ca^{2+} ACSF reversed established LTP. LTP was also significantly reduced in slices infected with an adenovirus containing a dominant-negative mutant N-cadherin cDNA. The HAV peptides, function-blocking Abs

and the Ad-cN390Δ adenovirus inhibited hippocampal LTP without changing the basal synaptic properties such as the input-output relations and paired-pulse facilitation. These findings suggest that these cadherin inhibitory reagents work by perturbing mechanisms involved in synaptic plasticity, rather than causing a general anti-adhesive and disruptive effect on the slice.

The fluorescent membrane label FM 1-43 was used to examine whether HAV peptides affect vesicle exocytosis in labeled presynaptic terminals. Cultured hippocampal neurons incubated with HAV peptides (1 mM) or scrambled peptides did not show difference in the dye destaining of synaptic terminals. Therefore, HAV peptides do not affect presynaptic vesicle exocytosis in hippocampal cultures.

6.3.2 Multifunctional cadherins

Cadherins expressed at synaptic sites are multifunctional. They are involved in the dynamic arrangements of cells during early morphogenetic events including synaptogenesis and forming functional neuronal networks (Takeichi, 1995). They may play a similar role in the adult nervous system, directing the growth of new synaptic connections (Colman, 1997; Fannon and Colman, 1996; Serafini, 1997; Uchida et al., 1996). They also participate in synaptic plasticity in the adult brain (see this thesis). The involvement of cadherins in synaptic plasticity in adult hippocampus suggests that learning-associated changes in the adult brain and morphogenetic events during the very early development of animals may use an overlapping set of molecular machinery. It is an interesting notion that the same set of signaling molecules, or even the same mechanisms, may be used for both development and adult learning (Kandel and Odell, 1992).

6.3.3 Cadherins in synaptic function

My data indicate that cadherins as local signaling molecules, make important contributions to activity-dependent changes in synaptic strength in the adult hippocampus. Pretreatment of hippocampal slices with function-blocking cadherin Abs or HAV-containing peptides attenuated hippocampal LTP without any changes in basal synaptic transmission, posttetanic potentiation, paired-pulse facilitation, NMDA receptor-mediated currents, or postsynaptic depolarization mechanisms. The lack of effect of these cadherin interfering reagents on the basal synaptic parameters suggests that these blockers are specific for inhibiting synaptic plasticity, rather than imparting a general anti-adhesive and disruptive effect on the slice. Consistent with this, data from the FM 1-43 experiments suggest HAV peptides do not affect presynaptic vesicle exocytosis in cultured hippocampal neurons.

The inhibition by the HAV peptides suggests that intercellular interactions between cadherins are critical for LTP formation. Cadherin-mediated interactions could be required for intracellular signaling on either side of the synapse, or to increase the area of contact between pre- and postsynaptic elements as suggested by the zipper motif of some cadherin-cadherin homodimers (Shapiro et al., 1995).

The inhibition of LTP by peptide inhibitors is dependent upon the concentration of HAV peptides. A concentration-dependent inhibition of LTP has also been reported for inhibitors of integrin, L1, and NCAM function (Luthi et al., 1994; Muller et al., 1996; Bahr et al., 1997). The inhibition by function-blocking cadherin Abs or HAV peptides is independent of LTP-inducing protocols, suggesting a fundamental role for cadherins in synaptic plasticity.

The inhibition of LTP by peptide inhibitors further indicates a time- and Ca^{2+}-dependent vulnerability. HAV peptides only inhibit LTP when present around the time of induction, but not after LTP has been successfully established. Similar observations have been made for integrins, L1, and NCAM (Luthi et al., 1994; Muller et al., 1996; Bahr et al., 1997), suggesting that the adhesion molecules may be involved in the very early steps associated with synaptic modification. Cadherin bonds are sensitive to dynamical changes in extracellular Ca^{2+}. Cadherin inhibitory reagents do not inhibit LTP when extracellular Ca^{2+} is elevated to 5 mM, possibly reflecting a relative protection of the cadherin bonds owing to a less dramatic reduction in cleft Ca^{2+} during stimulation. Delivery of HAV peptides in 0.5 mM $[Ca^{2+}]_o$ ACSF during the maintenance of LTP reduced potentiation, suggesting a role for cadherins in both the induction and expression of LTP. The sensitivity of cadherin bonds to extracellular Ca^{2+} dynamics may provide a mechanism for the direct coupling of cadherin-mediated adhesive or signaling events to synaptic activity.

My data also suggest that cadherin homophilic bonds at synaptic cleft may act as Ca2+-sensors (Tang et al., 1998). They are sensitive to dynamic changes in extracellular Ca2+ concentration. The Ca2+-sensing ability of synaptic cadherins may provide a mechanism for direct coupling of cadherin-mediated adhesion and signaling events to synaptic activity during learning and memory formation in the brain.

The small amount of residual enhancement observed in the presence of the function-blocking Abs and HAV peptides, or in slices infected with the Ad-cN390Δ virus may reflect the involvement of other classic cadherin subtypes (Fannon and Colman, 1996) or non-classic cadherins (Kohmura et al., 1998), or a cadherin-independent portion of plasticity, perhaps mediated by other cell adhesion molecules implicated in synaptic plasticity

(see Section 6.1). Together with these other studies showing the involvement of other cell adhesion molecules in synaptic plasticity, these data indicate that local, mechanical, or structural signals must also be considered as important regulators of synaptic plasticity.

More and more cadherin types have been reported at synaptic sites. These synaptic cadherins not only include classic cadherins (Fannon and Colman, 1996; Uchida et al., 1996, and this thesis), but some non-classic cadherins such as a cadherin-related neuronal receptor (CNR) as well (Kohmura et al., 1998). CNR has been speculated to play an analogous role in signaling and modulating NMDA receptor function, or in building specific synaptic connections (Miyakawa et al., 1997).

To address the questions of the relative contribution of each of these adhesion molecules in synaptic plasticity and whether or not they play redundant or independent roles in the modulation of synaptic transmission, more studies using approaches to turn on or off the function of certain adhesion type(s) seem necessary. These approaches may include conditional or multiple knockouts, and/or expression of dominant-negative proteins.

6.3.4 Concluding remarks

The emerging picture of synaptic junctions in the brain is that they are highly dynamic structures. The involvement of an increasing number of synaptic cell adhesion molecules (e.g., cadherin, integrins, Ig family members) in regulating synaptic efficacy has changed our view about their roles in synaptic function. The next several years will undoubtedly be an exciting period for the study of the various adhesion molecules in many aspects of synaptic function. These studies will help us further understand the mechanisms for learning and memory in the brain at the cellular and molecular level.

Appendix A. Centrifugal force-based cell adhesion assay

A centrifugal force-based cell adhesion assay was performed using LE cells (mouse L cell line expressing mouse E-cadherin) to quantitatively test the effect of anti-E-cadherin Ab and HAV peptides (sequence derived from mouse E-cadherin) on cell-cell adhesion. The expression of E-cadherin at cell-cell contacts of the LE cells was shown previously by Angres et al. (Angres et al., 1996).

The assay was adopted from the adhesion assay developed and described by McClay et al. (McClay et al., 1981) and Lotz et al. (Lotz et al., 1989). A schematic diagram is provided in Figure A-1. The detailed description of this method is provided in Appendix C-13.

The results of cell adhesion assay suggested a significant inhibition of cell adhesion by 1:100 anti-E-cadherin Ab (15.5%), 0.2 mM (12.5%), 1.0 mM SHAVS (18.2%), 0.2 mM (12.5%), 0.4 mM E-cad 17mer (39.1%), but not by 1:100 preimmune rat IgG, 0.2 mM VSHSA, 0.2 mM, 0.4 mM E-cadherin 17mer (Figure A-2).

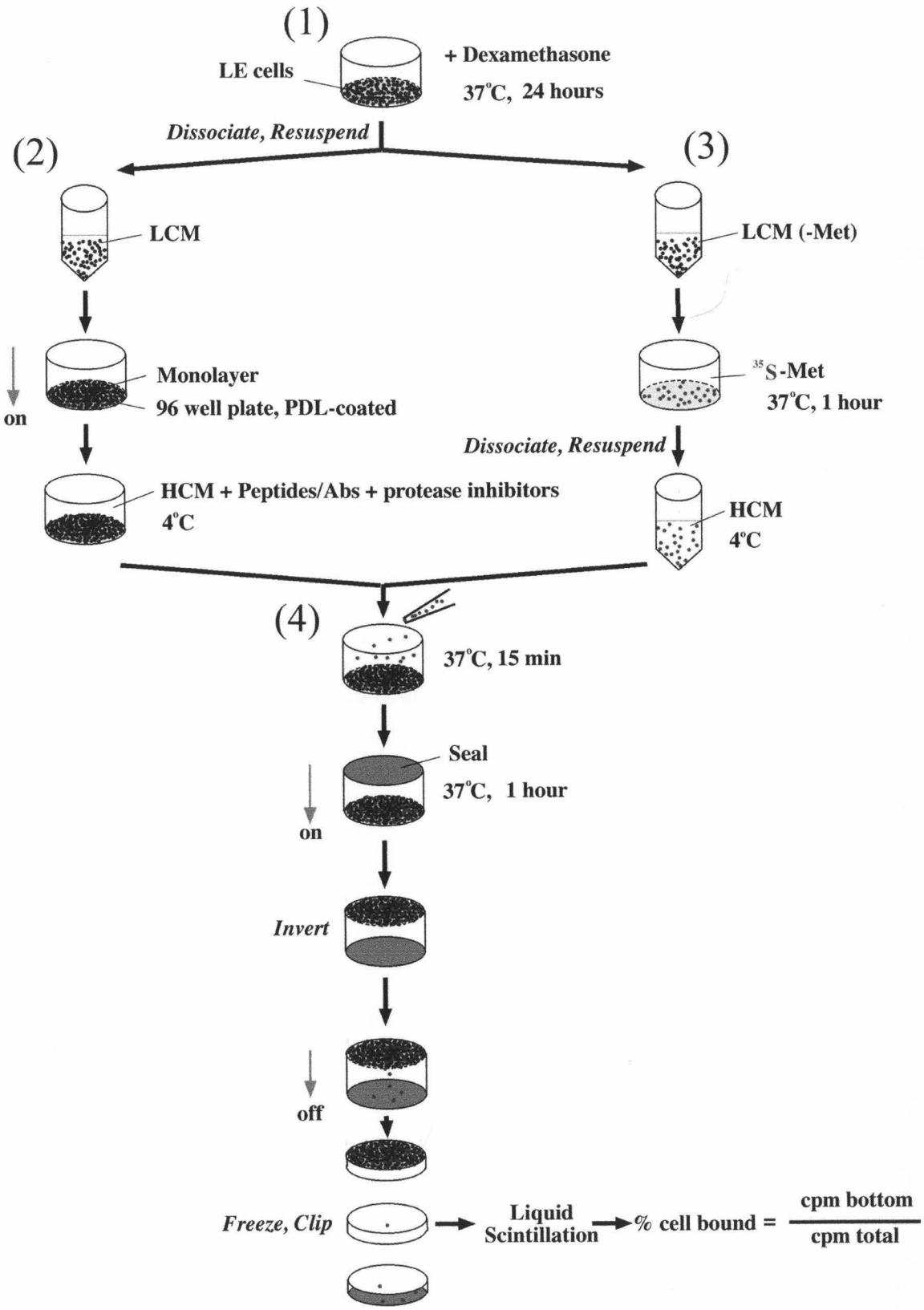

(1)
LE cells
+ Dexamethasone
37°C, 24 hours
Dissociate, Resuspend
(2)
(3)
LCM
LCM (-Met)
Monolayer
96 well plate, PDL-coated
on
35S-Met
37°C, 1 hour
Dissociate, Resuspend
HCM + Peptides/Abs + protease inhibitors
4°C
HCM
4°C
(4)
37°C, 15 min
Seal
37°C, 1 hour
on
Invert
off
Freeze, Clip
Liquid Scintillation
% cell bound = cpm bottom / cpm total

Figure A-1. Experiment design for centrifuge force-based cell adhesion assay.

The assay was adopted from the adhesion assay by McClay et al. (McClay et al., 1981) and Lotz et al. (Lotz et al., 1989).

(1) Preparation of LE cells expressing E-cadherin: expression of E-cadherin is induced by dexamethasone.

(2) Preparation of LE cell monolayer in a 96-well plate coated with Poly-D-Lysine.

(3) ^{35}S-L-Met labeling of LE cells.

(4) Cell adhesion assay. ^{35}S-labeled cells are placed with LE cell monolayer, and centrifuged on contact with monolayer. The plate is then inverted. Those ^{35}S-labeled cells that do not adhere to the monolayer are therefore centrifuged off. Radioactivity from different parts is measured. The percent cell bound is then calculated.

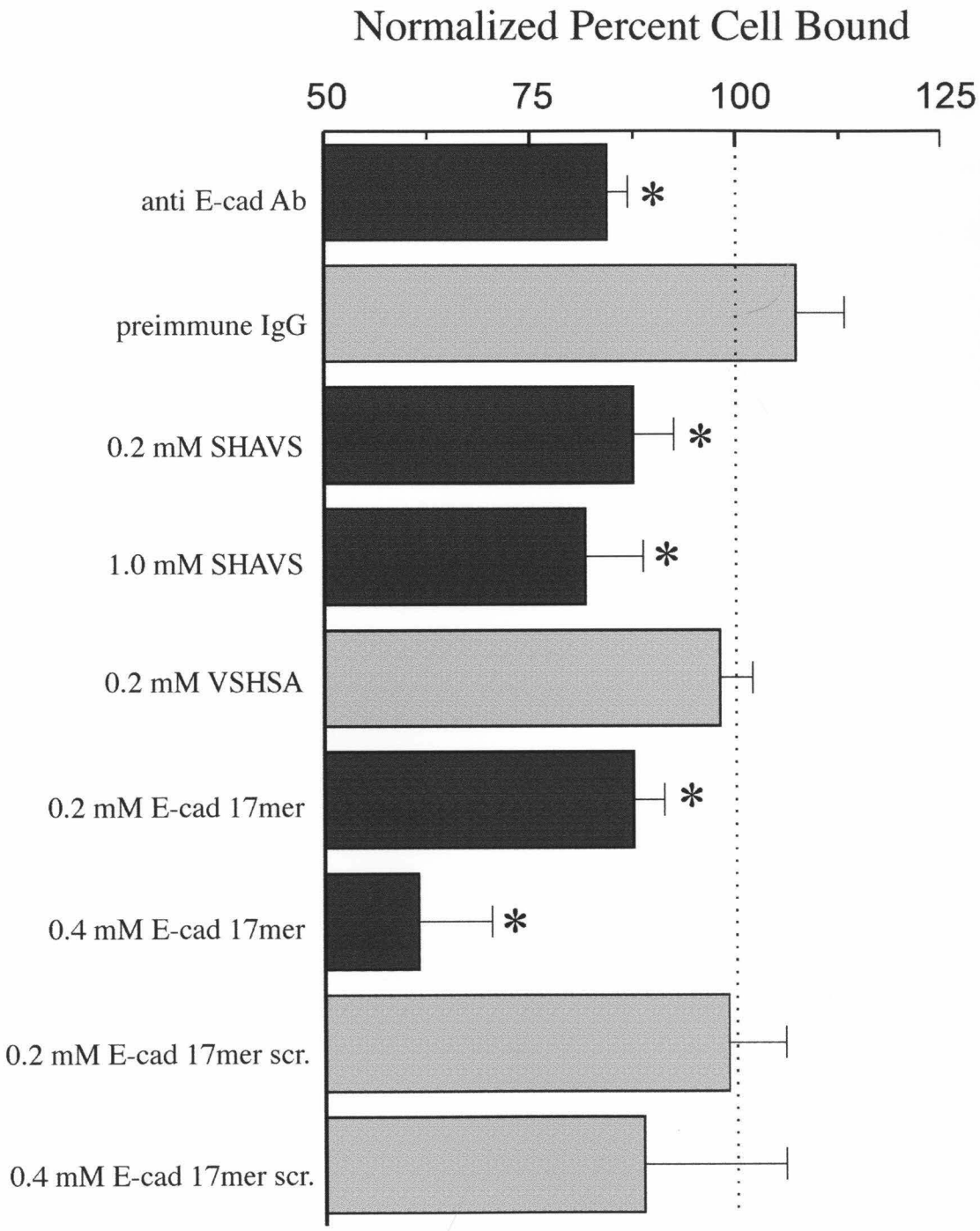

Normalized Percent Cell Bound
50
75
100
125
anti E-cad Ab
preimmune IgG
0.2 mM SHAVS
1.0 mM SHAVS
0.2 mM VSHSA
0.2 mM E-cad 17mer
0.4 mM E-cad 17mer
0.2 mM E-cad 17mer scr.
0.4 mM E-cad 17mer scr.
*
*
*
*
*

Figure A-2. Summary graph of adhesion assay on L cells expressing E-cadherin.

Normalized percent cell bound is the percent cell bound data of each experimental group normalized to the percent cell bound data of control group (i.e., cell medium only, without Ab or peptide). Asterisks indicate significant inhibition ($p < 0.05$, paired t-test) relative to control.

Appendix B. Similarities between different cadherin types across major different species

Cadherin types	Similarity (%)			
	EC	TM	CP	MP
Mouse-mouse				
N to E	46	44	62	49
N to P	43	22	56	43
E to P	53	41	80	58
Chicken-chicken				
N to L	46	50	63	50
Mouse-chicken				
N to N	89	100	99	92
E to L	58	79	89	65

EC: extracellular domain

TM: transmembrane domain

CP: cytoplasmic domain;

MP: mature protein

Appendix C. Materials and methods

1. Antibodies

(1) a mouse anti-N-cadherin monoclonal antibody (mAb): clone GC-4, Sigma, raised against purified chicken N-cadherin, reacts with the N-terminal half of the extracellular domain of N-cadherin. (2) a mouse anti-Pan cadherin mAb: clone CH-19, Sigma, raised against a synthetic peptide corresponding to the C-terminal 24 amino acids of chicken N-cadherin, reacts many members of the cadherin family (Geiger et al., 1990). (3) a rat anti-E-cadherin mAb: clone DECMA-1, Sigma, raised against a mouse embryonal carcinoma cell line. (4) a polyclonal rabbit antiserum (E2) to a recombinant E-cadherin cytoplasmic domain GST fusion protein (a gift from James Nelson) (5) a mouse anti-synapsin I mAb and a rabbit anti-synapsin I polyclonal antibody: gift from Mary Kennedy. (6) rabbit antiserum to a full length recombinant rat glypican GST fusion protein: gift from R. U. Margolis. (7) a polyclonal rabbit antiserum to a recombinant E-cadherin cytoplasmic domain GST fusion protein: gift from James Nelson. (8) rabbit α- and β-catenin antiserum: Sigma, raised against a synthetic peptide corresponding to amino acids 890-901 or 768-781 of human/mouse α- or β-catenin. (9) a mouse γ-catenin mAb: clone 15F11, Sigma, raised against recombinant chicken plakoglobin. (10) a mouse β-galactosidase mAb (IgG1): clone GAL-13, Sigma, β-D-Galactosidase purified from *E. Coli* was used as the immunogen. (11) a monoclonal rat anti-N-cadherin antibody: clone NCD-2, Zymed. It reacts with chicken N-cadherin and cN390Δ. (12) affinity purified rat or mouse IgG: Sigma. (13) FITC or Cy3-conjugated goat IgG to rabbit, mouse or rat IgG: Jackson

Labs, used as secondary antibodies. (14) alkaline phosphatase conjugated anti-digoxigenin antibody: BioRad, for *in situ* hybridization color reaction.

2. Peptides

HAV-containing peptides (5-mer and 17-mer) and their corresponding scrambled peptides were from the Caltech Peptide Synthesis Lab. The 17-mer peptide sequences used, derived from mouse N- and E-cadherin were as follows: E-cadherin, AKYILYSHAVSS NGEAV; N-cadherin, ARFHLRAHAVDINGNQV; and scrambled (derived from E-cadherin sequence), VAVLYEKSGIAYHNSAS.

3. cDNA clones

(1) a rat N-cadherin cDNA clone: 2.4 Kb insert in Bluescript® SK (cloning site: EcoRI-XhoI) with the 5' end corresponding to the middle region of rat N-cadherin EC4 domain, gift from S. Suzuki.

(2) a mouse E-cadherin cDNA clone: 0.5 Kb insert of the cytoplasmic domain in Bluescript® SK (cloning site: EcoRI), gift from S. Suzuki (Tanihara et al., 1994).

(3) a mouse full length E-cadherin cDNA clone: 4.3 Kb insert in Bluescript® SK (cloning site: EcoRI), gift from Masatoshi Takeichi (Nagafuchi et al., 1987).

(4) cN390Δ, a mutant chicken N-cadherin cDNA clone with a large portion encoding 390 amino acids in the extracellular domain deleted, gift from Masatoshi Takeichi (Fujimori and Takeichi, 1993).

4. Hippocampal slice preparation and storage

Young adult male Sprague-Dawley rats (6-8 weeks) or male BALB/C57I mice (5-7 weeks) were decapitated and the brain was removed and quickly placed in chilled and oxygenated (95% O_2 + 5% CO_2) artificial cerebrospinal fluid (ACSF) (119 mM NaCl; 2.5 mM KCl; 1.3 mM $MgSO_4$; 2.5 mM $CaCl_2$; 1.0 mM NaH_2PO_4; 26.2 mM $NaHCO_3$; 11.0 mM glucose). 500 μm transverse slices were dissected from isolated hippocampi using a Stoelting tissue chopper, and stored for at least 1.5 hours on a Millipore membrane placed over a petri dish containing ACSF and exposed to moistured oxygen circulating in an enclosed chamber at room temperature (RT).

5. Electrophysiological recordings

Field, intracellular, or whole-cell excitatory postsynaptic potentials/currents (EPSP/Cs) measured in stratum radiatum or in CA1 pyramidal cells, respectively, were evoked by stimulation of the Schaffer collateral-commissural afferents (1/15 Hz). In two pathway experiments, two bipolar stimulating electrodes were placed in stratum radiatum to stimulate two independent sets of afferents fibers (Fig. 2). The independence was ascertained by examining interactions in paired-pulse facilitation. Extracellular recording electrodes were filled with 3 M NaCl; intracellular recording electrodes were filled with 2 mM cesium acetate; whole-cell pipettes were filled with 100 mM cesium gluconate, 0.6 mM EGTA, 5 mM $MgCl_2$, 2 mM Mg-ATP, 0.3 mM Li-GTP, 40 mM HEPES (pH 7.3). Whole-cell recordings were made in the single-electrode voltage-clamp mode with 75-90% of series resistance compensation. Tetanic stimulation was delivered at the test intensity in 1-s trains at 100 Hz, with 1-4 trains delivered 30 s apart. Control and antibody or peptide-treated slices always received the same number of trains. Theta Burst Stimula-

tion (TBS) consisted of three applications (30 s apart) of the following: four bursts of stimuli, each of five pulses at 100 Hz with an interburst interval of 200 ms. Pairing was accomplished by sustained depolarization (to 0 mV) of the intracellularly recorded neuron by DC injection in conjunction with low frequency (1 Hz) stimulation of the test pathway for 60 s. NMDA receptor-mediated EPSP/Cs were pharmacologically isolated by including 10 μM CNQX, 10 μM glycine and 50 μM picrotoxin in 0.1 mM Mg^{2+}-containing ACSF, or in the normal ACSF and combined with a depolarization of the cells, respectively. 50 μM APV was applied at the conclusion of experiments to confirm that the recorded EPSP/Cs were mediated by NMDA receptor activation.

6. Antibody and peptide introduction

In antibody or 17-mer peptide incubation experiments, adjacent slices were placed in individual wells containing antibody or peptides diluted in 200 μl normal ACSF or altered ACSF containing 5.0 mM Ca^{2+} in high Ca^{2+} experiments. Following incubation for 2-3 hours, slices were transferred to a recording chamber and perfused with normal ACSF or high Ca^{2+} ACSF for 15-30 minutes before tetanic stimulation was delivered. 5-mer peptide was introduced directly into the perfusion media during recordings.

7. Recombinant adenovirus vectors and slice infection

Ad-lacZ adenovirus vector containing lacZ cDNA ($3x10^{10}$ pfu) and Ad-cN390Δ adenovirus vector containing cN390Δ mutant chicken N-cadherin cDNA ($3x10^{9}$ pfu) were from N. Davidson lab.

Acute hippocampal slices were obtained as usual. Adjacent slices were placed in individual wells with Ad-lacZ (10 μl) or Ad-cN390Δ adenovirus (20 μl) diluted in 200 μl slice

media (119 mM NaCl; 2.5 mM KCl; 1.3 mM $MgSO_4$; 2.5 mM $CaCl_2$; 1.0 mM NaH_2PO_4; 26.2 mM $NaHCO_3$; 20 mM glucose, 250 μM glutamine, 50 U/ml penicillin-streptomycin, and B-27) in a humidified 95% O_2/5% CO_2 atmosphere for 24-30 hours before recording or fixation. Half volume of slice media was replaced with fresh slice media every 12 hours.

8. Western blotting

SDS-PAGE was done using 3.5% stacking gel and 7.5% separating gel with 200 μg protein loaded in each lane. The gel was equilibrated for 25 minutes in transfer buffer (25 mM Tris base, 192 mM glycine, 10% ethanol, and 0.005% SDS) and transferred to Millipore Immobilon-P PVDF membrane. The membrane was sequentially washed at RT with blocking buffer (3% non-fat dry milk in PBS and 0.1% TWEEN-20) for 2 hours, 1:500 diluted anti-Pan cadherin mAb (Sigma) in blocking buffer for 1 hour, 4 times of PBS, 1:2000 diluted anti-mouse secondary (Amersham) in blocking buffer for 1 hour, and 4 times of PBS. The membrane was detected using Amersham ECL western blot reagents.

9. *In situ* hybridization

Non-radioactive *in situ* hybridization with digoxigenin (DIG)-labeled cRNA probes was used modifying the protocol of Schaeren-Wiemers et al., Brain Research Institute, Zurich, Switzerland (Schaerenwiemers and Gerfinmoser, 1993).

(1) Preparation of DIG-labeled cRNA probes (sense and antisense):

The three cDNAs for *in situ* hybridization were: a 2.4 kb rat N-cadherin fragment (Ncad-frag), a 4.3 kb mouse full-length E-cadherin (Ecad-full), and a 0.5 kb mouse E-cadherin fragment (Ecad-frag). They were all cloned into Bluescript® SK +/- phagemid vector.

These plasmids were linearized with restriction enzymes (Ncad-frag: EcoRI, XhoI; Ecad-full: XbaI, HindIII; Ecad-frag: XbaI, HindIII. All enzymes from Boehringer Mannheim) and extracted. To synthesize DIG-labeled cRNA probes, *in vitro* transcription (MEGAscript™ of Ambion) was performed at 37°C for 6 hours with DIG-labeled UTP (Boehringer Mannheim). The sense strands were used as controls. Reaction solution was treated with DNAse I to remove DNA templates, and with QuickSpin™ column (Boehringer Mannheim Corp.) to purify cRNA probes from unincorporated DIG and nucleotides. The labeled cRNA probes were finally hydrolyzed into ~100 bp fragments at 60°C with 8 mM $NaHCO_3$ and 12 mM Na_2CO_3. The purpose for alkali hydrolysis is to enhance penetration and avoid nonspecific background due to the length of the cRNA.

(2) Preparation of tissue sections:

Young adult male Sprague-Dawley rats (6-8 weeks) were decapitated and the brain was removed and quickly placed in chilled and oxygenated (95% O_2 + 5% CO_2) ACSF. Isolated hippocampi were immediately fixed on ice with 4% paraformaldehyde and 0.2% glutaraldehyde overnight, and then treated sequentially with PBS 3 times for 15 minutes, ice cold 30% sucrose and 0.4% paraformaldehyde in PBS overnight, and covered with Tissue-Tek embedding medium (Sakura) 30 minutes. Each fixed tissue was transferred into a plastic boat filled with Tissue-Tek medium and frozen on a dry ice slate. Frozen tissue can be stored at –80°C. Cryocuts (16 μm) were performed at –18°C and the cryosections were thaw-mounted on poly L-lysine-coated slides. Sections were equilibrated to RT for about 15 minutes, dried at 50°C for 5 minutes, and fixed with 4% paraformaldehyde in PBS for 10 minutes, washed three times with PBS for 15 minutes, and acetylated with 0.1 M Triethanolamine in 0.25% acetic anhydride for 10 minutes.

(3) Prehybridization, hybridization and washing of tissue sections:

For prehybridization, slides were treated with a prehybridization solution (50% formamide, 5 x SSC, 5 x Denhardt's, 0.25mg/ml brewer tRNA and 0.5mg/ml herring sperm DNA in dH2O) in a 5 x SSC (sodium chloride and sodium citrate solution) humidified chamber at RT for 2-6 hours. The hybridization mixture was prepared by adding 0.3-0.5 μg/ml DIG-labeled cRNA probes to the prehybridization solution. For hybridization, these slides were then treated with hybridization mixture at 70°C overnight. After hybridization, the slides were washed with 5 x SSC, 0.2 x SSC at 70°C for 2 hours, and 0.2 x SSC at RT for 5 minutes.

(4) Immunological detection of DIG-labeled hybrids:

The slides were rinsed with buffer 1 (0.1 M maleic acid and 0.15 M NaCl) for 5 minutes, blocked in buffer 2 (2% fetal calf serum (FCS) and 0.3% Triton-100 in buffer 1) for 1 hour, incubated with alkaline phosphatase conjugated anti-DIG antibody diluted in buffer 3 (1% FCS , 0.3% Trition-100 in buffer 1) for 2-4 hours, rinsed twice with buffer 1 for 30 minutes, and equilibrated with buffer 4 (0.1 M Tris-base, 0.1 M NaCl, 0.05 M MgCl2) for 10 minutes. To perform the color reaction, slides were then incubated with the substrate solution (0.45 mg/ml NBT, 0.175 mg/ml X-phosphate, 0.24 mg/ml levamisole, 0.1% TWEEN-20 in buffer 4) overnight in a dark, moist chamber with buffer 4. Color reaction was stopped by rinsing slides with PBS 3 times. Sections were mounted with Aqua-Mount and coverslip before photo images were taken.

10. Immunofluorescent staining and confocal microscopy

500 μm slices were fixed on ice with 4% paraformaldehyde and 0.2% glutaraldehyde for 4 hours and transferred to PBS. Sections (50 μm) were then cut using a vibrotome and a sapphire knife. The sections were then put on a rotary shaker and treated sequentially with 0.7% Triton-X in PBS, PBS, 0.1 M glycine in PBS, dH_2O, 1% $NaBH_4$, dH_2O, pre-block buffer (0.05% Triton-X, 5% goat serum in PBS), primary antibody in preblock at 4°C overnight, preblock buffer, FITC or Cy3 conjugated secondary antibody in preblock, preblock, and PBS. Cultured hippocampal cells (E18, 25-50 DIV) were fixed in -20°C methanol, and washed sequentially in Dulbecco's PBS, preblock buffer, primary antibody in preblock at 4°C overnight, preblock, FITC or Cy3 conjugated secondary antibodies (minimally cross-reactive to antigens in dual labeling) in preblock, preblock, and PBS. Hippocampal sections or culture coverslips were mounted in the mounting medium (80% glycerol and 4 mg/ml p-phenylenediamine in 0.1 M NaCarbonate buffer, pH 9.0) and viewed with a Zeiss LSM 310 laser-scan confocal microscope through either a 10x or a 63x oil-immersion lens. Cy3 was excited at 543 nm and Fluorescein at 488 nm. Images were recorded through standard emission filters at contrast settings for which the cross-over between the two channels was negligible.

11. FM1-43 staining, destaining, and measurement of fluorescence intensity

Cultured P2 hippocampal neurons (7 DIV) were stained with 10 μM FM1-43 (Molecular Probes, Oregon, USA) in 60 mM KCl solution at 37°C for 5 minutes. The neurons were then incubated with either scrambled peptides (1 mM) or HAV-containing peptides (1 mM) in normal ACSF or a modified ACSF containing 0 mM Ca^{2+} for 15-60 minutes

before destaining. Before destaining, the incubation solution was changed into peptides in normal ACSF if the Ca^{2+}-free ACSF had been used earlier. Destaining was performed by giving 20 μl puff of 1 M KCl to the neurons. Images for destaining were taken 3 minutes after stimulation. Images were obtained using Olympus IX70 confocal microscope through 40X lens with a zoom of 1.5. Fluorescence was acquired by excitation at 488 nm using a 510-nm emission filter. The percent change in fluorescence in each labeled synaptic terminal along 2-3 prominent dendrites was measured for each image. The average percent change in fluorescence intensity following destaining was compared between HAV peptide and scrambled peptide-treated neurons.

12. Data analysis

We analyzed the initial slope of the field EPSP and the slope and amplitude of the intracellular EPSP. Ensemble average were constructed using all data pointers, aligned with respect to the time of LTP induction. A between-experiment comparison examining whether the HAV peptides or Ab-treated slice differed significantly from its associated control experiments in the magnitude of potentiation measured 50-60 minutes after tetanus. The Ab-treated and control slices are same day, adjacent slices. In the 2-pathway experiments examining the effect of HAV peptides in low Ca^{2+} ACSF on LTP during maintenance, only experiments with the control pathways recovered to 85% or their baseline after washout were included.

13. Centrifugal force-based cell adhesion assay

(1) Preparation of LE cells expressing E-cadherin:

LE cells (L cell line expressing E-cadherin under dexamethasone-inducible promoter) from W. James Nelson's laboratory were routinely cultured in Dulbecco's modified Eagle's medium (DME, Gibco) (Angres et al., 1996). To induce E-cadherin expression, LE cells were replated in culture dishes with 1 μM dexamethasone in DME + 20% FCS in 37°C for 24 hours. Cells were then dissociated and resuspended with HDF solution (8 g/L NaCl, 0.4 g/L KCl, 1g/L D-glucose, 0.35 g/L $NaHCO_3$, 0.2 g/L EDTA). Some cells were then used for preparing monolayer; the rest were used for ^{35}S-L-Met labeling.

(2) Preparation of LE cell monolayer:

A 96-well plate coated with Poly-D-Lysine (PDL, Mr>130K) in 0.5 Na-carbonate buffer (pH 9.5) was used to make a monolayer of LE cells. Each well was equilibrated with "assaying low calcium medium (LCM)" (0.4 g/L KCl, 0.2 g/L $MgSO_4.7H_2O$, 6.66 g/L NaCl, 1 g/L D-glucose, amino acids containing 15 mg/L L-methionine, vitamins, 10 mM Na-HEPES, 5 μM $CaCl_2$, 0.14 g/L $NaH_2PO_4 \cdot H_2O$, pH 7.0). LE cells resuspended in "assaying LCM" were added into well ($1.6x10^5$ cells/well), and centrifuged onto the well bottom at 50 g. The plate was kept in 37°C for 20 minutes, washed gently with the "assaying LCM" 3 times, and kept in 4°C before adhesion assay.

(3) ^{35}S-L-Met labeling:

LE cells were resuspended in "labeling LCM" (0.4 g/L KCl, 0.2 g/L $MgSO_4.7H_2O$, 5.96 g/L NaCl, 1 g/L D-glucose, 0.01 g/L phenol red, amino acids without L-methionine, vitamins, 1 g/L $NaHCO_3$, 10 mM Na-HEPES, 5 μM $CaCl_2$, 0.14 g/L $NaH_2PO_4 \cdot H_2O$, pH 7.0), and incubated with 1μCi/μl ^{35}S-L-Met at 37°C for 1 hour. Labeled LE cells were resuspended in ice cold HCM containing protease inhibitors (1 μg/ml pepstatin A, 50 μg/ml TLCK, 100 μg/ml TPCK and 50 μg/ml PMSF).

(4) Adhesion assay:

The "assaying LCM" in the "monolayer" plate was exchanged into HCM ("assaying LCM" with 1.8 mM $CaCl_2$) containing 2x antibody or peptide with peptide inhibitors (see above). Equal volume of cell suspension (HCM containing 10^5 of ^{35}S-L-Met-labeled cells) was added to each well. The plate was kept in 37°C for 15 minutes, centrifuged at 16 g to make ^{35}S-labeled cells on contact with monolayer, then kept in 37°C for 1-2 hours. Another 96-well plate with wells filled with corresponding HCM containing 1x antibody or peptide was assembled with the "monolayer" plate with a tight seal. The sealed two-plate was then inverted, centrifuged off ^{35}S-labeled cells that do not adhere to the monolayer at 500 g, and quickly frozen in a slurry of dry ice-ethanol. The top, bottom, and middle parts of each well were then clipped off using a dog toenail clipper, and put into separate scintillation vials for liquid scintillation assay. The percent cell bound was calculated as a ratio of the cpm from the well bottom of the cpm from the whole well.

References

Abeliovich, A., Paylor, R., Chen, C., Kim, J. J., Wehner, J. M., and Tonegawa, S. (1993). PKC-γ Mutant Mice Exhibit Mild Deficits in Spatial and Contextual Learning. Cell *75*, 1263-1271.

Aberle, H., Schwartz, H., and Kemler, R. (1996). Cadherin-Catenin Complex: Protein Interactions and Their Implications for Cadherin Function. Journal of Cellular Biochemistry *61*, 514-523.

Adams, C. L., and Nelson, W. J. (1998). Cytomechanics of Cadherin-Mediated Cell-Cell Adhesion. Current Opinion in Cell Biology *10*, 572-577.

Adams, C. L., Nelson, W. J., and Smith, S. J. (1996). Quantitative Analysis of Cadherin-Catenin-Actin Reorganization during Development of Cell-Cell Adhesion. Journal of Cell Biology *135*, 1899-1911.

Aiba, A., Chen, C., Herrup, K., Rosenmund, C., Stevens, C. F., and Tonegawa, S. (1994). Reduced Hippocampal Long-Term Potentiation and Context-Specific Deficit in Associative Learning in mGluR1 Mutant Mice. Cell *79*, 365-375.

Aiba, A., Kano, M., Chen, C., Stanton, M. E., Fox, G. D., Herrup, K., Zwingman, T. A., and Tonegawa, S. (1994). Deficient Cerebellar Long-Term Depression and Impaired Motor Learning in mGluR1 Mutant Mice. Cell *79*, 377-388.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Waston, J. D. (1994). Molecular Biology of the Cell, 3rd edition.

Andersen, P., and Moser, E. I. (1995). Brain Temperature and Hippocampal Function. Hippocampus *5*, 491-498.

Angres, B., Barth, A., and Nelson, W. J. (1996). Mechanism for Transition from Initial to Stable Cell-Cell Adhesion: Kinetic Analysis of E-Cadherin-Mediated Adhesion Using a Quantitative Adhesion Assay. Journal of Cell Biology *134*, 549-557.

Arancio, O., Kiebler, M., Lee, C. J., Levram, V., Tsien, R. Y., Kandel, E. R., and Hawkins, R. D. (1996). Nitric Oxide Acts Directly in the Presynaptic Neuron to Produce Long-Term Potentiation in Cultured Hippocampal Neurons. Cell *87*, 1025-1035.

Asztely, F., Erdemli, G., and Kullmann, D. M. (1997). Extrasynaptic Glutamate Spillover in the Hippocampus: Dependence on Temperature and the Role of Active Glutamate Uptake. Neuron *18*, 281-293.

Bahr, B. A., Staubli, U., Xiao, P., Chun, D., Ji, Z. X., Esteban, E. T., and Lynch, G. (1997). Arg-Gly-Asp-Ser-Selective Adhesion and the Stabilization of Long-Term Potentiation: Pharmacological Studies and the Characterization of a Candidate Matrix Receptor. Journal of Neuroscience *17*, 1320-1329.

Bailey, C. H., Chen, M., Keller, F., and Kandel, E. R. (1992). Serotonin-Mediated Endocytosis of apCAM: an Early Step of Learning-Related Synaptic Growth in *Aplysia*. Science *256*, 645-649.

Bailey, C. H., Kaang, B. K., Chen, M., Martin, K. C., Lim, C. S., Casadio, A., and Kandel, E. R. (1997). Mutation in the Phosphorylation Sites of Map Kinase Blocks Learning-Related Internalization of apCAM in *Aplysia* Sensory Neurons. Neuron *18*, 913-924.

Balsamo, J., Arregui, C., Leung, T. C., and Lilien, J. (1998). The Non-receptor Protein-Tyrosine-Phosphatase PTP1B Binds to the Cytoplasmic Domain of N-Cadherin and Regulates the Cadherin-Actin Linkage. Journal of Cell Biology *143*, 523-532.

Balsamo, J., Leung, T. C., Ernst, H., Zanin, M. K. B., Hoffman, S., and Lilien, J. (1996). Regulated Binding of a PTP1B-Like Phosphatase to N-Cadherin: Control of Cadherin-Mediated Adhesion by Dephosphorylation of β-Catenin. Journal of Cell Biology *134*, 801-813.

Barnes, C. A. (1988). Spatial Learning and Memory Processes: the Search for Their Neurobiological Mechanisms in the Rat. Trends in Neurosciences *11*, 163-169.

Barth, A. I., Nathke, I. S., and Nelson, W. J. (1997). Cadherins, Catenins and APC Protein: Interplay Between Cytoskeletal Complexes and Signaling Pathways. Current Opinion in Cell Biology *9*, 683-690.

Bastmeyer, M., Ott, H., Leppert, C. A., and Stuermer, C. A. O. (1995). Fish E587 Glycoprotein, a Member of the L1 Family of Cell Adhesion Molecules, Participates in Axonal Fasciculation and the Age-Related Order of Ganglion Cell Axons in the Goldfish Retina. Journal of Cell Biology *130*, 969-976.

Becker, T. C., Noel, R. J., Coats, W. S., Gomezfoix, A. M., Alam, T., Gerard, R. D., and Newgard, C. B. (1994). Use of Recombinant Adenovirus for Metabolic Engineering of Mammalian Cells. Methods in Cell Biology *43*, 161-189.

Beesley, P. W., Mummery, R., and Tibaldi, J. (1995). N-Cadherin is a Major Glycoprotein Component of Isolated Rat Forebrain Postsynaptic Densities. Journal of Neurochemistry *64*, 2288-2294.

Beesley, P. W., Mummery, R., Tibaldi, J., Chapman, A. P., Smith, S. J., and Rider, C. C. (1995). The Postsynaptic Density: Putative Involvement in Synapse Stabilization via Cadherins and Covalent Modification by Ubiquitination. Biochemical Society Transactions *23*, 59-64.

Behrens, J., Vakaet, L., Friis, R., Winterhager, E., Vanroy, F., Mareel, M. M., and Birchmeier, W. (1993). Loss of Epithelial Differentiation and Gain of Invasiveness Correlates with Tyrosine Phosphorylation of the E-Cadherin β-Catenin Complex in Cells Transformed with a Temperature-Sensitive v-src Gene. Journal of Cell Biology *120*, 757-766.

Bekkers, J. M., Richerson, G. B., and Stevens, C. F. (1990). Origin of Variability in Quantal Size in Cultured Hippocampal Neurons and Hippocampal Slices. Proceedings of the National Academy of Sciences of the United States of America *87*, 5359-5362.

Benson, D. L., and Tanaka, H. (1998). N-Cadherin Redistribution during Synaptogenesis in Hippocampal Neurons. Journal of Neuroscience *18*, 6892-6904.

Bergles, D. E., and Jahr, C. E. (1997). Synaptic Activation of Glutamate Transporters in Hippocampal Astrocytes. Neuron *19*, 1297-1308.

Berkner, K. L. (1988). Development of Adenovirus Vectors for the Expression of Heterologous Genes. Biotechniques *6*, 616-Continues.

Berndorff, D., Gessner, R., Kreft, B., Schnoy, N., Lajouspetter, A. M., Loch, N., Reutter, W., Hortsch, M., and Tauber, R. (1994). Liver-Intestine Cadherin: Molecular Cloning and Characterization of a Novel Ca^{2+}-Dependent Cell Adhesion Molecule Expressed in Liver and Intestine. Journal of Cell Biology *125*, 1353-1369.

Betz, W. J., and Bewick, G. S. (1992). Optical Analysis of Synaptic Vesicle Recycling at the Frog Neuromuscular Junction. Science *255*, 200-203.

Betz, W. J., Mao, F., and Bewick, G. S. (1992). Activity-Dependent Fluorescent Staining and Destaining of Living Vertebrate Motor Nerve Terminals. Journal of Neuroscience *12*, 363-375.

Bixby, J. L., Lilien, J., and Reichardt, L. F. (1988). Identification of the Major Proteins That Promote Neuronal Process Outgrowth on Schwann Cells *in vitro*. Journal of Cell Biology *107*, 353-361.

Blaschuk, O. W., Pouliot, Y., and Holland, P. C. (1990). Identification of a Conserved Region Common to Cadherins and Influenza Strain A Hemagglutinins. Journal of Molecular Biology *211*, 679-682.

Blaschuk, O. W., Sullivan, R., David, S., and Pouliot, Y. (1990). Identification of a Cadherin Cell Adhesion Recognition Sequence. Developmental Biology *139*, 227-229.

Bliss, T. V. P., and Lomo, T. (1973). Long-lasting Potentiation of Synaptic Transmission in the Dentate Area of the Anaesthetized Rabbit following Stimulation of the Perforant Path. J. Physiol., London *232*, 331-356.

Bliss, T. V. P., and Collingridge, G. L. (1993). A Synaptic Model of Memory: Long-Term Potentiation in the Hippocampus. Nature *361*, 31-39.

Bohme, G. A., Bon, C., Stutzmann, J. M., Doble, A., and Blanchard, J. C. (1991). Possible Involvement of Nitric Oxide in Long-Term Potentiation. European Journal of Pharmacology *199*, 379-381.

Bonhoeffer, T., Staiger, V., and Aertsen, A. (1989). Synaptic Plasticity in Rat Hippocampal Slice Cultures: Local Hebbian Conjunction of Presynaptic and Postsynaptic Stimulation Leads to Distributed Synaptic Enhancement. Proceedings of the National Academy of Sciences of the United States of America *86*, 8113-8117.

Boyd, I. A., and Martin, A. R. (1956). The End-Plate Potential in mammalian muscle. Journal of Physiology Paris *132*, 74-91.

Bradley, R. S., Espeseth, A., and Kintner, C. (1998). NF-Protocadherin, a Novel Member of the Cadherin Superfamily, is Required for *Xenopus* Ectodermal Differentiation. Current Biology *8*, 325-334.

Bradykalnay, S. M., Rimm, D. L., and Tonks, N. K. (1995). The Receptor Protein Tyrosine Phosphatase PTPμ Associates with Cadherins and Catenins. Molecular Biology of the Cell *6*, 676-676.

Brittis, P. A., Lemmon, V., Rutishauser, U., and Silver, J. (1995). Unique Changes of Ganglion Cell Growth Cone Behavior following Cell Adhesion Molecule Perturbations: a Time-Lapse Study of the Living Retina. Molecular and Cellular Neuroscience *6*, 433-449.

Buchs, P. A., and Muller, D. (1996). Induction of Long-Term Potentiation is Associated with Major Ultrastructural-Changes of Activated Synapses. Proceedings of the National Academy of Sciences of the United States of America *93*, 8040-8045.

Buchs, P. A., Stoppini, L., Parducz, A., Siklos, L., and Muller, D. (1994). A New Cytochemical Method for the Ultrastructural-Localization of Calcium in the Central Nervous System. Journal of Neuroscience Methods *54*, 83-93.

Byers, S., Amaya, E., Munro, S., and Blaschuk, O. (1992). Fibroblast Growth Factor Receptors Contain a Conserved HAV Region Common to Cadherins and Influenza Strain A Hemagglutinins: a Role in Protein-Protein Interactions. Developmental Biology *152*, 411-414.

Castro, C. A., Silbert, L. H., McNaughton, B. L., and Barnes, C. A. (1989). Recovery of Spatial Learning Deficits after Decay of Electrically Induced Synaptic Enhancement in the Hippocampus. Nature *342*, 545-548.

Ceccarelli, B., Hurlbut, W. P., and Mauro, A. (1973). Turnover of Transmitter and Synaptic Vesicles at the Frog Neuromuscular Junction. Journal of Cell Biology *57*, 499-524.

Cepek, K. L., Shaw, S. K., Parker, C. M., Russell, G. J., Morrow, J. S., Rimm, D. L., and Brenner, M. B. (1994). Adhesion Between Epithelial Cells and T-Lymphocytes Mediated by E-Cadherin and the $\alpha^{E}\beta^{7}$ Integrin. Nature *372*, 190-193.

Chang, F. L. F., and Greenough, W. T. (1984). Transient and Enduring Morphological Correlates of Synaptic Activity and Efficacy Change in the Rat Hippocampal Slice. Brain Research *309*, 35-46.

Chen, B. M., and Grinnell, A. D. (1994). Regulation of Transmitter Release by Muscle Length in Frog Motor Nerve Terminals: Dynamics of the Effect and the Role of Integrin-ECM Interactions. Advances in Second Messenger and Phosphoprotein Research *29*, 383-398.

Chen, B. M., and Grinnell, A. D. (1995). Integrins and Modulation of Transmitter Release from Motor Nerve Terminals by Stretch. Science *269*, 1578-1580.

Chen, C., and Tonegawa, S. (1997). Molecular Genetic Analysis of Synaptic Plasticity, Activity-Dependent Neural Development, Learning, and Memory in the Mammalian Brain. Annual Review of Neuroscience *20*, 157-184.

Chen, H. Y., Paradies, N. E., Fedorchaiken, M., and Brackenbury, R. (1997). E-Cadherin Mediates Adhesion and Suppresses Cell Motility via Distinct Mechanisms. Journal of Cell Science *110*, 345-356.

Cheng, J., Wu, K., Armanini, M., Orourke, N., Dowbenko, D., and Lasky, L. A. (1997). A Novel Protein Tyrosine Phosphatase Related to the Homotypically Adhering κ-Receptors and μ-Receptors. Journal of Biological Chemistry *272*, 7264-7277.

Chuah, M. I., David, S., and Blaschuk, O. (1991). Differentiation and Survival of Rat Olfactory Epithelial Neurons in Dissociated Cell Culture. Developmental Brain Research *60*, 123-132.

Clark, E. A., and Brugge, J. S. (1995). Integrins and Signal Transduction Pathways: the Road Taken. Science *268*, 233-239.

Colman, D. R. (1997). Neurites, Synapses, and Cadherins Reconciled. Molecular and Cellular Neuroscience *10*, 1-6.

Conquet, F., Bashir, Z. I., Davies, C. H., Daniel, H., Ferraguti, F., Bordi, F., Franzbacon, K., Reggiani, A., Matarese, V., Conde, F., Collingridge, G. L., and Crepel, F. (1994). Motor Deficit and Impairment of Synaptic Plasticity in Mice Lacking mGluR1. Nature *372*, 237-243.

Cremer, H., Lange, R., Christoph, A., Plomann, M., Vopper, G., Roes, J., Brown, R., Baldwin, S., Kraemer, P., Scheff, S., Barthels, D., Rajewsky, K., and Wille, W. (1994). Inactivation of the N-CAM Gene in Mice Results in Size Reduction of the Olfactory Bulb and Deficits in Spatial Learning. Nature *367*, 455-459.

Dalseg, A. M., Gaardsvoll, H., and Bock, E. (1993). Molecular Biology of Cadherins in the Nervous System. Molecular Neurobiology *7*, 207-228.

Damsky, C. H., Richa, J., Solter, D., Knudsen, K., and Buck, C. A. (1983). Identification and Purification of a Cell Surface Glycoprotein Mediating Intercellular Adhesion in Embryonic and Adult Tissue. Cell *34*, 455-466.

Daniel, J. M., and Reynolds, A. B. (1995). The Tyrosine Kinase Substrate $P120^{CAS}$ Binds Directly to E-Cadherin but not to the Adenomatous Polyposis Coli Protein or α-Catenin. Molecular and Cellular Biology *15*, 4819-4824.

Dantzig, A. H., Hoskins, J., Tabas, L. B., Bright, S., Shepard, R. L., Jenkins, I. L., Duckworth, D. C., Sportsman, J. R., Mackensen, D., Rosteck, P. R., and Skatrud, P. L. (1994). Association of Intestinal Peptide Transport with a Protein Related to the Cadherin Superfamily. Science *264*, 430-433.

Davies, S. N., Lester, R. A. J., Reymann, K. G., and Collingridge, G. L. (1989). Temporally Distinct Presynaptic and Postsynaptic Mechanisms Maintain Long-Term Potentiation. Nature *338*, 500-503.

Davis, G. W., Schuster, C. M., and Goodman, C. S. (1996). Genetic Dissection of Structural and Functional Components of Synaptic Plasticity. III. CREB is Necessary for Presynaptic Functional Plasticity. Neuron *17*, 669-679.

Dedhar, S., and Hannigan, G. E. (1996). Integrin Cytoplasmic Interactions and Bidirectional Transmembrane Signaling. Current Opinion in Cell Biology *8*, 657-669.

Desmond, N. L., and Levy, W. B. (1986). Changes in the Numerical Density of Synaptic Contacts with Long-Term Potentiation in the Hippocampal Dentate Gyrus. Journal of Comparative Neurology *253*, 466-475.

Desmond, N. L., and Levy, W. B. (1986). Changes in the Postsynaptic Density with Long-Term Potentiation in the Dentate Gyrus. Journal of Comparative Neurology *253*, 476-482.

Desmond, N. L., and Levy, W. B. (1988). Synaptic Interface Surface Area Increases with Long-Term Potentiation in the Hippocampal Dentate Gyrus. Brain Research *453*, 308-314.

Desmond, N. L., and Levy, W. B. (1990). Morphological Correlates of Long-Term Potentiation Imply the Modification of Existing Synapses, not Synaptogenesis, in the Hippocampal Dentate Gyrus. Synapse *5*, 139-143.

Doherty, P., Rowett, L. H., Moore, S. E., Mann, D. A., and Walsh, F. S. (1991). Neurite Outgrowth in Response to Transfected N-CAM and N-Cadherin Reveals Fundamental Differences in Neuronal Responsiveness to CAMs. Neuron *6*, 247-258.

Doherty, P., Williams, E., and Walsh, F. S. (1995). A Soluble Chimeric Form of the L1 Glycoprotein Stimulates Neurite Outgrowth. Neuron *14*, 57-66.

Dolphin, A. C., Errington, M. L., and Bliss, T. V. P. (1982). Long-Term Potentiation of the Perforant Path *in vivo* is Associated with Increased Glutamate Release. Nature *297*, 496-498.

Doyle, E., Nolan, P. M., Bell, R., and Regan, C. M. (1992). Intraventricular Infusions of Anti-Neural Cell Adhesion Molecules in a Discrete Posttraining Period Impair Consolidation of a Passive Avoidance Response in the Rat. Journal of Neurochemistry *59*, 1570-1573.

Drazba, J., and Lemmon, V. (1990). The Role of Cell Adhesion Molecules in Neurite Outgrowth on Muller Cells. Developmental Biology *138*, 82-93.

Durand, G. M., Kovalchuk, Y., and Konnerth, A. (1996). Long-Term Potentiation and Functional Synapse Induction in Developing Hippocampus. Nature *381*, 71-75.

Egelman, D. M., and Montague, P. R. (1998). Computational Properties of Peri-Dendritic Calcium Fluctuations. Journal of Neuroscience *18*, 8580-8589.

Engert, F., and Bonhoeffer, T. (1997). Synapse Specificity of Long-Term Potentiation Breaks Down at Short Distances. Nature *388*, 279-284.

Espeseth, A., Johnson, E., and Kintner, C. (1995). *Xenopus* F-Cadherin, a Novel Member of the Cadherin Family of Cell Adhesion Molecules, is Expressed at Boundaries in the Neural Tube. Molecular and Cellular Neuroscience *6*, 199-211.

Fagotto, F., Funayama, N., Gluck, U., and Gumbiner, B. M. (1996). Binding to Cadherins Antagonizes the Signaling Activity of β-Catenin during Axis Formation in *Xenopus*. Journal of Cell Biology *132*, 1105-1114.

Fannon, A. M., and Colman, D. R. (1996). A Model for Central Synaptic Junctional Complex Formation Based on the Differential Adhesive Specificities of the Cadherins. Neuron *17*, 423-434.

Fannon, A. M., Sherman, D. L., Ilyinagragerova, G., Brophy, P. J., Friedrich, V. L., and Colman, D. R. (1995). Novel E-Cadherin-Mediated Adhesion in Peripheral Nerve: Schwann Cell Architecture is Stabilized by Autotypic Adherens Junctions. Journal of Cell Biology *129*, 189-202.

Fields, R. D., and Itoh, K. (1996). Neural Cell Adhesion Molecules in Activity-Dependent Development and Synaptic Plasticity. Trends in Neurosciences *19*, 473-480.

Fitzsimonds, R. M., Song, H. J., and Poo, M. M. (1997). Propagation of Activity-Dependent Synaptic Depression in Simple Neural Networks. Nature *388*, 439-448.

Frerking, M., and Wilson, M. (1996). Saturation of Postsynaptic Receptors at Central Synapses. Current Opinion in Neurobiology *6*, 395-403.

Friedlander, D. R., Mege, R. M., Cunningham, B. A., and Edelman, G. M. (1989). Cell Sorting-Out is Modulated by both the Specificity and Amount of Different Cell Adhesion Molecules (CAMs) Expressed on Cell Surfaces. Proceedings of the National Academy of Sciences of the United States of America *86*, 7043-7047.

Fuchs, M., Muller, T., Lerch, M. M., and Ullrich, A. (1996). Association of Human Protein-Tyrosine-Phosphatase-Kappa with Members of the Armadillo Family. Journal of Biological Chemistry *271*, 16712-16719.

Fujimori, T., and Takeichi, M. (1993). Disruption of Epithelial Cell-Cell Adhesion by Exogenous Expression of a Mutated Nonfunctional N-Cadherin. Molecular Biology of the Cell *4*, 37-47.

Garrod, D., Chidgey, M., and North, A. (1996). Desmosomes: Differentiation, Development, Dynamics and Disease. Current Opinion in Cell Biology *8*, 670-678.

Geiger, B., Volberg, T., Ginsberg, D., Bitzur, S., Sabanay, I., and Hynes, R. O. (1990). Broad-Spectrum Pan-Cadherin Antibodies, Reactive with the C-Terminal 24-Amino Acid Residues of N-Cadherin. Journal of Cell Science *97*, 607-614.

Geinisman, Y. (1993). Perforated Axospinous Synapses with Multiple, Completely Partitioned Transmission Zones: Probable Structural Intermediates in Synaptic Plasticity. Hippocampus *3*, 417-434.

Geinisman, Y., Detoledomorrell, L., and Morrell, F. (1991). Induction of Long-Term Potentiation is Associated with an Increase in the Number of Axospinous Synapses with Segmented Postsynaptic Densities. Brain Research *566*, 77-88.

Geinisman, Y., Detoledomorrell, L., Morrell, F., Heller, R. E., Rossi, M., and Parshall, R. F. (1993). Structural Synaptic Correlate of Long-Term Potentiation: Formation of Axo-

spinous Synapses with Multiple, Completely Partitioned Transmission Zones. Hippocampus *3*, 435-446.

Geinisman, Y., Morrell, F., and Detoledomorrell, L. (1992). Increase in the Number of Axospinous Synapses with Segmented Postsynaptic Densities following Hippocampal Kindling. Brain Research *569*, 341-347.

Gerlai, R. (1996). Gene-Targeting Studies of Mammalian Behavior: is It the Mutation or the Background Genotype. Trends in Neurosciences *19*, 177-181.

Gilbertson-beadling, S. K., and Fisher, C. (1993). A Potential Role for N-Cadherin in Mediating Endothelial Cell-Smooth Muscle Cell Interactions in the Rat Vasculature. Laboratory Investigation *69*, 203-209.

Glanzman, D. L., Kandel, E. R., and Schacher, S. (1990). Target-Dependent Structural-Changes Accompanying Long-Term Synaptic Facilitation in *Aplysia* Neurons. Science *249*, 799-802.

Glanzman, D. L., Mackey, S. L., Hawkins, R. D., Dyke, A. M., Lloyd, P. E., and Kandel, E. R. (1989). Depletion of Serotonin in the Nervous System of *Aplysia* Reduces the Behavioral Enhancement of Gill Withdrawal as well as the Heterosynaptic Facilitation Produced by Tail Shock. Journal of Neuroscience *9*, 4200-4213.

Grant, S. G. N., Odell, T. J., Karl, K. A., Stein, P. L., Soriano, P., and Kandel, E. R. (1992). Impaired Long-Term Potentiation, Spatial Learning, and Hippocampal Development in Fyn Mutant Mice. Science *258*, 1903-1910.

Green, E. J., McNaughton, B. L., and Barnes, C. A. (1990). Exploration-Dependent Modulation of Evoked Responses in Fascia-Dentata: Dissociation of Motor, EEG, and Sensory Factors and Evidence for a Synaptic Efficacy Change. Journal of Neuroscience *10*, 1455-1471.

Grotewiel, M. S., Beck, C. D. O., Wu, K. H., Zhu, X. R., and Davis, R. L. (1998). Integrin-Mediated Short-Term Memory in *Drosophila*. Nature *391*, 455-460.

Gumbiner, B. M. (1996). Cell Adhesion: the Molecular Basis of Tissue Architecture and Morphogenesis. Cell *84*, 345-357.

Gustafsson, B., Wigstrom, H., Abraham, W. C., and Huang, Y. Y. (1987). Long-Term Potentiation in the Hippocampus Using Depolarizing Current Pulses as the Conditioning Stimulus to Single Volley Synaptic Potentials. Journal of Neuroscience *7*, 774-780.

Haley, J. E., Wilcox, G. L., and Chapman, P. F. (1992). The Role of Nitric Oxide in Hippocampal Long-Term Potentiation. Neuron *8*, 211-216.

Hamaguchi, M., Matsuyoshi, N., Ohnishi, Y., Gotoh, B., Takeichi, M., and Nagai, Y. (1993). $p60^{v\text{-}src}$ Causes Tyrosine Phosphorylation and Inactivation of the N-Cadherin Catenin Cell Adhesion System. EMBO Journal *12*, 307-314.

Harris, K. M., and Landis, D. M. D. (1986). Membrane-Structure at Synaptic Junctions in Area CA1 of the Rat Hippocampus. Neuroscience *19*, 857-Continues.

Harris, K. M., and Stevens, J. K. (1989). Dendritic Spines of Ca-1 Pyramidal Cells in the Rat Hippocampus: Serial Electron-Microscopy with Reference to Their Biophysical Characteristics. Journal of Neuroscience *9*, 2982-2997.

Hatta, K., and Takeichi, M. (1986). Expression of N-Cadherin Adhesion Molecules Associated with Early Morphogenetic Events in Chick Development. Nature *320*, 447-449.

Hawkins, R. D., and Kandel, E. R. (1984). is There a Cell Biological Alphabet for Simple Forms of Learning. Psychological Review *91*, 375-391.

Hay, E. D. (1991). Cell Biology of the Extracellular Matrix, edited by E. D. Hay. New York: Plenum, 1-4.

Hebb, D. O. (1949). The Organization of Behavior. New York: Wiley.

Hermiston, M. L., and Gordon, J. I. (1995). *In vivo* Analysis of Cadherin Function in the Mouse Intestinal Epithelium: Essential Roles in Adhesion, Maintenance of Differentiation, and Regulation of Programmed Cell Death. Journal of Cell Biology *129*, 489-506.

Hermiston, M. L., and Gordon, J. I. (1995). Inflammatory Bowel Disease and Adenomas in Mice Expressing a Dominant Negative N-Cadherin. Science *270*, 1203-1207.

Herndon, M. E., and Lander, A. D. (1990). A Diverse Set of Developmentally Regulated Proteoglycans is Expressed in the Rat Central Nervous System. Neuron *4*, 949-961.

Hertig, C. M., Butz, S., Koch, S., Eppenbergereberhardt, M., Kemler, R., and Eppenberger, H. M. (1996). N-Cadherin in Adult Rat Cardiomyocytes in Culture. II. Spatio-temporal Appearance of Proteins Involved in Cell-Cell Contact and Communication:

Formation of Two Distinct N-Cadherin/Catenin Complexes. Journal of Cell Science *109*, 11-20.

Hessler, N. A., Shirke, A. M., and Malinow, R. (1993). The Probability of Transmitter Release at a Mammalian Central Synapse. Nature *366*, 569-572.

Heuser, J. E., and Reese, T. S. (1973). Evidence for Recycling of Synaptic Vesicle Membrane at the Frog Neuromuscular Junction. Journal of Cell Biology *57*, 315-344.

Heynen, A. J., Abraham, W. C., and Bear, M. F. (1996). Bidirectional Modification of CA1 Synapses in the Adult Hippocampus *in vivo*. Nature *381*, 163-166.

Hinck, L., Nathke, I. S., Papkoff, J., and Nelson, W. J. (1994). β-Catenin: a Common Target for the Regulation of Cell Adhesion by Wnt-1 and Src Signaling Pathways. Trends in Biochemical Sciences *19*, 538-542.

Hinck, L., Nathke, I. S., Papkoff, J., and Nelson, W. J. (1994). Dynamics of Cadherin/Catenin Complex Formation: Novel Protein Interactions and Pathways of Complex Assembly. Journal of Cell Biology *125*, 1327-1340.

Hisajima, H., Saito, H., Abe, K., and Nishiyama, N. (1992). Effects of Acidic Fibroblast Growth Factor on Hippocampal Long-Term Potentiation in Fasted Rats. Journal of Neuroscience Research *31*, 549-553.

Holst, B. D., Vanderklish, P. W., Krushel, L. A., Zhou, W., Langdon, R. B., McWhirter, J. R., Edelman, G. M., and Crossin, K. L. (1998). Allosteric Modulation of AMPA-Type Glutamate Receptors Increases Activity of the Promoter for the Neural Cell Adhesion Molecule, N-CAM. Proceedings of the National Academy of Sciences of the United States of America *95*, 2597-2602.

Hoschuetzky, H., Aberle, H., and Kemler, R. (1994). β-Catenin Mediates the Interaction of the Cadherin Catenin Complex with Epidermal Growth Factor Receptor. Journal of Cell Biology *127*, 1375-1380.

Hosokawa, T., Rusakov, D. A., Bliss, T. V. P., and Fine, A. (1995). Repeated Confocal Imaging of Individual Dendritic Spines in the Living Hippocampal Slice: Evidence for Changes in Length and Orientation Associated with Chemically-Induced LTP. Journal of Neuroscience *15*, 5560-5573.

Hotchin, N. A., and Hall, A. (1995). The Assembly of Integrin Adhesion Complexes Requires both Extracellular Matrix and Intracellular Rho/Rac GTPases. Journal of Cell Biology *131*, 1857-1865.

Huang, A. M., Wang, H. L., Tang, Y. P., and Lee, E. H. Y. (1998). Expression of Integrin-Associated Protein Gene Associated with Memory Formation in Rats. Journal of Neuroscience *18*, 4305-4313.

Huber, A. H., Nelson, W. J., and Weis, W. I. (1997). Three-Dimensional Structure of the Armadillo Repeat Region of β-Catenin. Cell *90*, 871-882.

Hughes, P. E., Diazgonzalez, F., Leong, L., Wu, C. Y., McDonald, J. A., Shattil, S. J., and Ginsberg, M. H. (1996). Breaking the Integrin Hinge: a Defined Structural Constraint Regulates Integrin Signaling. Journal of Biological Chemistry *271*, 6571-6574.

Hunkapiller, T., and Hood, L. (1989). Diversity of the Immunoglobulin Gene Superfamily. Advances in Immunology *44*, 1-63.

Hyafil, F., Babinet, C., and Jacob, F. (1981). Cell-Cell Interaction in Early Embryogenesis: A Molecular Approach to the Role of Calcium. Cell *26*, 447-454.

Inoue, A., and Sanes, J. R. (1997). Lamina-Specific Connectivity in the Brain: Regulation by N-Cadherin, Neurotrophins, and Glycoconjugates. Science *276*, 1428-1431.

Inuzuka, H., Miyatani, S., and Takeichi, M. (1991). R-Cadherin: a Novel Ca^{2+}-Dependent Cell Cell Adhesion Molecule Expressed in the Retina. Neuron *7*, 69-79.

Inuzuka, H., Redies, C., and Takeichi, M. (1991). Differential Expression of R-Cadherin and N-Cadherin in Neural and Mesodermal Tissues during Early Chicken Development. Development *113*, 959-967.

Isaac, J. T. R., Crair, M. C., Nicoll, R. A., and Malenka, R. C. (1997). Silent Synapses during Development of Thalamocortical Inputs. Neuron *18*, 269-280.

Isaac, J. T. R., Nicoll, R. A., and Malenka, R. C. (1995). Evidence for Silent Synapses: Implications for the Expression of LTP. Neuron *15*, 427-434.

Itoh, K., Ozaki, M., Stevens, B., and Fields, R. D. (1997). Activity-Dependent Regulation of N-Cadherin in Drg Neurons: Differential Regulation of N-Cadherin, NCAM, and L1 by Distinct Patterns of Action Potentials. Journal of Neurobiology *33*, 735-748.

Itoh, K., Stevens, B., Schachner, M., and Fields, R. D. (1995). Regulated Expression of the Neural Cell Adhesion Molecule L1 by Specific Patterns of Neural Impulses. Science *270*, 1369-1372.

Iwai, Y., Usui, T., Hirano, S., Steward, R., Takeichi, M., and Uemura, T. (1997). Axon Patterning Requires *D*N-Cadherin, a Novel Neuronal Adhesion Receptor, in the *Drosophila* Embryonic CNS. Neuron *19*, 77-89.

Jiang, R. L., and Gridley, T. (1997). Gene Targeting: Things Go Better with CRE. Current Biology *7*, R321-R323.

Jones, E. Y. (1996). Three-Dimensional Structure of Cell Adhesion Molecules. Current Opinion in Cell Biology *8*, 602-608.

Jou, T. S., Stewart, D. B., Stappert, J., Nelson, W. J., and Marrs, J. A. (1995). Genetic and Biochemical Dissection of Protein Linkages in the Cadherin-Catenin Complex. Proceedings of the National Academy of Sciences of the United States of America *92*, 5067-5071.

Kandel, E. R., Schwartz, J.H., and Jessel, T.M. (1991). Principles of Neural Science, 3rd Ed. New York : Elsevier, c1991.

Kandel, E. R., and Odell, T. J. (1992). Are Adult Learning Mechanisms Also Used for Development. Science *258*, 243-245.

Kang, H. J., Welcher, A. A., Shelton, D., and Schuman, E. M. (1997). Neurotrophins and Time: Different Roles for TrkB Signaling in Hippocampal Long-Term Potentiation. Neuron *19*, 653-664.

Kantor, D. B., Lanzrein, M., Stary, S. J., Sandoval, G. M., Smith, W. B., Sullivan, B. M., Davidson, N., and Schuman, E. M. (1996). A Role for Endothelial NO Synthase in LTP Revealed by Adenovirus-Mediated Inhibition and Rescue. Science *274*, 1744-1748.

Karecla, P. I., Green, S. J., Bowden, S. J., Coadwell, J., and Kilshaw, P. J. (1996). Identification of a Binding-Site for Integrin $\alpha^E\beta^7$ in the N-Terminal Domain of E-Cadherin. Journal of Biological Chemistry *271*, 30909-30915.

Karthikeyan, L., Flad, M., Engel, M., Meyerputtlitz, B., Margolis, R. U., and Margolis, R. K. (1994). Immunocytochemical and *in situ* Hybridization Studies of the Heparan-

Sulfate Proteoglycan, Glypican, in Nervous Tissue. Journal of Cell Science *107*, 3213-3222.

Kassner, P. D., Kawaguchi, S., and Hemler, M. E. (1994). Minimum α-Chain Cytoplasmic Tail Sequence Needed to Support Integrin-Mediated Adhesion. Journal of Biological Chemistry *269*, 19859-19867.

Katz, B. (1969). The Release of Neural Transmitter Substances, Liverpool University Press, Liverpool.

Kauer, J. A., Malenka, R. C., and Nicoll, R. A. (1988). A Persistent Postsynaptic Modification Mediates Long-Term Potentiation in the Hippocampus. Neuron *1*, 911-917.

Kelly, R. B. (1993). Storage and Release of Neurotransmitters. Cell *72*, 43-53.

Kemler, R. (1992). Classical cadherins. Sem. Cell Biol. *3*, 149-155.

Kintner, C. (1992). Regulation of Embryonic Cell Adhesion by the Cadherin Cytoplasmic Domain. Cell *69*, 225-236.

Knudsen, K. A., Soler, A. P., Johnson, K. R., and Wheelock, M. J. (1995). Interaction of α-Actinin with the Cadherin/Catenin Cell-Cell Adhesion Complex via α-Catenin. Journal of Cell Biology *130*, 67-77.

Knudsen, K. A., and Wheelock, M. J. (1992). Plakoglobin, or an 83 kD Homolog Distinct from β-Catenin, Interacts with E-Cadherin and N-Cadherin. Molecular Biology of the Cell *3*, A215-A215.

Koch, A. W., Pokutta, S., Lustig, A., and Engel, J. (1997). Calcium-Binding and Homoassociation of E-Cadherin Domains. Biochemistry *36*, 7697-7705.

Koch, P. J., and Franke, W. W. (1994). Desmosomal Cadherins: Another Growing Multigene Family of Adhesion Molecules. Current Opinion in Cell Biology *6*, 682-687.

Kohmura, N., Senzaki, K., Hamada, S., Kai, N., Yasuda, R., Watanabe, M., Ishii, H., Yasuda, M., Mishina, M., and Yagi, T. (1998). Diversity Revealed by a Novel Family of Cadherins Expressed in Neurons at a Synaptic Complex. Neuron *20*, 1137-1151.

Konorski, J. (1948). Conditioned Reflexes and Neuron Organisation. Cambridge University Press, Cambridge.

Korinek, V., Barker, N., Morin, P. J., Vanwichen, D., Deweger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. (1997). Constitutive Transcriptional Activation by a β-Catenin-Tcf Complex in APC(-/-) Colon-Carcinoma. Science *275*, 1784-1787.

Korn, H., and Faber, D. S. (1991). Quantal Analysis and Synaptic Efficacy in the CNS. Trends in Neurosciences *14*, 439-445.

Korn, H., and Faber, D. S. (1998). Quantal Analysis and Long-Term Potentiation. Comptes Rendus De L Academie Des Sciences Serie III Sciences De La Vie Life Sciences *321*, 125-130.

Krnjevic, K., Morris, M. E., and Reiffenstein, R. J. (1982). Stimulation-Evoked Changes in Extracellular K^+ and Ca^{2+} in Pyramidal Layers of the Rats Hippocampus. Canadian Journal of Physiology and Pharmacology *60*, 1643-1657.

Kullmann, D. M. (1994). Amplitude Fluctuations of Dual Component EPSCs in Hippocampal Pyramidal Cells: Implications for Long-Term Potentiation. Neuron *12*, 1111-1120.

Kullmann, D. M., Erdemli, G., and Asztely, F. (1996). LTP of AMPA and NMDA Receptor-Mediated Signals: Evidence for Presynaptic Expression and Extrasynaptic Glutamate Spill-Over. Neuron *17*, 461-474.

Kypta, R. M., Su, H., and Reichardt, L. F. (1996). Association Between a Transmembrane Protein Tyrosine Phosphatase and the Cadherin-Catenin Complex. Journal of Cell Biology *134*, 1519-1529.

Laflamme, S. E., Thomas, L. A., Yamada, S. S., and Yamada, K. M. (1994). Single Subunit Chimeric Integrins as Mimics and Inhibitors of Endogenous Integrin Functions in Receptor Localization, Cell Spreading and Migration, and Matrix Assembly. Journal of Cell Biology *126*, 1287-1298.

Larkman, A., Stratford, K., and Jack, J. (1991). Quantal Analysis of Excitatory Synaptic Action and Depression in Hippocampal Slices. Nature *350*, 344-347.

Larson, J., and Lynch, G. (1986). Induction of Synaptic Potentiation in Hippocampus by Patterned Stimulation Involves Two Events. Science *232*, 985-988.

Larue, L., Ohsugi, M., Hirchenhain, J., and Kemler, R. (1994). E-Cadherin Null Mutant Embryos Fail to Form a Trophectoderm Epithelium. Proceedings of the National Academy of Sciences of the United States of America *91*, 8263-8267.

Lee, K. S., Schottler, F., Oliver, M., and Lynch, G. (1980). Brief Bursts of High-Frequency Stimulation Produce Two Types of Structural Changes in Rat Hippocampus. J. Neurophysiol. *44*, 247-258.

Lemmon, V., Farr, K. L., and Lagenaur, C. (1989). L1-Mediated Axon Outgrowth Occurs via a Homophilic Binding Mechanism. Neuron *2*, 1597-1603.

Letourneau, P. C., Condic, M. L., and Snow, D. M. (1994). Interactions of Developing Neurons with the Extracellular Matrix. Journal of Neuroscience *14*, 915-928.

Liao, D., Jones, A., and Malinow, R. (1992). Direct Measurement of Quantal Changes underlying Long-Term Potentiation in CA1 Hippocampus. Neuron *9*, 1089-1097.

Liao, D. Z., Hessler, N. A., and Malinow, R. (1995). Activation of Postsynaptically Silent Synapses during Pairing-Induced LTP in CA1 Region of Hippocampal Slice. Nature *375*, 400-404.

Lilien, J., Balsamo, J., Hoffman, S., and Eisenberg, C. (1997). β-Catenin is a Target for Extracellular Signals Controlling Cadherin Function: the Neurocan-GalNAcPTase Connection. Current Topics in Developmental Biology *35*, 161-189.

Lin, X. Y., and Glanzman, D. L. (1994). Long-Term Potentiation of *Aplysia* Sensorimotor Synapses in Cell Culture: Regulation by Postsynaptic Voltage. Proceedings of the Royal Society of London Series B Biological Sciences *255*, 113-118.

Linden, D. J. (1997). Long-Term Potentiation of Glial Synaptic Currents in Cerebellar Culture. Neuron *18*, 983-994.

Linnemann, D., Gaardsvoll, H., Dalseg, A. M., Zhernosekov, D., Lundgren, T., Edvardsen, K., and Bock, E. (1994). Characterization of N-Cadherin Messenger-RNA and Polypeptide Expression in Rat. International Journal of Developmental Neuroscience *12*, 441-450.

Lisman, J. E., and Harris, K. M. (1993). Quantal Analysis and Synaptic Anatomy: Integrating two Views of Hippocampal Plasticity. Trends in Neurosciences *16*, 141-147.

Litwack, E. D., Stipp, C. S., Kumbasar, A., and Lander, A. D. (1994). Neuronal Expression of Glypican, a Cell Surface Glycosylphosphatidylinositol-Anchored Heparan Sulfate Proteoglycan, in the Adult Rat Nervous System. Journal of Neuroscience *14*, 3713-3724.

Lotz, M. M., Burdsal, C. A., Erickson, H. P., and McClay, D. R. (1989). Cell Adhesion to Fibronectin and Tenascin: Quantiative Measurements of Initial Binding and Subsequent Strengthening Responses. J. Cell Biol. *109*, 1795-1805.

Luscher, C., Malenka, R. C., and Nicoll, R. A. (1998). Monitoring Glutamate Release during LTP with Glial Transporter Currents. Neuron *21*, 435-441.

Luthi, A., Laurent, J. P., Figurov, A., Muller, D., and Schachner, M. (1994). Hippocampal Long-Term Potentiation and Pleural Cell Adhesion Molecules L1 and NCAM. Nature *372*, 777-779.

Ma, L., Zablow, L., R., K. E., and Siegelbaum, S. A. (1999). Cyclic AMP Induces Functional Presynaptic Boutons in Hippocampal CA3-CA1 Neuronal Cultures. Nature Neuroscience *2*, 24-30.

Madison, D. V., Schuman, E. M. (1991). LTP, Post or Pre? A Look at the Evidence for the Locus of Long-Term Potentiation. The New Biologist *3*: 549-557.

Mahoney, P. A., Weber, U., Onofrechuk, P., Biessmann, H., Bryant, P. J., and Goodman, C. S. (1991). The Fat Tumor Suppressor Gene in *Drosophila* Encodes a Novel Member of the Cadherin Gene Superfamily. Cell *67*, 853-868.

Malinow, R. (1994). LTP: Desperately Seeking Resolution. Science *266*, 1195-1196.

Manabe, T., and Nicoll, R. A. (1994). Long-Term Potentiation: Evidence Against an Increase in Transmitter Release Probability in the CA1 Region of the Hippocampus. Science *265*, 1888-1892.

Manabe, T., Renner, P., and Nicoll, R. A. (1992). Postsynaptic Contribution to Long-Term Potentiation Revealed by the Analysis of Miniature Synaptic Currents. Nature *355*, 50-55.

Matsuyoshi, N., Hamaguchi, M., Taniguchi, S., Nagafuchi, A., Tsukita, S., and Takeichi, M. (1992). Cadherin-Mediated Cell-Cell Adhesion is Perturbed by *v-src* Tyrosine Phosphorylation in Metastatic Fibroblasts. Journal of Cell Biology *118*, 703-714.

Mayford, M., Barzilai, A., Keller, F., Schacher, S., and Kandel, E. R. (1992). Modulation of an NCAM-Related Adhesion Molecule with Long-Term Synaptic Plasticity in *Aplysia*. Science *256*, 638-644.

Mayford, M., Mansuy, I. M., Muller, R. U., and Kandel, E. R. (1997). Memory and Behavior: a Second-Generation of Genetically Modified Mice. Current Biology *7*, R580-R589.

Mbalaviele, G., Chen, H., Boyce, B. F., Mundy, G. R., and Yoneda, T. (1995). The Role of Cadherin in the Generation of Multinucleated Osteoclasts from Mononuclear Precursors in Murine Marrow. Journal of Clinical Investigation *95*, 2757-2765.

McClay, D. R., Wessel, G. M., and Marchase, R. B. (1981). Intercellular Recognition: Quantitation of Initial Binding Events. Proc. Natl. Acad. Sci. USA *78*, 4975-4979.

McCrea, P. D., and Gumbiner, B. M. (1991). Purification of a 92-kDa Cytoplasmic Protein Tightly Associated with the Cell-Cell Adhesion Molecule E-Cadherin (Uvomorulin): Characterization and Extractability of the Protein Complex from the Cell Cytostructure. Journal of Biological Chemistry *266*, 4514-4520.

McCrea, P. D., Turck, C. W., and Gumbiner, B. (1991). A Homolog of the Armadillo Protein in *Drosophila* (Plakoglobin) Associated with E-Cadherin. Science *254*, 1359-1361.

McHugh, T. J., Blum, K. I., Tsien, J. Z., Tonegawa, S., and Wilson, M. A. (1996). Impaired Hippocampal Representation of Space in CA1-Specific NMDAR1 Knockout Mice. Cell *87*, 1339-1349.

McNaughton, B. L., Barnes, C. A., Rao, G., Baldwin, J., and Rasmussen, M. (1986). Long-Term Enhancement of Hippocampal Synaptic Transmission and the Acquisition of Spatial Information. Journal of Neuroscience *6*, 563-571.

McNeill, H., Ryan, T. A., Smith, S. J., and Nelson, W. J. (1993). Spatial and Temporal Dissection of Immediate and Early Events following Cadherin-Mediated Epithelial Cell Adhesion. Journal of Cell Biology *120*, 1217-1226.

Mege, R. M., Goudou, D., Diaz, C., Nicolet, M., Garcia, L., Geraud, G., and Rieger, F. (1992). N-Cadherin and N-CAM in Myoblast Fusion: Compared Localization and Effect of Blockade by Peptides and Antibodies. Journal of Cell Science *103*, 897-906.

Meyer, R. A., Laird, D. W., Revel, J. P., and Johnson, R. G. (1992). Inhibition of Gap Junction and Adherens Junction Assembly by Connexin and A-CAM Antibodies. Journal of Cell Biology *119*, 179-189.

Miller, J. R., and Moon, R. T. (1996). Signal Transduction Through β-Catenin and Specification of Cell Fate during Embryogenesis. Genes & Development *10*, 2527-2539.

Miller, P. D., Chung, W. W., Lagenaur, C. F., and Dekosky, S. T. (1993). Regional Distribution of Neural Cell Adhesion Molecule (N-CAM) and L1 in Human and Rodent Hippocampus. Journal of Comparative Neurology *327*, 341-349.

Miyakawa, T., Yagi, T., Kitazawa, H., Yasuda, M., Kawai, N., Tsuboi, K., and Niki, H. (1997). Fyn Kinase as a Determinant of Ethanol Sensitivity: Relation to NMDA-Receptor Function. Science *278*, 698-701.

Miyatani, S., Shimamura, K., Hatta, M., Nagafuchi, A., Nose, A., Matsunaga, M., Hatta, K., and Takeichi, M. (1989). Neural Cadherin: Role in Selective Cell-Cell Adhesion. Science *245*, 631-635.

Montarolo, P. G., Goelet, P., Castellucci, V. F., Morgan, J., Kandel, E. R., and Schacher, S. (1986). A Critical Period for Macromolecular-Synthesis in Long-Term Heterosynaptic Facilitation in *Aplysia*. Science *234*, 1249-1254.

Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K. W. (1997). Activation of β-Catenin-Tcf Signaling in Colon Cancer by Mutations in β-Catenin or APC. Science *275*, 1787-1790.

Moser, E., Moser, M. B., and Andersen, P. (1993). Synaptic Potentiation in the Rat Dentate Gyrus during Exploratory Learning. Neuroreport *5*, 317-320.

Moser, E. I., and Andersen, P. (1994). Conserved Spatial Learning in Cooled Rats in Spite of Slowing of Dentate Field Potentials. Journal of Neuroscience *14*, 4458-4466.

Moser, E. I., Krobert, K. A., Moser, M. B., and Morris, R. G. M. (1998). Impaired Spatial Learning after Saturation of Long-Term Potentiation. Science *281*, 2038-2042.

Muller, D., Joly, M., and Lynch, G. (1988). Contributions of Quisqualate and NMDA Receptors to the Induction and Expression of LTP. Science *242*, 1694-1697.

Muller, D., Wang, C., Skibo, G., Toni, N., Cremer, H., Calaora, V., Rougon, G., and Kiss, J. Z. (1996). PSA-NCAM is Required for Activity-Induced Synaptic Plasticity. Neuron *17*, 413-422.

Munemitsu, S., Albert, I., Rubinfeld, B., and Polakis, P. (1996). Deletion of an Amino-Terminal Sequence Stabilizes β-Catenin *in vivo* and Promotes Hyperphosphorylation of the Adenomatous Polyposis-Coli Tumor Suppressor Protein. Molecular and Cellular Biology *16*, 4088-4094.

Murphy, G. G., and Glanzman, D. L. (1997). Mediation of Classical-Conditioning in *Aplysia* californica by Long-Term Potentiation of Sensorimotor Synapses. Science *278*, 467-471.

Murthy, V. N. (1997). Synaptic Plasticity: Neighborhood Influences. Current Biology *7*, R512-R515.

Nadel, L., and Moscovitch, M. (1997). Memory Consolidation, Retrograde Amnesia and the Hippocampal Complex. Current Opinion in Neurobiology *7*, 217-227.

Nagafuchi, A., Shirayoshi, Y., Yasuda, K., and Takeichi, M. (1987). Transformation of Cell Adhesion Property by Exogenously Introduced E-Cadherin cDNA. Cell Structure and Function *12*, 628-628.

Nagafuchi, A., Takeichi, M., and Tsukita, S. (1991). The 102 kD Cadherin-Associated Protein: Similarity to Vinculin and Posttranscriptional Regulation of Expression. Cell *65*, 849-857.

Nagar, B., Overduin, M., Ikura, M., and Rini, J. M. (1996). Structural Basis of Calcium-Induced E-Cadherin Rigidification and Dimerization. Nature *380*, 360-364.

Nakic, M., Mitrovic, N., Sperk, G., and Schachner, M. (1996). Kainic Acid Activates Transient Expression of Tenascin-C in the Adult Rat Hippocampus. Journal of Neuroscience Research *44*, 355-362.

Nathke, I. S., Adams, C. L., Polakis, P., Sellin, J. H., and Nelson, W. J. (1996). The Adenomatous Polyposis-Coli Tumor Suppressor Protein Localizes to Plasma-Membrane Sites Involved in Active Cell Migration. Journal of Cell Biology *134*, 165-179.

Nathke, I. S., Hinck, L., Swedlow, J. R., Papkoff, J., and Nelson, W. J. (1994). Defining Interactions and Distributions of Cadherin and Catenin Complexes in Polarized Epithelial Cells. Journal of Cell Biology *125*, 1341-1352.

Nicholson, C., ten Bruggencate, G., Stockle, H., and Steinberg, R. (1978). Calcium and Potassium Changes in Extracellular Microenvironment of Cat Cerebellar Cortex. Journal of Neurophysiology *41*, 1026-1039.

Nose, A., Nagafuchi, A., and Takeichi, M. (1988). Expressed Recombinant Cadherins Mediate Cell Sorting in Model Systems. Cell *54*, 993-1001.

Nose, A., and Takeichi, M. (1986). A Novel Cadherin Cell Adhesion Molecule: Its Expression Patterns Associated with Implantation and Organogenesis of Mouse Embryos. Journal of Cell Biology *103*, 2649-2658.

Nose, A., Tsuji, K., and Takeichi, M. (1990). Localization of Specificity Determining Sites in Cadherin Cell Adhesion Molecules. Cell *61*, 147-155.

O'Dell, T. J., Hawkins, R. D., Kandel, E. R., and Arancio, O. (1991). Tests of the Roles of Two Diffusible Substances in Long-Term Potentiation: Evidence for Nitric Oxide as a Possible Early Retrograde Messenger. Proceedings of the National Academy of Sciences of the United States of America *88*, 11285-11289.

O'Dell, T. J., Huang, P. L., Dawson, T. M., Dinerman, J. L., Snyder, S. H., Kandel, E. R., and Fishman, M. C. (1994). Endothelial NOS and the Blockade of LTP by NOS Inhibitors in Mice Lacking Neuronal NOS. Science *265*, 542-546.

Oka, H., Shiozaki, H., Kobayashi, K., Inoue, M., Tahara, H., Kobayashi, T., Takatsuka, Y., Matsuyoshi, N., Hirano, S., Takeichi, M., and Mori, T. (1993). Expression of E-Cadherin Cell Adhesion Molecules in Human Breast-Cancer Tissues and Its Relationship to Metastasis. Cancer Research *53*, 1696-1701.

Orsulic, S., and Peifer, M. (1996). An *in vivo* Structure-Function Study of Armadillo, the β-Catenin Homolog, Reveals both Separate and Overlapping Regions of the Protein Required for Cell Adhesion and for Wingless Signaling. Journal of Cell Biology *134*, 1283-1300.

Overduin, M., Harvey, T. S., Bagby, S., Tong, K. I., Yau, P., Takeichi, M., and Ikura, M. (1995). Solution Structure of the Epithelial Cadherin Domain Responsible for Selective Cell Adhesion. Science *267*, 386-389.

Ozawa, M., Engel, J., and Kemler, R. (1990). Single Amino Acid Substitutions in One Ca^{2+} Binding Site of Uvomorulin Abolish the Adhesive Function. Cell *63*, 1033-1038.

Parkin, A. J. (1996). The Amnesic Patient HM. in Classic Case Studies in Neuropsychology. Edited by Code C. Hove, UK: Lawrence Erlbaum.

Peifer, M. (1995). Cell Adhesion and Signal Transduction: the Armadillo Connection. Trends in Cell Biology *5*, 224-229.

Peng, X., Bahr, B. A., Staubli, U., Vanderklish, P. W., and Lynch, G. (1991). Evidence That Matrix Recognition Contributes to Stabilization but not Induction of LTP. Neuroreport *2*, 461-464.

Perl, A. K., Wilgenbus, P., Dahl, U., Semb, H., and Christofori, G. (1998). A Causal Role for E-Cadherin in the Transition from Adenoma to Carcinoma. Nature *392*, 190-193.

Persohn, E., and Schachner, M. (1987). Immunoelectron Microscopic Localization of the Neural Cell Adhesion Molecules L1 and N-CAM during Postnatal-Development of the Mouse Cerebellum. Journal of Cell Biology *105*, 569-576.

Pokutta, S., Herrenknecht, K., Kemler, R., and Engel, J. (1994). Conformational-Changes of the Recombinant Extracellular Domain of E-Cadherin Upon Calcium Binding. European Journal of Biochemistry *223*, 1019-1026.

Pollack, A. L., Barth, A. I. M., Altschuler, Y., Nelson, W. J., and Mostov, K. E. (1997). Dynamics of β-Catenin Interactions with APC Protein Regulate Epithelial Tubulogenesis. Journal of Cell Biology *137*, 1651-1662.

Pouliot, Y. (1992). Phylogenetic Analysis of the Cadherin Superfamily. Bioessays *14*, 743-748.

Radice, G. L., Rayburn, H., Matsunami, H., Knudsen, K. A., Takeichi, M., and Hynes, R. O. (1997). Developmental Defects in Mouse Embryos Lacking N-Cadherin. Developmental Biology *181*, 64-78.

Ramon y Cajal, S. (1894). La fine structure des centres nerveux. Proc. R. Soc. Lond. *55*, 444-468.

Ranscht, B., and Bronnerfraser, M. (1991). T-Cadherin Expression Alternates with Migrating Neural Crest Cells in the Trunk of the Avian Embryo. Development *111*, 15-22.

Ranscht, B., and Dourszimmermann, M. T. (1991). T-Cadherin, a Novel Cadherin Cell Adhesion Molecule in the Nervous System Lacks the Conserved Cytoplasmic Region. Neuron *7*, 391-402.

Redies, C. (1997). Cadherins and the Formation of Neural Circuitry in the Vertebrate CNS. Cell and Tissue Research *290*, 405-413.

Redies, C., Inuzuka, H., and Takeichi, M. (1992). Restricted Expression of N-Cadherin and R-Cadherin on Neurites of the Developing Chicken CNS. Journal of Neuroscience *12*, 3525-3534.

Redies, C., and Takeichi, M. (1996). Cadherins in the Developing Central Nervous System: an Adhesive Code for Segmental and Functional Subdivisions. Developmental Biology *180*, 413-423.

Redman, S. (1990). Quantal Analysis of Synaptic Potentials in Neurons of the Central Nervous System. Physiological Reviews *70*, 165-198.

Riehl, R., Johnson, K., Bradley, R., Grunwald, G. B., Cornel, E., Lilienbaum, A., and Holt, C. E. (1996). Cadherin Function is Required for Axon Outgrowth in Retinal Ganglion Cells *in vivo*. Neuron *17*, 837-848.

Rimm, D. L., Koslov, E. R., Kebriaei, P., Cianci, C. D., and Morrow, J. S. (1995). α_1^E-Catenin is an Actin-Binding and Actin-Bundling Protein Mediating the Attachment of F-Actin to the Membrane Adhesion Complex. Proceedings of the National Academy of Sciences of the United States of America *92*, 8813-8817.

Ringwald, M., Schuh, R., Vestweber, D., Eistetter, H., Lottspeich, F., Engel, J., Dolz, R., Jahnig, F., Epplen, J., Mayer, S., Muller, C., and Kemler, R. (1987). The Structure of Cell Adhesion Molecule Uvomorulin: Insights Into the Molecular Mechanism of Ca^{2+}-Dependent Cell Adhesion. EMBO Journal *6*, 3647-3653.

Robinson, G. B. (1992). Maintained Saturation of Hippocampal Long-Term Potentiation Does not Disrupt Acquisition of the 8-Arm Radial Maze. Hippocampus *2*, 389-395.

Rose, S. (1995). The Rise of Neurogenetic Determinism. Nature *373*, 380-382.

Rosenmund, C., Clements, J. D., and Westbrook, G. L. (1993). Nonuniform Probability of Glutamate Release at a Hippocampal Synapse. Science *262*, 754-757.

Roskelley, C. D., Srebrow, A., and Bissell, M. J. (1995). A Hierarchy of ECM-Mediated Signaling Regulates Tissue-Specific Gene Expression. Current Opinion in Cell Biology *7*, 736-747.

Routtenberg, A. (1995). Knockout Mouse Fault Lines. Nature *374*, 314-315.

Rubinfeld, B., Robbins, P., Elgamil, M., Albert, I., Porfiri, E., and Polakis, P. (1997). Stabilization of β-Catenin by Genetic-Defects in Melanoma Cell Lines. Science *275*, 1790-1792.

Rubinfeld, B., Souza, B., Albert, I., Muller, O., Chamberlain, S. H., Masiarz, F. R., Munemitsu, S., and Polakis, P. (1993). Association of the APC Gene Product with β-Catenin. Science *262*, 1731-1734.

Rubinfeld, B., Souza, B., Albert, I., Munemitsu, S., and Polakis, P. (1995). The APC Protein and E-Cadherin Form Similar but Independent Complexes with α-Catenin, β-Catenin, and Plakoglobin. Journal of Biological Chemistry *270*, 5549-5555.

Ryan, T. A., Reuter, H., Wendland, B., Schweizer, F. E., Tsien, R. W., and Smith, S. J. (1993). The Kinetics of Synaptic Vesicle Recycling Measured at Single Presynaptic Boutons. Neuron *11*, 713-724.

Ryan, T. A., and Smith, S. J. (1995). Vesicle Pool Mobilization during Action Potential Firing at Hippocampal Synapses. Neuron *14*, 983-989.

Saffell, J. L., Williams, E. J., Mason, I. J., Walsh, F. S., and Doherty, P. (1997). Expression of a Dominant-Negative FGF Receptor Inhibits Axonal Growth and FGF Receptor Phosphorylation Stimulated by CAMs. Neuron *18*, 231-242.

Sakimura, K., Kutsuwada, T., Ito, I., Manabe, T., Takayama, C., Kushiya, E., Yagi, T., Aizawa, S., Inoue, Y., Sugiyama, H., and Mishina, M. (1995). Reduced Hippocampal LTP and Spatial Learning in Mice Lacking NMDA Receptor ε-1 Subunit. Nature *373*, 151-155.

Sako, Y., Nagafuchi, A., Tsukita, S., Takeichi, M., and Kusumi, A. (1998). Cytoplasmic Regulation of the Movement of E-Cadherin on the Free Cell Surface as Studied by Optical Tweezers and Single Particle Tracking: Corralling and Tethering by the Membrane Skeleton. Journal of Cell Biology *140*, 1227-1240.

Salomon, D., Ayalon, O., Patelking, R., Hynes, R. O., and Geiger, B. (1992). Extrajunctional Distribution of N-Cadherin in Cultured Human Endothelial Cells. Journal of Cell Science *102*, 7-17.

Sano, K., Tanihara, H., Heimark, R. L., Obata, S., Davidson, M., Stjohn, T., Taketani, S., and Suzuki, S. (1993). Protocadherins: a Large Family of Cadherin-Related Molecules in Central Nervous System. EMBO Journal *12*, 2249-2256.

Sanson, B., White, P., and Vincent, J. P. (1996). Uncoupling Cadherin-Based Adhesion from Wingless Signaling in *Drosophila*. Nature *383*, 627-630.

Sarantis, M., Ballerini, L., Miller, B., Silver, R. A., Edwards, M., and Attwell, D. (1993). Glutamate Uptake from the Synaptic Cleft Does not Shape the Decay of the Non-NMDA Component of the Synaptic Current. Neuron *11*, 541-549.

Scanziani, M., Malenka, R. C., and Nicoll, R. A. (1996). Role of Intercellular Interactions in Heterosynaptic Long-Term Depression. Nature *380*, 446-450.

Schaerenwiemers, N., and Gerfinmoser, A. (1993). A Single Protocol to Detect Transcripts of Various Types and Expression Levels in Neural Tissue and Cultured Cells: in-Situ Hybridization Using Digoxigenin-Labeled cRNA Probes. Histochemistry *100*, 431-440.

Schneider, R. (1992). The Human Protooncogene Ret: a Communicative Cadherin. Trends in Biochemical Sciences *17*, 468-469.

Schulze, C., and Firth, J. A. (1993). Immunohistochemical Localization of Adherens Junction Components in Blood-Brain-Barrier Microvessels of the Rat. Journal of Cell Science *104*, 773-782.

Schuman, E. M., and Madison, D. V. (1991). A Requirement for the Intercellular Messenger Nitric Oxide in Long-Term Potentiation. Science *254*, 1503-1506.

Schuman, E. M., and Madison, D. V. (1994). Locally Distributed Synaptic Potentiation in the Hippocampus. Science *263*, 532-536.

Schuman, E. M., Meffert, M. K., Schulman, H., and Madison, D. V. (1994). An ADP-Ribosyltransferase as a Potential Target for Nitric Oxide Action in Hippocampal Long-Term Potentiation. Proceedings of the National Academy of Sciences of the United States of America *91*, 11958-11962.

Schuster, C. M., Davis, G. W., Fetter, R. D., and Goodman, C. S. (1996). Genetic Dissection of Structural and Functional Components of Synaptic Plasticity. I. Fasciclin-II Controls Synaptic Stabilization and Growth. Neuron *17*, 641-654.

Schuster, C. M., Davis, G. W., Fetter, R. D., and Goodman, C. S. (1996). Genetic Dissection of Structural and Functional Components of Synaptic Plasticity. II. Fasciclin-II Controls Presynaptic Structural Plasticity. Neuron *17*, 655-667.

Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995). Integrins: Emerging Paradigms of Signal Transduction. Annual Review of Cell and Developmental Biology *11*, 549-599.

Scott-Burden, T. (1994). Extracellular Matrix: the Cellular Environment. News in Physiological Sciences *9*, 110-115.

Scoville, W. B., and Milner, B. (1957). Loss of Recent Memory after Bilateral Hippocampus Lesions. J. Neurol. Neurosurg. Psychiatr. *20*, 11-21.

Serafini, T. (1997). An Old Friend in a New Home: Cadherins at the Synapse. Trends in Neurosciences *20*, 322-323.

Shapiro, L., Fannon, A. M., Kwong, P. D., Thompson, A., Lehmann, M. S., Grubel, G., Legrand, J. F., Alsnielsen, J., Colman, D. R., and Hendrickson, W. A. (1995). Structural Basis of Cell-Cell Adhesion by Cadherins. Nature *374*, 327-337.

Shapiro, L., Kwong, P. D., Fannon, A. M., Colman, D. R., and Hendrickson, W. A. (1995). Considerations on the Folding Topology and Evolutionary Origin of Cadherin Domains. Proceedings of the National Academy of Sciences of the United States of America *92*, 6793-6797.

Sharp, P. E., McNaughton, B. L., and Barnes, C. A. (1989). Exploration-Dependent Modulation of Evoked-Responses in Fascia Dentata: Fundamental Observations and Time Course. Psychobiology *17*, 257-269.

Sherrington, C. S. (1897). The Central Nervous System. Part III of M. Foster, A Text Book of Physiology, 7th ed. London: Macmillan.

Shimoyama, Y., Hirohashi, S., Hirano, S., Noguchi, M., Shimosato, Y., Takeichi, M., and Abe, O. (1989). Cadherin Cell Adhesion Molecules in Human Epithelial Tissues and Carcinomas. Cancer Research *49*, 2128-2133.

Shirayoshi, Y., Nose, A., Iwasaki, K., and Takeichi, M. (1986). N-Linked Oligosaccharides are not Involved in the Function of a Cell-Cell Binding Glycoprotein E-Cadherin. Cell Structure and Function *11*, 245-252.

Silva, A. J., Paylor, R., Wehner, J. M., and Tonegawa, S. (1992). Impaired Spatial Learning in α-Calcium-Calmodulin Kinase-II Mutant Mice. Science *257*, 206-211.

Silva, A. J., Stevens, C. F., Tonegawa, S., and Wang, Y. Y. (1992). Deficient Hippocampal Long-Term Potentiation in α-Calcium-Calmodulin Kinase-II Mutant Mice. Science *257*, 201-206.

Skrede, K. K., and Malthe-Sorenssen, D. (1981). Increased Resting and Evoked Release of Transmitter following Repetitive Electrical Tetanization in Hippocampus: A Biochemical Correlate to Long-Lasting Synaptic Potentiation. Brain Research *208*, 436-441.

Smith, S. J. (1992). Do Astrocytes Process Neural Information. Progress in Brain Research *94*, 119-136.

Son, H., Hawkins, R. D., Martin, K., Kiebler, M., Huang, P. L., Fishman, M. C., and Kandel, E. R. (1996). Long-Term Potentiation is Reduced in Mice That are Doubly Mutant in Endothelial and Neuronal Nitric Oxide Synthase. Cell *87*, 1015-1023.

Sorra, K. E., Fiala, J. C., and Harris, K. M. (1998). Critical-Assessment of the Involvement of Perforations, Spinules, and Spine Branching in Hippocampal Synapse Formation. Journal of Comparative Neurology *398*, 225-240.

Sorra, K. E., and Harris, K. M. (1998). Stability in Synapse Number and Size at Two Hr after Long-Term Potentiation in Hippocampal Area CA1. Journal of Neuroscience *18*, 658-671.

Squire, L. R. (1992). Declarative and Nondeclarative Memory: Multiple Brain Systems Supporting Learning and Memory. Journal of Cognitive Neuroscience *4*, 232-243.

Stanley, J. R. (1995). Autoantibodies Against Adhesion Molecules and Structures in Blistering Skin Diseases. Journal of Experimental Medicine *181*, 1-4.

Staubli, U., Chun, D., and Lynch, G. (1998). Time-Dependent Reversal of Long-Term Potentiation by an Integrin Antagonist. Journal of Neuroscience *18*, 3460-3469.

Staubli, U., Vanderklish, P., and Lynch, G. (1990). An Inhibitor of Integrin Receptors Blocks Long-Term Potentiation. Behavioral and Neural Biology *53*, 1-5.

Steinberg, M. S., and Takeichi, M. (1994). Experimental Specification of Cell Sorting, Tissue Spreading, and Specific Spatial Patterning by Quantitative Differences in Cadherin Expression. Proceedings of the National Academy of Sciences of the United States of America *91*, 206-209.

Stevens, C. F. (1993). Quantal Release of Neurotransmitter and Long-Term Potentiation. Cell *72*, 55-63.

Stevens, C. F., and Wang, Y. Y. (1994). Changes in Reliability of Synaptic Function as a Mechanism for Plasticity. Nature *371*, 704-707.

Su, L. K., Vogelstein, B., and Kinzler, K. W. (1993). Association of the APC Tumor-Suppressor Protein with Catenins. Science *262*, 1734-1737.

Suzuki, S., Sano, K., and Tanihara, H. (1991). Diversity of the Cadherin Family: Evidence for Eight New Cadherins in Nervous Tissue. Cell Regulation *2*, 261-270.

Takeichi, M. (1977). Functional Correlation between Cell Adhesive Properties and Some Cell Surface Proteins. Journal of Cell Biology *75*, 464-474.

Takeichi, M. (1987). Cadherins: a Molecular Family Essential for Selective Cell Cell Adhesion and Animal Morphogenesis. Trends in Genetics *3*, 213-217.

Takeichi, M. (1988). The Cadherins: Cell Cell Adhesion Molecules Controlling Animal Morphogenesis. Development *102*, 639-Continues.

Takeichi, M. (1990). Cadherins: a Molecular Family Important in Selective Cell-Cell Adhesion. Annual Review of Biochemistry *59*, 237-252.

Takeichi, M. (1991). Cadherin Cell Adhesion Receptors as a Morphogenetic Regulator. Science *251*, 1451-1455.

Takeichi, M. (1995). Morphogenetic Roles of Classic Cadherins. Current Opinion in Cell Biology *7*, 619-627.

Takeichi, M., Inuzuka, H., Shimamura, K., Matsunaga, M., and Nose, A. (1990). Cadherin-Mediated Cell Cell Adhesion and Neurogenesis. Neuroscience Research, S92-S96.

Takeichi, M., Uemura, T., Iwai, Y., Uchida, N., Inoue, T., Tanaka, T., and Suzuki, S. C. (1997). Cadherins in Brain Patterning and Neural Network Formation. Cold Spring Harbor Symposia on Quantitative Biology *62*, 505-510.

Tamura, K., Shan, W. S., Hendrickson, W. A., Colman, D. R., and Shapiro, L. (1998). Structure-Function Analysis of Cell Adhesion by Neural (N-) Cadherin. Neuron *20*, 1153-1163.

Tang, C. M., Margulis, M., Shi, Q. Y., and Fielding, A. (1994). Saturation of Postsynaptic Glutamate Receptors after Quantal Release of Transmitter. Neuron *13*, 1385-1393.

Tang, L. X., Hung, C. P., and Schuman, E. M. (1998). A Role For the Cadherin Family of Cell-Adhesion Molecules in Hippocampal Long-Term Potentiation. Neuron *20*, 1165-1175.

Tanihara, H., Kido, M., Obata, S., Heimark, R. L., Davidson, M., Stjohn, T., and Suzuki, S. (1994). Characterization of Cadherin-4 and Cadherin-5 Reveals New Aspects of Cadherins. Journal of Cell Science *107*, 1697-1704.

Tanzi, E. (1893). I fatti i le induzioni nell'odierna istologia del sistema nervoso. Riv Sperim Freniatria Med Leg *19*, 419-472.

Tomschy, A., Fauser, C., Landwehr, R., and Engel, J. (1996). Homophilic Adhesion of E-Cadherin Occurs by a Cooperative Two-Step Interaction of N-Terminal Domains. EMBO Journal *15*, 3507-3514.

Tong, G., Malenka, R. C., and Nicoll, R. A. (1996). Long-Term Potentiation in Cultures of Single Hippocampal Granule Cells: a Presynaptic Form of Plasticity. Neuron *16*, 1147-1157.

Torres, M. A., Yangsnyder, J. A., Purcell, S. M., Demarais, A. A., McGrew, L. L., and Moon, R. T. (1996). Activities of the Wnt-1 Class of Secreted Signaling Factors are Antagonized by the Wnt-5a Class and by a Dominant-Negative Cadherin in Early *Xenopus* Development. Journal of Cell Biology *133*, 1123-1137.

Tsien, J. Z., Chen, D. F., Gerber, D., Tom, C., Mercer, E. H., Anderson, D. J., Mayford, M., Kandel, E. R., and Tonegawa, S. (1996). Subregion-Restricted and Cell Type-Restricted Gene Knockout in Mouse Brain. Cell *87*, 1317-1326.

Tsien, J. Z., Huerta, P. T., and Tonegawa, S. (1996). The Essential Role of Hippocampal CA1 NMDA Receptor-Dependent Synaptic Plasticity in Spatial Memory. Cell *87*, 1327-1338.

Uchida, N., Honjo, Y., Johnson, K. R., Wheelock, M. J., and Takeichi, M. (1996). The Catenin Cadherin Adhesion System is Localized in Synaptic Junctions Bordering Transmitter Release Zones. Journal of Cell Biology *135*, 767-779.

Van Harreveld, A., and Fifkova, E. (1975). Swelling of Dendritic Spines in the Fascia Dentata after Stimulation of the Perforant Fibers as a Mechanism of Post-Tetanic Potentiation. Exp. Neurol. *49*, 736-749.

Vaughn, D. E., and Bjorkman, P. J. (1996). The (Greek) Key to Structures of Neural Adhesion Molecules. Neuron *16*, 261-273.

Vestweber, D., and Kemler, R. (1985). Identification of a Putative Cell Adhesion Domain of Uvomorulin. EMBO Journal *4*, 3393-3398.

Volberg, T., Geiger, B., Dror, R., and Zick, Y. (1991). Modulation of Intercellular Adherens-Type Junctions and Tyrosine Phosphorylation of Their Components in Rsv-Transformed Cultured Chick Lens Cells. Cell Regulation *2*, 105-120.

Volk, T., Cohen, O., and Geiger, B. (1987). Formation of Heterotypic Adherens-Type Junctions Between L-CAM-Containing Liver Cells and A-CAM-Containing Lens Cells. Cell *50*, 987-994.

Volk, T., and Geiger, B. (1986). A-CAM: a 135-kD Receptor of Intercellular Adherens Junctions. II. Antibody-Mediated Modulation of Junction Formation. Journal of Cell Biology *103*, 1451-1464.

Volk, T., Volberg, T., Sabanay, I., and Geiger, B. (1990). Cleavage of A-CAM by Endogenous Proteinases in Cultured Lens Cells and in Developing Chick-Embryos. Developmental Biology *139*, 314-326.

Voronin, L. L. (1983). Long-Term Potentiation in the Hippocampus. Neuroscience *10*, 1051-1069.

Wagner, A. P., Beck, K. D., and Reck, G. (1992). Neural Cell Adhesion Molecule (NCAM) and N-Cadherin Messenger-RNA during Development and Aging: Selective Reduction in the 7.4-Kb and 6.7-Kb NCAM Messenger-RNA Levels in the Hippocampus of Adult and Old Rats. Mechanisms of Ageing and Development *62*, 201-208.

Walsh, F. S., and Doherty, P. (1997). Neural Cell Adhesion Molecules of the Immunoglobulin Superfamily: Role in Axon Growth and Guidance. Annual Review of Cell and Developmental Biology *13*, 425-456.

Willems, J., Bruyneel, E., Noe, V., Slegers, H., Zwijsen, A., Mege, R. M., and Mareel, M. (1995). Cadherin-Dependent Cell-Aggregation is Affected by Decapeptide Derived from Rat Extracellular-Superoxide Dismutase. Febs Letters *363*, 289-292.

Williams, E. J., Doherty, P., Turner, G., Reid, R. A., Hemperly, J. J., and Walsh, F. S. (1992). Calcium Influx Into Neurons Can Solely Account for Cell Contact Dependent Neurite Outgrowth Stimulated by Transfected L1. Journal of Cell Biology *119*, 883-892.

Williams, E. J., Furness, J., Walsh, F. S., and Doherty, P. (1994). Activation of the FGF Receptor Underlies Neurite Outgrowth Stimulated by L1, N-CAM, and N-Cadherin. Neuron *13*, 583-594.

Wu, G. Y., Malinow, R., and Cline, H. T. (1996). Maturation of a Central Glutamatergic Synapse. Science *274*, 972-976.

Yamada, K. M., and Miyamoto, S. (1995). Integrin Transmembrane Signaling and Cytoskeletal Control. Current Opinion in Cell Biology *7*, 681-689.

Yamagata, M., Herman, J. P., and Sanes, J. R. (1995). Lamina-Specific Expression of Adhesion Molecules in Developing Chick Optic Tectum. Journal of Neuroscience *15*, 4556-4571.

Yap, A. S., Brieher, W. M., Pruschy, M., and Gumbiner, B. M. (1997). Lateral Clustering of the Adhesive Ectodomain: a Fundamental Determinant of Cadherin Function. Current Biology *7*, 308-315.

Yap, A. S., Niessen, C. M., and Gumbiner, B. M. (1998). The Juxtamembrane Region of the Cadherin Cytoplasmic Tail Supports Lateral Clustering, Adhesive Strengthening, and Interaction with P129(Ctn). Journal of Cell Biology *141*, 779-789.

Zhang, Z. H., Vuori, K., Wang, H. G., Reed, J. C., and Ruoslahti, E. (1996). Integrin Activation by R-Ras. Cell *85*, 61-69.

Zhuo, M., Hu, Y. H., Schultz, C., Kandel, E. R., and Hawkins, R. D. (1994). Role of Guanylyl Cyclase and cGMP-Dependent Protein-Kinase in Long-Term Potentiation. Nature *368*, 635-639.

Zimmer, A. (1996). Gene Targeting and Behavior: a Genetic Problem Requires a Genetic Solution. Trends in Neurosciences *19*, 470-470.

Zola-Morgan, S., and Squire, L. R. (1993). Neuroanatomy of Memory. Annual Review of Neuroscience *16*, 547-563.