

## Curriculum Vitae

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- BE, Kao JPY, Thompson SM (in review) Evidence of calcium-permeable AMPA receptors in dendritic spines of CA1 pyramidal neurons. *J Neuroscience*
2. \*Santos MD, \***Mohammadi MH**, Yang S, Liang CW, Kao JP, Alger BE, Thompson SM, Tang CM. (2012) Dendritic hold and read: a gated mechanism for short term information storage and retrieval. *PLoS One* 7(5). (\*equal contribution)
  3. \*Liang CW, \***Mohammadi M**, Santos MD, Tang CM. (2011) Patterned photostimulation with digital micromirror devices to investigate dendritic integration across branch points. *J Vis Exp.* 2011 Mar 2;(49). (\*equal contribution)
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  5. \*Bowen SE, \***Mohammadi MH**, Batis JC, Hannigan JH. (2007) Gestational toluene exposure effects on spontaneous and amphetamine-induced locomotor behavior in rats. *Neurotoxicol Teratol.* 2007 Mar-Apr;29(2):236-46. (\*equal contribution)
  6. Bowen SE, Batis JC, **Mohammadi MH**, Hannigan JH. (2005) Abuse pattern of gestational toluene exposure and early postnatal development in rats. *Neurotoxicol Teratol.* 2005 Jan-Feb;27(1):105-16.

## **Selected Abstracts and Presentations**

### **PUBLISHED ABSTRACTS**

1. Santos, M.D., **Mohammadi, M.H.**, Thompson, S.M., & Tang, C-M. dendritic hold and read”, a novel form of short-term neural plasticity on CA1 pyramidal neurons. 2008 meeting of Society for Neuroscience, Washington, DC
2. Edwards, D.A., **Mohammadi, M.H.**, & Alger, B.E. Metaplastic control of the hippocampal endocannabinoid system. Presented at 2006 meeting of Society for Neuroscience, Atlanta, GA
3. **Mohammadi, M.H.**, Hannigan, J.H., & Bowen, S.E. Biological effects of prenatal exposure to toluene in rats. Presented at the 2004 annual meeting of the Behavioral Toxicology Society
4. Batis, J.C., **Mohammadi, M.H.**, Hannigan, J.H. & Bowen S.E. Early postnatal development following binge gestational toluene exposure in rats. Presented at the 2004 conference of the Behavioral Toxicology Society.
5. **Mohammadi, M.H.**, Hannigan, J.H., & Bowen, S.E. Dextra-amphetamine induced locomotor activity in rats exposed to toluene in utero. Presented at the 2004 conference for The College on Problems for Drug Dependence, San Juan, Puerto Rico
6. Batis, J.C., **Mohammadi, M.H.**, Ban, R.B., Hannigan, J.H., & Bowen, S.E. Dose-dependent impairment of Water maze reversal learning following maternal toluene abuse. Presented at the 2004 conference for The College on Problems for Drug Dependence, San Juan, Puerto Rico
7. **Mohammadi, M.H.**, Batis, J.C., Hannigan, J.H., & Bowen, S.E. Long-term effects of prenatal exposure to toluene on spontaneous locomotor activity.

Poster and talk presented at the 2003 International Society for Developmental Psychobiology, New Orleans, LA.

9. Bowen, S.E., **Mohammadi, M.H.**, Batis, J.C., & Hannigan, J. H. Effects of Prenatal Exposure to
10. Toluene on locomotor activity in rats. Poster presented at the 2003 conference of the Behavioral Toxicology Society, Philadelphia, PA.

### **INVITED TALKS**

1. **Mohammadi, M.H.**, Santos, M.D., & Tang, C.M.. Dendritic Hold and Read, A novel form of short-term neural plasticity on CA1 pyramidal neurons. Invited speaker and session chair at Gordon Research Conference on Dendrites, Lucca, Italy
2. **Mohammadi, M.H.**, Hannigan, J.H., & Bowen, S.E. Effects of prenatal exposure to toluene on biological development in the rat. Presented at an inter-lab exchange at the University of Michigan, Ann Arbor, MI.
3. **Mohammadi, M.H.**, Batis, J.C., Hannigan, J.H., & Bowen, S.E. Long-term effects of prenatal exposure to toluene on spontaneous locomotor activity. Poster and talk presented at the 2003 International Society for Developmental Psychobiology, New Orleans, LA.

## **Abstract**

Title of Thesis: Dendritic hold and read: A novel form of short term information storage and retrieval.

Name: Michael H. Mohammadi, Doctor of Philosophy, 2012

Dissertation directed by: Cha-Min Tang, MD/PHD

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### **Abstract:**

The discovery of the dual gating of the NMDA receptor was a major turning point in the field of learning and memory. Much of the excitement was directed to the ability of the NMDA receptor to act as a coincidence detector, as well as the unusually high permeability to calcium. These unique features of the NMDA receptor provided the means at the molecular level to implement Hebb's hypothesis for synaptic plasticity. In the late 1980's it was proposed that the NMDA receptor itself could hold information in one of two ways. One school of thought believed that the slow unbinding of glutamate allowed the NMDA receptors to maintain a long lasting conductance that enables a reverberating feedback circuit to sustain spiking behavior after the input into the network has ended. Bekkers and Stevens (1990) proposed an alternate hypothesis; that information could be stored by the bound-but-blocked (non-conducting) state of the NMDA receptors. Neither group experimentally tested their theoretical consideration. Suspecting that Bekkers and Stevens failed to consider a concept that was not available to them, electrical compartmentalization of distal dendrites and regenerative NMDA spikes, I reexamined their predictions with inclusion of factors required for dendritic spikes during signal read-out. Here I show that the dual gating of the NMDA receptor can hold information in the form of bound glutamate on a large population of receptors for 100's of ms, which can be read-out by a local depolarization. Key to this model is the electrical compartmentalization of a thin terminal dendrite, which allows for a regenerative all-or-none spike. The experimental observation called, "dendritic hold and read" or DHR, is a mechanism that can support a novel form of short-term memory.

Dendritic hold and read: A novel form of information storage and retrieval

by  
MICHAEL HASAN MOHAMMADI

Dissertation submitted to the faculty of the Graduate School of the  
University of Maryland, Baltimore in partial fulfillment  
of the requirements for the degree Doctor of Philosophy

2012

To my parents, Valiollah and Brigitte Mohammadi  
Everything I am is because of your love and guidance.  
Thank you for always pushing me to succeed and to dream.

## Acknowledgments

I am very grateful to my advisor Dr. Cha-Min Tang for his patience, support and technical wizardry which has helped me transition to a position in the microscopy industry. I thank my committee, Drs. Brad Alger, Thomas Blanpied, Joseph P.Y. Kao and Scott Thompson. Each a great mind and leader in his respective field and very supportive in my finishing this thesis. I would also thank my lab mates, especially Drs. Daniel Santos, Conrad Liang, David Edwards, Chris Reich, Miranda Karson, Kevin Wittington and Carlos LaFourcade for their training, encouragement and support.

I am very thankful to my parents for providing my sister, brother and me with an amazing environment to grow, learn and dream. Special thanks to my sister Azita who spent her childhood teaching me the importance of education and never giving up on my goals and dreams. To my brother Jamie, though you're blissfully unaware, you are the reason I pursued a career in science and you're my daily reminder to always love.

I have many friends to thank. Dr. Gregory Bissonette was especially helpful giving comments on many drafts and always acting as a sounding board for ideas. Drs. Jon Dilgen, Dan Andersen, Tony Gover, Peter Murray and Chris Antolik as well as Kim McKay for their support as well as many discussions both in and out of the lab. Thank you Nicole Gerbino for being very loving and supportive in the last six months of writing, you gave me constant encouragement when things seemed impossible. Finally, to Nadia and my three-legged Raja, the greatest huskies in the world and the best companions a guy could ask for. I lost Raja to cancer in the final edits of this thesis but he was my source of strength the last three years and I am thankful to have shared six and a half wonderful years with him and hopefully many more to come with Nadia.

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**Chapter I**  
**Introduction**

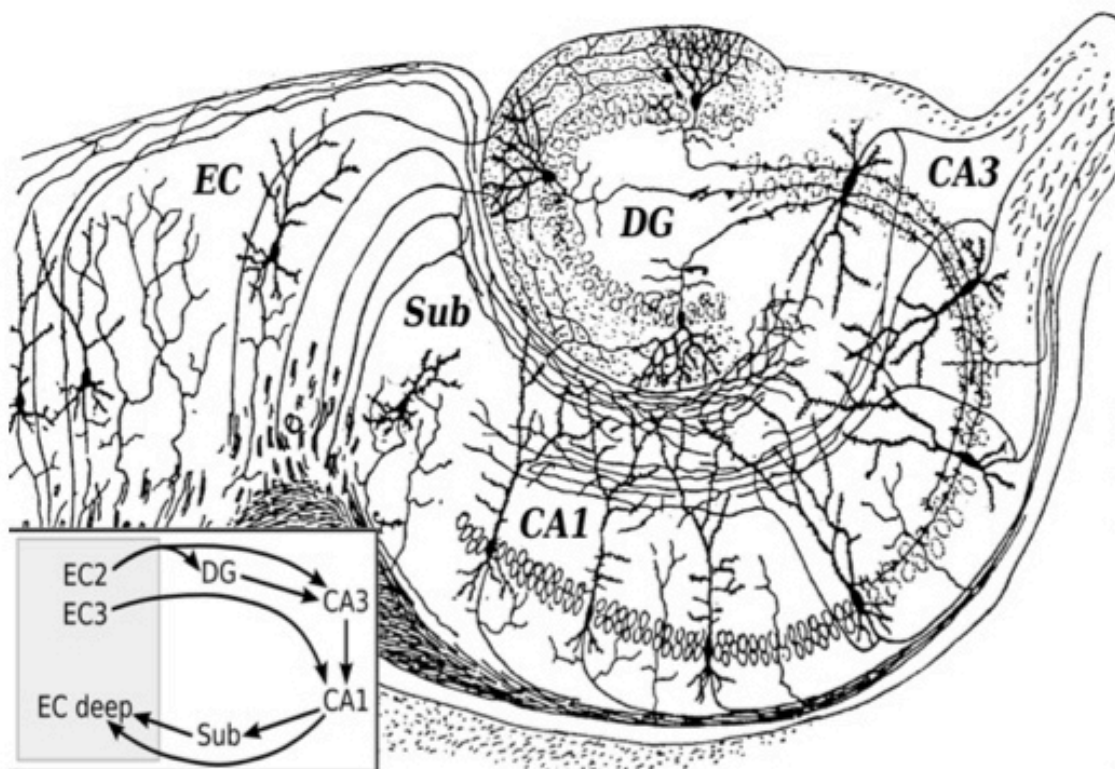
## 1.1 Overview

The brain contains an estimated 100 billion neurons which form complex networks that consist of more than  $\sim 10^{14}$  synapses. Together, these synapses process information in both space (neurons to networks) and time (milliseconds to years). Perhaps the most profound aspect of these millions of billions of connections is their ability to process and filter large amounts of information and modify behavior while retaining information on both very short and very long-term time scales. The way in which the hippocampus accomplishes learning and memory is the subject of intense interest and debate (for reviews see Citri & Malenka 2008; Dan & Poo 2004; Zucker & Regher 2002). Here I will present one potential component of this complex process.

## 1.2 The Hippocampus

The hippocampus is located in the basal-medial area of the temporal lobe and is named for its anatomical resemblance to a sea horse (“hippos” is a horse, and “kampos” is a sea monster, the combined is a greek mythological “sea horse”). The hippocampus is a very anatomically identifiable brain area (Figure 1-1). The hippocampus proper consists of the dentate gyrus (DG), areas ‘*cornu ammonis*’ 1 (CA1), CA2, CA3, and the less talked about CA4 (Lorente de No 1934) as well as the subiculum. The flow of information in the hippocampus forms a trisynaptic circuit. Information arrives to the DG from the entorhinal cortex (EC) (Amaral et al. 1991). Neurons in the EC also synapse directly onto both CA3 and CA1 pyramidal neurons. DG mossy fiber axons then terminate on the apical dendrites of CA3 pyramidal neurons CA3 pyramidal neurons then project via the Schaffer collateral (SC) pathway to the basal and apical

dendrites of CA1 pyramidal neurons. Area CA1 axons project to the subiculum and the deep layers in the EC. Some CA1 axons also project longitudinally to the fimbria. The hippocampus also receives subcortical inputs from the amygdala, thalamus, medial septum, ventral tegmental area, raphe nuclei, as well as a variety of other regions (Amaral and Witter 1989).



**Figure 1-1: Outline of the hippocampus based on a drawing by Ramon y Cahal.** Insert shows network connections (Entorhinal cortex, EC; Dentate Gyrus, DG; Subiculum, Sub; Cornu ammonis, CA). Ramon y Cajal, 1917

The hippocampus is an important area for memory storage and retrieval (Bird and Burgess 2008). Damage to the hippocampus can inhibit the consolidation of memories (Scoville & Milner 1957). Initial work done by Scoville and Milner (1957) describe a patient known as H.M. (identified post-mortem in 2008 as Henry Molaison,

for review see Squire 2009), who had a portion of his hippocampus removed as a treatment for severe epilepsy. Following his treatment, H.M. had lost the ability to consolidate new short-term memories to long-term memory, while showing no deficits in episodic or procedural memory. Scoville and Milner concluded that the hippocampus is essential for memory formation and consolidation. Subsequent work showed that damage to the hippocampus can also result in retrograde amnesia, in which patients lose their ability to remember past events (Squire 2004). Other studies have demonstrated a role of the hippocampus in spatial memory processing and storage (Eichenbaum et al. 1992). Recent work has identified the hippocampus as an area that participates in the processing of emotions (Joels et al. 2008; Joels 2009).

In preclinical research, the hippocampus has received a considerable amount of attention for its role in a variety of neuropathologies including epilepsy (for review see Avoli, 2007), Parkinson disease (Dickson et al, 2009), schizophrenia (Bogerts and Luszcz 1999), and Alzheimer's disease (Salawu et al. 2011) as well as a variety of memory deficits (for review, see Nakazawa et al. 2004).

In the rodent, the entire hippocampus can be easily removed *in tact* and when cut into transverse sections maintains a very specific anatomy. Each slice maintains a well-defined architecture with each cell type being very similar both morphologically and functionally. This allows for general interpretations when analyzing the function of individual neurons as well as network behaviors. The hippocampus' importance in a variety of clinical pathologies, as well as the simplicity of its preparation, makes the hippocampus an ideal system for studying basic questions in synaptic signaling.

### 1.3 Anatomy of a CA1 pyramidal neuron

The basic structure of a neuron was first described in detail by Santiago Ramon y Cajal in the early 20<sup>th</sup> century. Each neuron contains three essential parts: a cell body (soma), axon, and dendrites (Figure 1-2). A major function of the soma is to integrate information arriving from the cell's dendrites as well as to house the nucleus and cellular machinery.

Dendrites are highly branched processes extending from the soma that serve to receive information from other cells. The axon (not shown in Figure 1-2) is a very long and thin process that extends from the soma to transfer the signal generated in the soma to presynaptic terminals. A layer of myelin surrounds the axon, insulating the thin projections and preventing signal decay or degradation.

With sufficient input ("threshold" achieved through the opening of voltage gated ion channels) the soma will initiate Na<sup>+</sup> dependent action potentials that propagate down the axon (unidirectional flow of information) (Cash and Yuste 1999). With the exception of a few circumstances (e.g. back propagating action potentials (bAP) (Stuart and Sakmann 1994) the directional flow of information postulated by Ramon y Cajal remains accepted in large part.

In area CA1 of the hippocampus dendrites are categorized by their morphology and location (for instance proximal (near the soma) or distal (farther away). Apical dendrites comprise of the main apical trunk as well as the thinner, primary receptive surfaces of terminal dendrites.

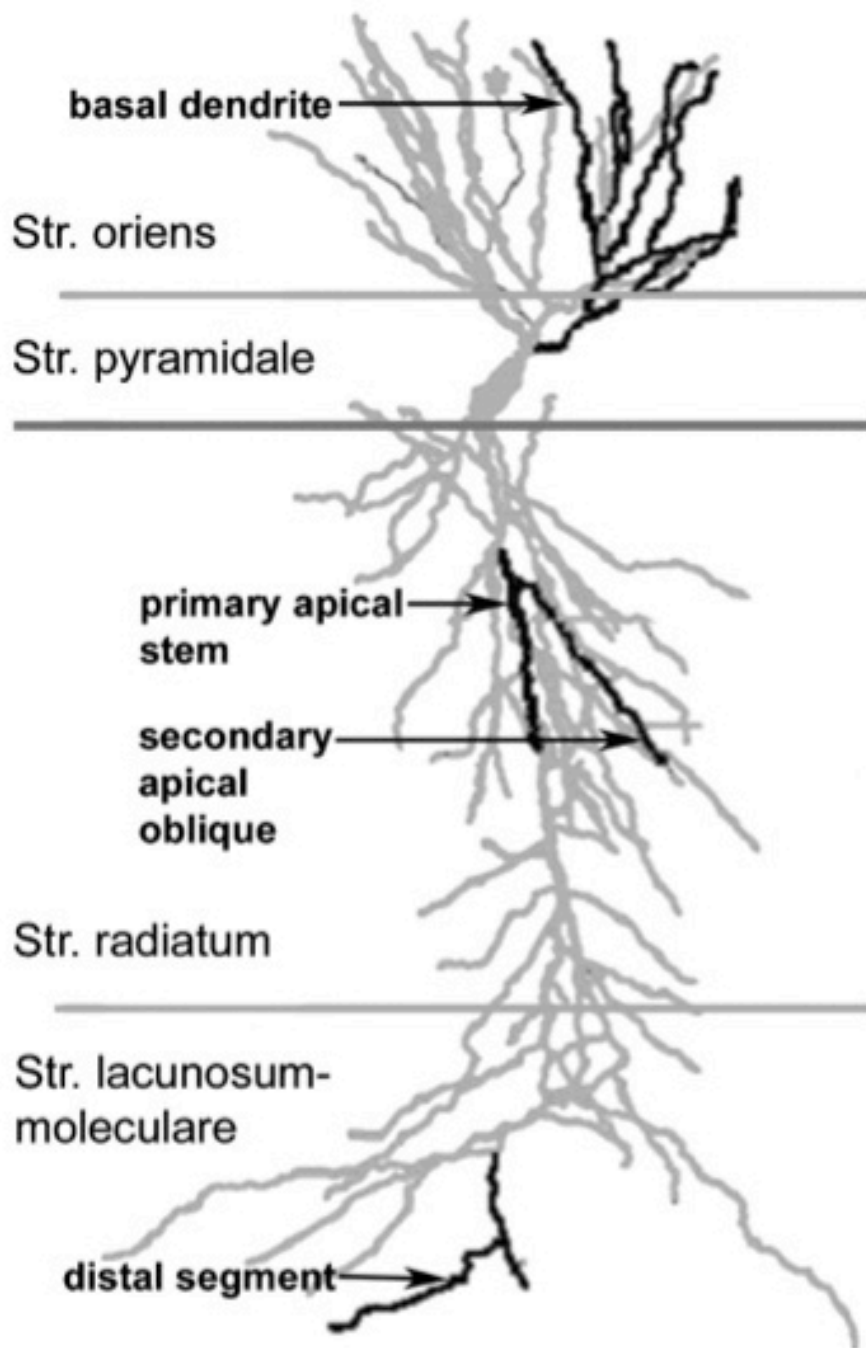


Figure 1-2. Anatomy of a CA1 pyramidal neuron. (PNAS 2003)

CA1 pyramidal neurons project both apical (long) and basal (short, highly branched) dendrites that receive input primarily from area CA3 (Amaral et al. 1991). These dendrites express a variety of voltage-gated ion channels including  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  channels which function to mediate the cells basal excitability, participate in excitatory post-synaptic potential (EPSP) generation, and play a role in synaptic plasticity (Magee and Johnston 1995). For instance, back-propagating action potentials that travel into the dendrites cause the influx of calcium through voltage gated  $\text{Ca}^{2+}$  channels which, when paired with presynaptic release of transmitter, invokes a form of short-term plasticity called spike-timing dependent plasticity (STDP, Campanac & Debanne 2008).

#### **1.4 CA1 Pyramidal neuron dendrites**

Dendrites are the primary receptive surface of a neuron (Magee 1999). Hippocampal pyramidal neurons typically receive tens of thousands of excitatory (glutamatergic) inputs and less than 2000 inhibitory (GABAergic) inputs (Gulyás et al. 1999). A majority of the excitatory inputs occur on dendritic spines while the inhibitory inputs synapse primarily on the apical trunk and soma (Megías et al. 2001).

The apical dendrites of CA1 neurons project into the layers of Strata radiatum, lucidum, lacunosum and moleculare. The primary input to CA1 pyramidal neuron dendrites arrive via area CA3 axons via SC pathway. These glutamatergic synapses contain both ionotropic (ion channel gated) as well as a variety of metabotropic glutamate receptors (mGluRs, g-protein linked activating second messenger pathways or indirectly acting on ion channels) (Arrigoni and Greene 2004).

Dendrites are complex in both their morphology and their function. Dendrites participate in signal processing as well as the integration of numerous types of inputs. The work of Wilfrid Rall in the 1950's was essential in our understanding of information flow on dendrites. Rall's 'cable theory' of how electrical information travels down a dendrite in a passive and active (compartmental) states has shaped our understanding of dendrite signal processing. His initial model analogized a dendrite to a series of interconnected cylindrical cables with basic membrane properties such as resistivity and capacitance. Through his modeling of dendritic conductances and integration (Rall 1959; Rall 1969) we now know that dendrites actively participate in signal propagation and summation in concurrent inputs.

Dendrites function as integrators of signals over space and time (Liang 2007). The organization of the highly complex dendritic tree plays an important role in summation of signals over both domains. When considered as a passive cable, the distribution of various ion channels along the dendrite ( $\text{Na}^+$ ,  $\text{Ca}^+$ ,  $\text{K}^+$ ) modulate intrinsic excitability and can help overcome signal decay (Magee 1999).

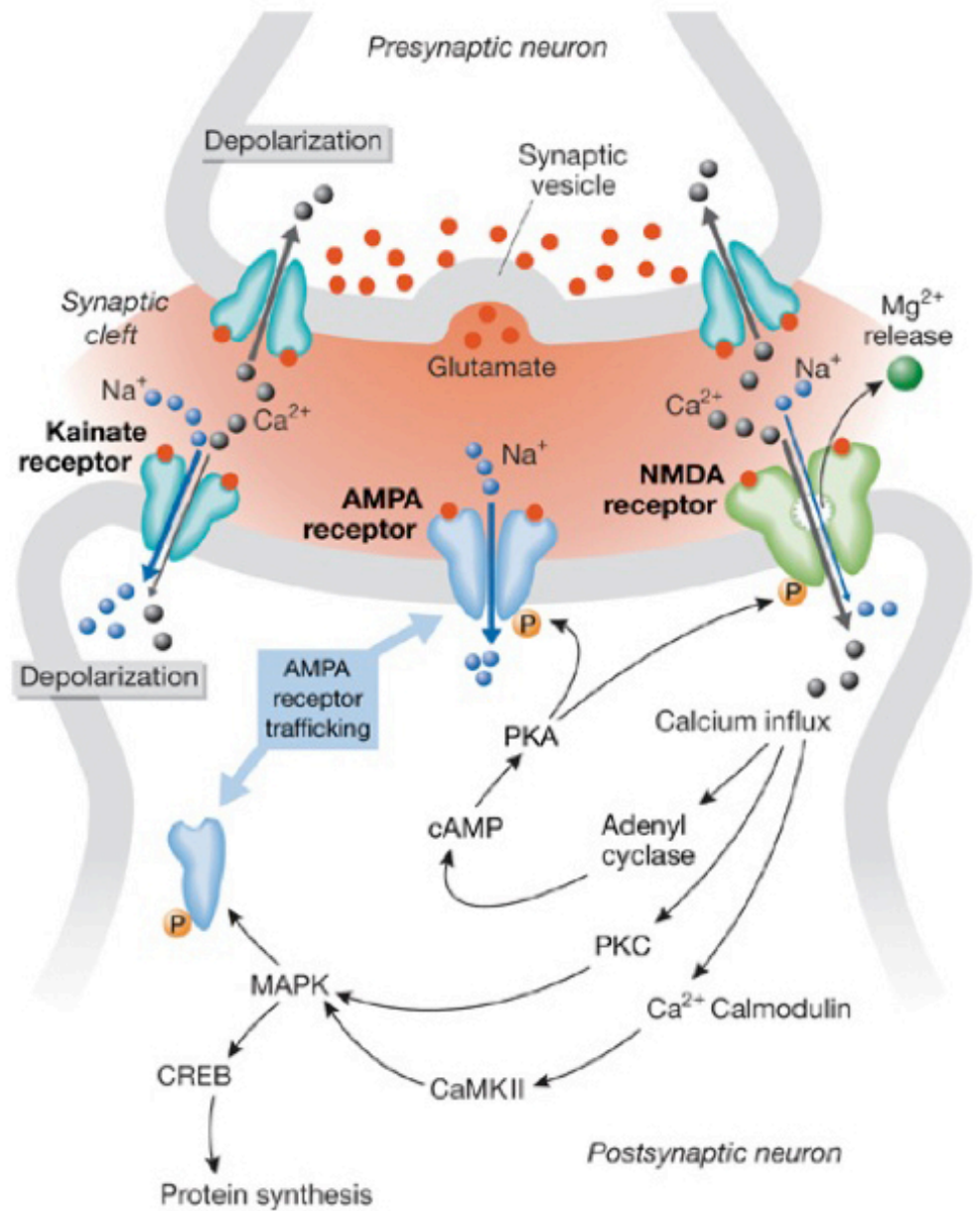
Synapses are localized regions of communication between two neurons. They are said to be plastic, that is, they exhibit the ability to modulate efficacy in response to past experience. This idea was first introduced by Donald Hebb (1949) in which he famously stated that 'when the axon of cell A is near enough to excite cell B or repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased' (Hebb 1949). This basic mechanism of 'fire together, wire together' has led

to our understanding of many different forms of plasticity, including both short-term and long-term potentiation and depression.

### **1.5 Exciting world of glutamate**

Glutamate was first discovered as an excitatory neurotransmitter in the 1950s (Hayashi 1952). By the 1970's it had been established as the primary excitatory neurotransmitter in the CNS (Meldrum 2000). Most fast synaptic transmission in the CNS is mediated by the binding of glutamate to its ionotropic receptors (Watkins et al. 1981; Seeburg 1993; Yamakura and Shimoji 1999). These receptors are defined by their specific pharmacologies and include the low-affinity  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainate receptors, and the high affinity *N*-methyl-D-aspartate (NMDA) receptors (Figure 1-3) (Cotman et al. 1988).

AMPA receptors are composed of four types of subunits (GluA1-GluA4) and are gated by the binding of ligand (primarily glutamate) onto at least two of the four available subunits (Dingledine et al. 1999). The primary binding domain comprises the N-terminal domain plus the extracellular loops (Dingledine and Conn 2000). AMPA receptors have a very low affinity for glutamate and display very fast gating kinetics (Meldrum 2000). The fast rise time of an excitatory postsynaptic potential (EPSP) is a result of activation of AMPA receptors. Interestingly, only those AMPA receptors lacking the GluA2 subunit conduct  $\text{Ca}^{2+}$  ions (Hume et al. 1991) whereas those containing GluA2 and other subunits will only gate  $\text{K}^+$  and  $\text{Na}^+$  (Isaac et al. 2007). AMPA receptors play an important role in the initial depolarization that is required for long-term potentiation (LTP) (Collingridge et al. 1992).



**Figure 1-3. Types of glutamate receptors (image from Voglis & Tavernarakis, 2006).** The primary ionotropic glutamate receptors in the CNS. Kainate and AMPA receptors act to depolarize the neuron. The NMDA receptor is more likely to be located extrasynaptically where glutamate spillover may bind and a subsequent depolarization via Kainate/AMPA receptors will open the cation channel. Ca<sup>2+</sup> influx can trigger downstream second messenger system, leading to changes in protein synthesis and receptor expression (for review see Citri and Malenka 2008). Image reprinted with permission from Nature Publishing Group standard use policy.

NMDA receptors consist of heteromeric combinations of the NR1 subunit and one or more GluN2(A-D) subunits (Laube et al. 1998). Changes in the GluN2 subunit

have profound effects on channel gating, affinity to glutamate and kinetics. NMDA receptors have a very high affinity for glutamate ( $K_d = 0.001 \mu\text{M}$ ; Dingledine et al. 1999). They are expressed both in the postsynaptic junction (Nusser 1999) and the extrasynaptic space (Tovar and Westbrook 2002; Petralia 2012).

The NMDA receptor possesses two important characteristics: 1) high permeability to  $\text{Ca}^{2+}$ , and 2) dual gating requirement. That is, current can flow through the NMDA receptor only when glutamate is bound to it and the membrane is subsequently depolarized. The depolarization is required to free the  $\text{Mg}^{2+}$  ion that blocks the channel at rest (Mayer et al. 1984; Nowak et al. 1984). Additionally, NMDA receptor activation requires the binding of a co-agonist, glycine or d-serine (Johnson & Ascher 1987). The unique dual-gating requirement of the NMDA receptor (requiring pre-synaptic glutamate release with post-synaptic depolarization) makes it an ideal molecular candidate for mediating Hebbian plasticity at a synapse.

## **1.6 Synaptic plasticity**

The NMDA receptor has been shown to play an important role in a particular form of information storage on dendrites known as LTP (Harris et al. 1984; for review see Citri & Malenka 2008). LTP is an activity-dependent form of synaptic plasticity in which repeated activation of the NMDA receptor results in a long-term increase in synaptic efficacy at a particular synapse. The initial binding of glutamate to AMPAR receptors opens the channel pore to allow  $\text{Na}^+$  to enter the cell (Borges and Dingledine 1998), depolarizing the membrane and causing NMDA receptors that have glutamate bound to open and let  $\text{Ca}^{2+}$  into the cell.  $\text{Ca}^{2+}$  entry activates a variety of second

messengers, including protein kinase A (PKA) (Abel et al. 1997), calcium/calmodulin-dependent protein kinase II (CaMKII) (Huang et al. 1994), cyclic adenosine monophosphate (cAMP)(Huang and Kandel 1994) and protein kinase C (PKC) (Malinow et al. 1989) (for overview of signaling see Figure 1-3). Activation of these, as well as other enzymatic pathways results in a long-term increase in the number of AMPARs at the synapse, as well as structural changes of the synapse that results in potentiation of those particular inputs at that particular synapse. The ability of a synapse to modify its strength is thought to underlie learning and memory (for overview see Neves et al. 2008).

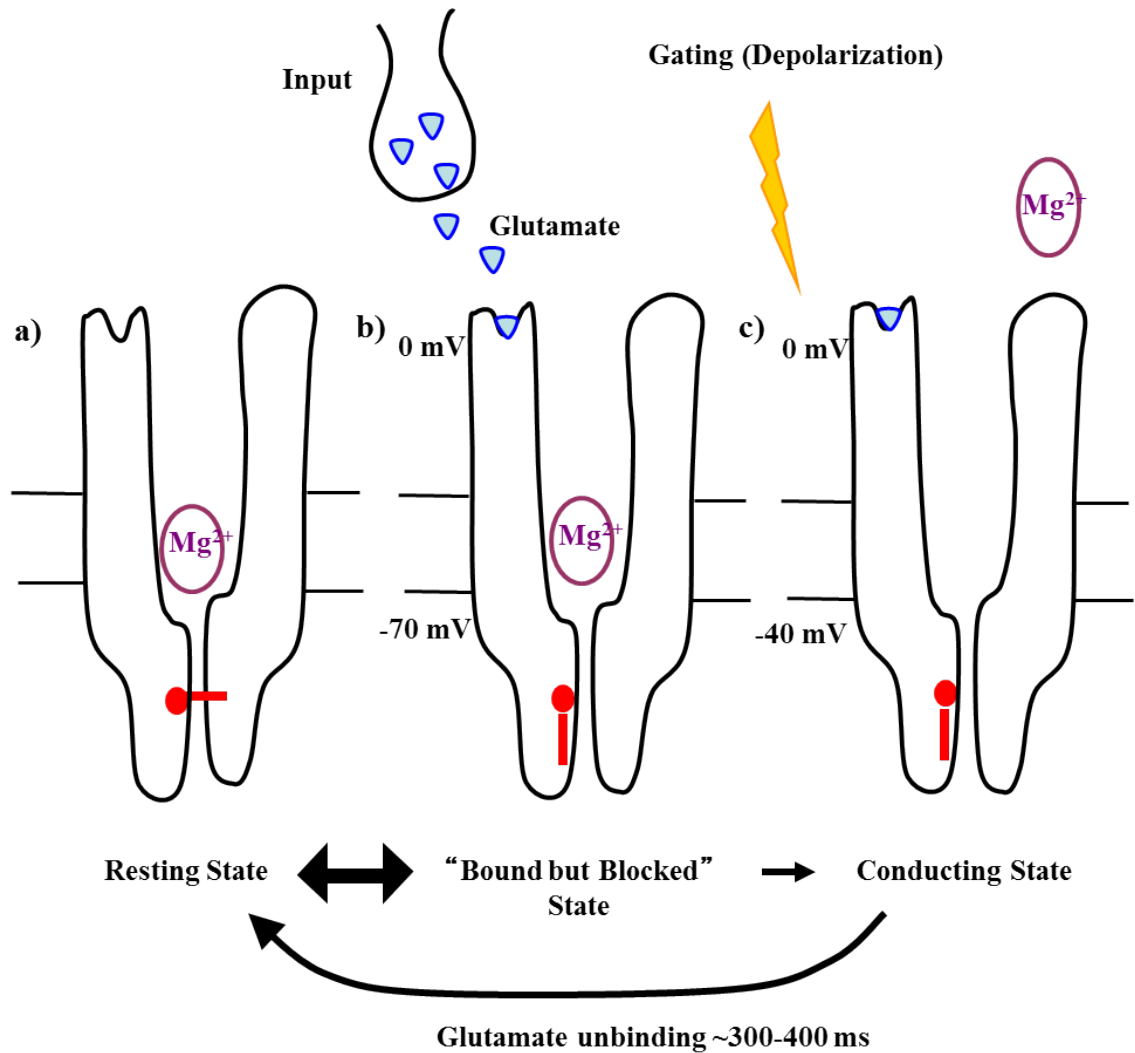
The NMDA receptor is important in a form of short-term plasticity known as spike-timing depended plasticity (STDP) at the CA3/CA1 synapse (Dan and Poo, 2004). STDP is a form of information storage in which the pairing of a pre-synaptic release of transmitter and post-synaptic depolarization results in changes in efficacy of the synapse (Magee and Johnston, 1997). In STDP, the post-synaptic depolarization acts to relieve the  $Mg^{2+}$  block from the NMDA receptor which leads to increased  $Ca^{2+}$  influx and subsequent activation of a variety of  $Ca^{2+}$  dependent second messengers (Gerkin et al. 2010). The key requirement for STDP is the timing of the pre-synaptic action potential with the post-synaptic depolarization (Kampa et al. 2007).

In dendrites, this depolarization is thought to come in the form of a back-propagating action potential (Kampa et al. 2007). Information storage during STDP occurs over a critical window in the 10's of milliseconds time scale and is bidirectional (in hippocampus first demonstrated by Debanne et al. 1998; Zhang et al. 1998; Magee and Johnston 1997; Sjostrom et al. 2001). Timing of the induction of STDP has

implications in long-term plasticity (Dan and Poo 2004). If the postsynaptic depolarization precedes presynaptic firing, long-term depression (LTD) is observed whereas if the postsynaptic depolarization follows transmitter release LTP occurs (Malenka and Siegelbaum 2001). Importantly, STDP is a graded phenomenon in that amplitude of potentiation/depression follows a linear scale dependent upon the time window in which it occurs (Polsky et al. 2009).

### **1.7 NMDA receptor gating as a mechanism for short term plasticity**

In 1990, Bekkers and Stevens proposed a novel role of the NMDA receptor in the storage of information. Based on the dual-gating requirement of the NMDA receptor, they suggested that information could be stored in a long-lived quasi-stable, “bound-but-blocked” state (Figure 1-4). This state exists following the binding of glutamate to the ligand binding domain of the NMDA receptor but prior to a depolarization that releases the  $Mg^{2+}$  block, or following the repolarization of the membrane after a fast EPSP that allows the  $Mg^{2+}$  to reenter the channel pore (Figure 1-4b). Glutamate remains bound to the NMDA receptor GLUN2A subunit for up to 100 ms, whereas glutamate can remain bound to the GLUN2B subunit for up to 1000 ms (Dingledine et al. 1999). Bekkers and Stevens suggested that the bound-but-blocked state may serve as a means to integrate glutamate signals over time. In theory, the bound-but-blocked state is an attractive short term memory mechanism. It would be highly specific in space and in time, and would be energy efficient. Further, such a mechanism for information storage would be ideal to handle large volumes of inputs and could be ‘read-out’ via any local, regenerative depolarization. Unfortunately, due



**Figure 1-4. Simplified cartoon of NMDA receptor dual gating.** a) A resting NMDA receptor at -70 mV is blocked by a  $Mg^{2+}$  ion. b) glutamate is released from a presynaptic neuron (or in our case by UV microphotolysis of caged-glutamate) and binds to the NMDA receptor. The receptor is now “bound” with glutamate but “blocked” by  $Mg^{2+}$ . c) In the presence of bound glutamate the membrane potential is depolarized to -40 mV, freeing the  $Mg^{2+}$  and allowing the channel to enter a conducting state. Note that glutamate may stay bound for ~300-400 ms making this “bound but blocked” state an ideal way for a dendrite to biophysically hold information.

to limitations of experimental design, specifically a way to control precisely the location and timing of glutamate stimulation on a particular synapse or dendrite, this hypothesis was never satisfactorily tested. It is also possible that the hypothesis was tested in ways that were too insensitive to reveal the resurrection of the bound-but-blocked NMDARs.

More specifically, the strength of the signal generated from the unblocking receptors is much weaker than the strength of triggering depolarization. This leads to the idea that the unblocking of NMDARs may not be observed as a local graded event, but require their all-or-none recruitment to be detected.

### **1.8 Dendritic compartmentalization and the dendritic spike**

Thin terminal apical dendrites on CA1 pyramidal neurons act as compartments with high input resistance and a variety of voltage gated ion channels that modulate the intrinsic excitability of the compartment (Wei et al., 2001; Liang, 2007). This idea is a critically important theoretical foundation for this thesis. The IV curve for NMDA receptors has a very shallow negative slope. This was the reason given by many of the leaders in the NMDA field in the late 1980s why regenerative depolarization was felt not to be possible. This point was never explicitly stated in print, but was widely held during personal discussion in scientific meetings (personal communication, Dr. Tang). Schiller et al. (2000) showed that the thin basal dendrites of neocortical could sustain NMDA spikes. Wei et al. (2001) showed that it was also possible on the apical dendrites of CA1 pyramidal neurons, albeit with the help of voltage gated calcium channels. Regenerative depolarization of NMDARs is possible on these dendrites because they are electrically very tight (i.e. high input resistance). Small inward currents result in significant membrane depolarization. But if the electrical compartment has low input resistance such as the soma or apical trunk, the inward current of the unblocked NMDA receptors will not be able to result in significant depolarization to sustain a regenerative depolarization by the shallow negative slope of the NMDA IV

curve. A regenerative NMDA spike allows the retrieved memory “signal” to rise clearly above the “noise” using a very weak triggering “gating” signal. Without a regenerative NMDA spike (i.e. when NMDA receptors are recruited in a graded manner) the retrieved signal would be significantly lower than the gating depolarization. For a memory system to be effective, the retrieved signal needs to be above the background synaptic activity and needs to be on the same order of magnitude if not greater than the “gating” signal. These are the reasons for why the terminal dendrites are the only locus possible for memory retrieval using the unblocking of NMDA receptors as the mechanism.

### **1.9 The “dendritic hold and read” phenomenon**

Previous studies in the Tang laboratory suggested that the dual gating property of NMDA receptors, combined with the properties of a thin dendrite as an electrical compartment, could serve a mnemonic function in ways other than coincident detection mediated synaptic plasticity. Coincidence detection had dominated thinking in the field of learning and memory (Tabone and Ramaswami 2012). Signals that were not coincident in time were assumed to be filtered out. The Tang laboratory set out to test the null hypothesis that non-coincident signals may serve as a form of information storage and readout by specifically setting up conditions in which excitatory inputs to a dendrite were provided hundreds of milliseconds prior to a second input directed at a separate location on the same dendrite. The experiment asked whether information from of the first stimulus (a photolytically released bolus of glutamate given for  $< 1$  ms across the length of a CA1 pyramidal neuron dendrite) could be recalled by an unrelated

second signal (photolytically released glutamate given for 10+ ms on two regionally distinct spots) separated far in time (Santos et al. 2007; Figure 1-5). The yellow circles marked the location of the first stimulus and the blue arrows marked the timing of the first stimulus. The red circles mark the location of the second interrogation signal and the red arrows marked its timing.

### **1.10 General Hypothesis**

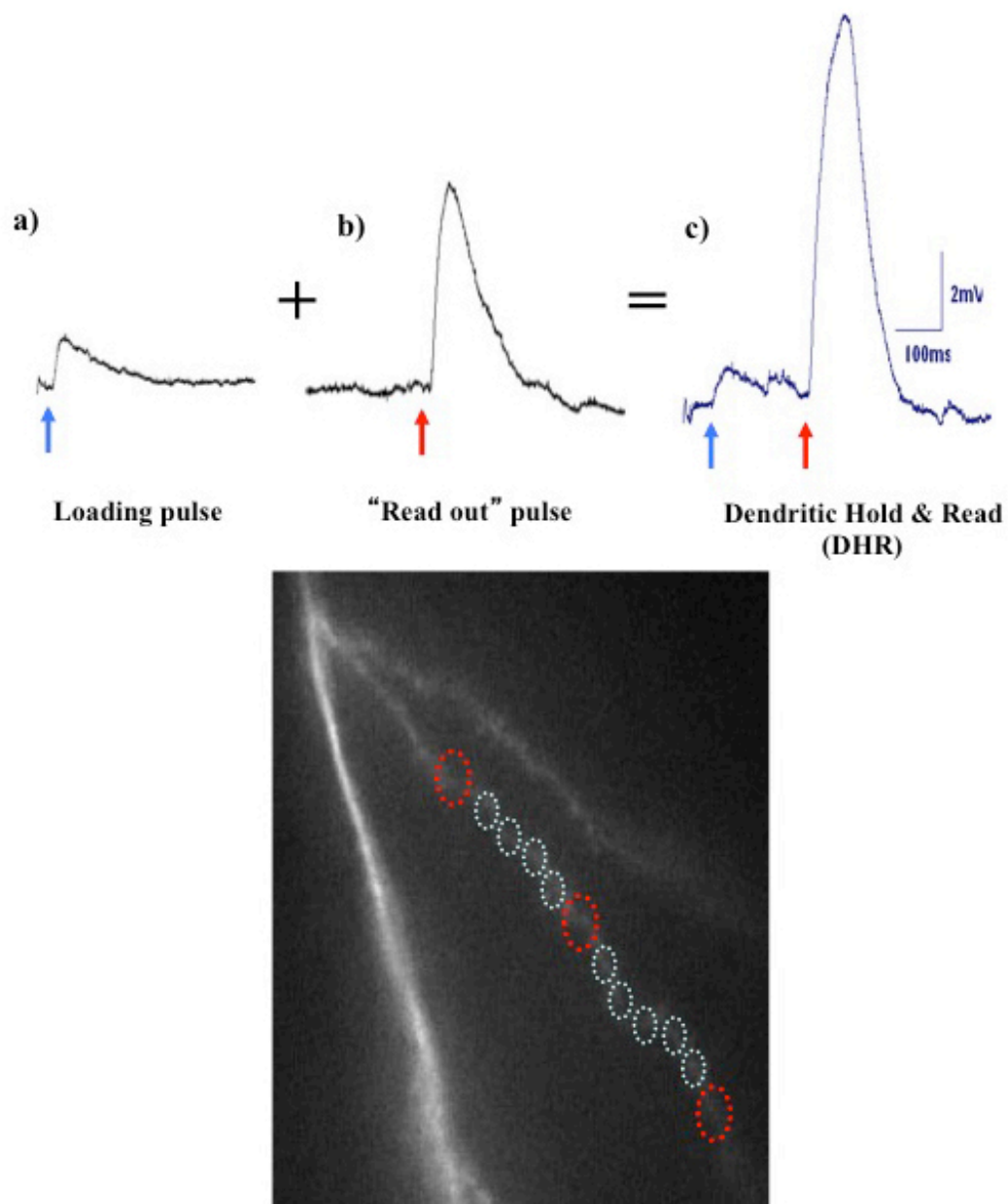
This thesis addresses the mechanism and physiological relevance of DHR. The experiments were organized to test several hypotheses that are discussed in two separate chapters.

**Hypothesis 1 (Chapter 3):** The information holding step in DHR is mediated by the “glutamate bound” but “Mg<sup>2+</sup>-blocked” state of NMDA receptors. *Information is defined here as the history of prior glutamate exposure (assumed to be equivalent to excitatory synaptic activity) on a dendritic branch.*

**Hypothesis 2 (Chapter 4):** DHR requires electrical compartmentalization in the form of a thin terminal dendrite.

**Hypothesis 3 (Chapter 4):** DHR is an all-or-none phenomenon.

**Hypothesis 4 (Chapter 4):** DHR is a physiological phenomenon.



**Figure 1-5. Dendritic Hold & Read (DHR).** a) A brief pulse of UV light (blue arrow, 0.5-1.0 ms) released a small amount of glutamate along the length of a CA1 pyramidal neuron dendrite (small blue/yellow spots) resulting in an EPSP. b) A longer (>10 ms) pulse of UV light is directed towards 3 large spots (red circles) to cause a large local depolarization. c) when a loading-pulse (a) precedes a “read-out” pulse (b) potentiation of the “read-out” pulse is observed. The phenomenon is repeatable.

**Chapter II**  
**General Methods**

## **2.1 Preparation of organotypic hippocampal slices**

Organotypic hippocampal slices were prepared according to the rollertube method of Gähwiler et al. 2001. Briefly, hippocampi were dissected from 4-8 day old rat pups (Figure 2-1) in accordance with a protocol approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee (IACUC). Sterile 400  $\mu$ M thick slices were cut using a Vibratome and attached to glass cover slips in a fibrin clot, formed by a mixture of 50% fibrinogen solution (Tisseal, Baxter Biosciences) and 50% chicken plasma. Coverslips with slices were placed in sealed test tubes containing 750  $\mu$ L of medium (25% horse serum, 25% Hanks' balanced salt solution, and 50% Eagle's basal medium supplemented with glucose). Slices were maintained in vitro for >12 days to allow for synaptic maturation (McKinney et al. 1999).

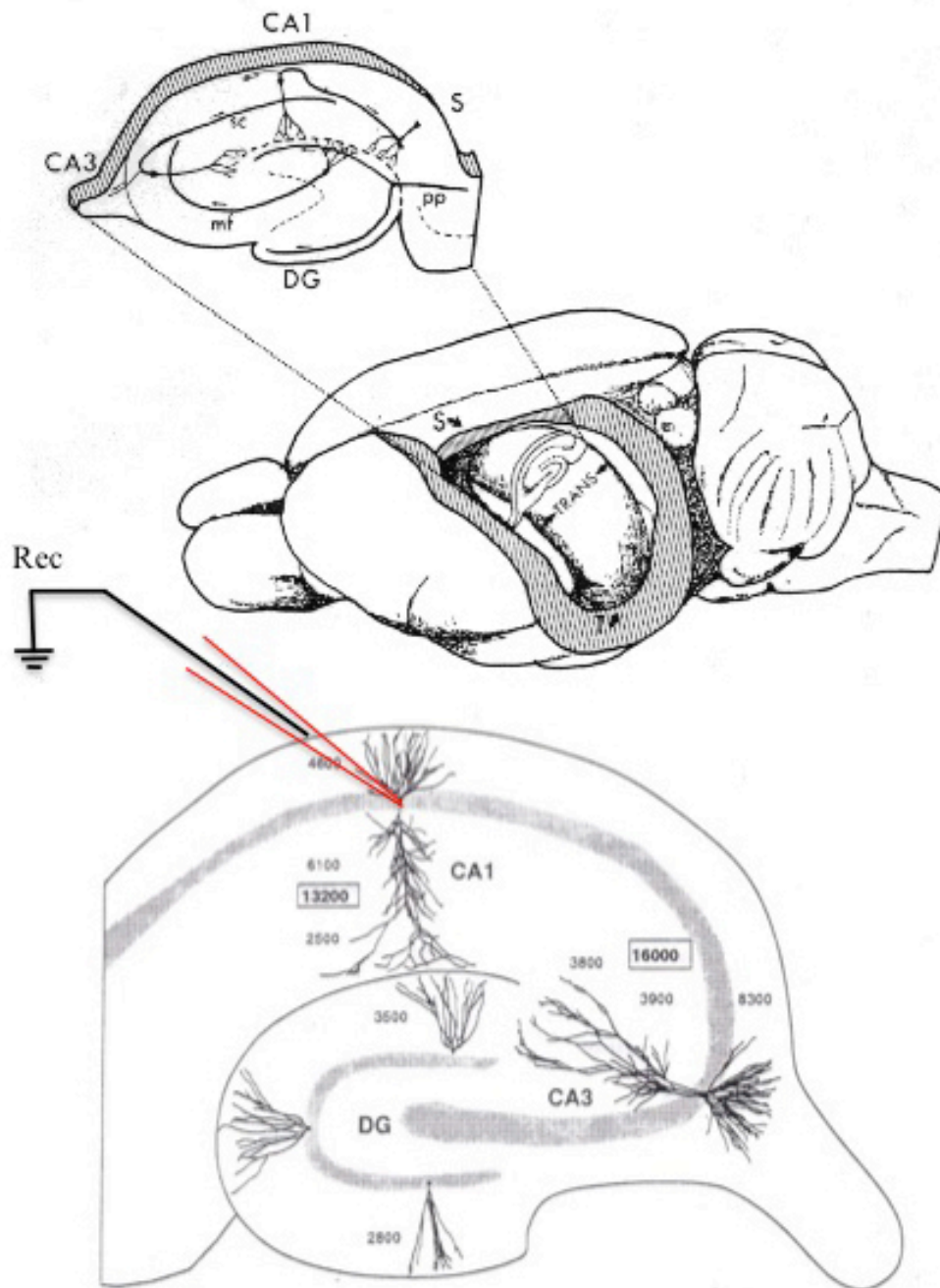
## **2.2 Preparation of acute hippocampal slices**

Hippocampal slices were prepared from 2-3 week old Sprague-Dawley rats in accordance with a protocol approved by the University Of Maryland School Of Medicine IACUC. Rats were deeply anesthetized and in-tact hippocampus were quickly removed and placed in chilled artificial cerebral spinal fluid (aCSF) containing (mM): 145 mM NaCl, 3 mM KCl, 10 mM HEPES, 2 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM glucose, and 0.1 mM glycine. They were then cut into 400  $\mu$ M coronal slices using a vibrating blade microtome. Slices were transferred to a holding chamber containing aCSF at room temperature that was bubbled with carbogen (95% O<sub>2</sub> / 5% CO<sub>2</sub>) for at least 1 hour prior to recording.

### 2.3 Whole-cell electrophysiology

Slices were transferred to a recording chamber and constantly perfused with carbogen-saturated aCSF which contained 500  $\mu\text{M}$  caged glutamate ( $\gamma$ -glutamate or *N*-Nmoc-glutamate). Unless otherwise stated, all experiments were performed at temperatures between 32-35 °C. Whole-cell recordings were done "blind" using an Axon Instruments Axoclamp 700B Amplifier and pClamp Version 10.2 software was used for data acquisition. Glass recording pipettes (resistance 3-6 megaOhms) were filled with an internal saline solution containing (mM): 135  $\text{KCH}_3\text{SO}_3$ , 10 HEPES, 10 NaCl, 1  $\text{MgCl}_2$ , 0.1 mM  $\text{K}_4\text{BAPTA}$ , 2 mM  $\text{Mg}^{2+}$ -ATP, and 10 mM Phosphocreatine, buffered to pH 7.3 with KOH. Alexa 594 (100  $\mu\text{M}$ ) was included in the internal solution to allow for visualization of the dendrites. Tetrodotoxin (TTX, 1  $\mu\text{M}$ ) was included in the aCSF for most experiments but was not added during experiments where field stimulation was used in place of photolysis. Unless otherwise noted, all recordings were done in the presence of the  $\text{GABA}_A$  antagonist bicuculline methiodide (10-20  $\mu\text{M}$ ) and  $\text{GABA}_B$  antagonist CGP-35348 (5-20  $\mu\text{M}$ ). Following a 10-minute baseline, an I-V curve was then established to determine passive properties of the cell. Recordings were done in "current-clamp" configuration and cells were held between -65 to -73 mV.

All chemicals and drugs, including ifenprodil and Ro25-6981, were obtained from Sigma-Aldrich. NVP-AAM007 was obtained from Novartis Pharma AG, Base; Switzerland.



**Figure 2-1. Anatomy of area CA1 recording.** I record from CA1 pyramidal neurons (“Rec” is the recording electrode) using the whole-cell patch clamp method. Primary inputs to CA1 arrive from area CA3 via the Schaffer Collateral (SC) pathway. These are glutamatergic synapses. Images taken from *Synaptic Organization of the Brain*, 4<sup>th</sup> ed, pg. 418 and 420.

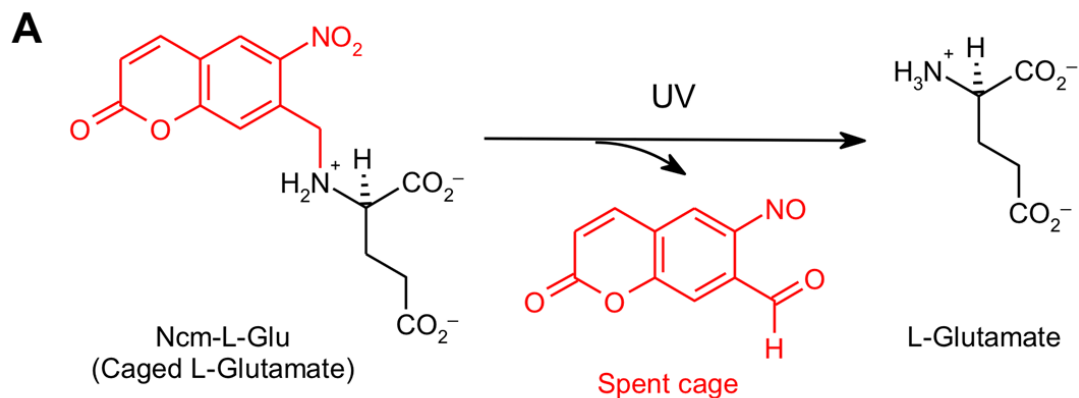
## 2.4 Preparation of caged glutamate, *N*-[(6-nitrocoumar-7-yl)methyl]-*L*-glutamic acid (*N*-Ncm-Glu)

Caged glutamate (Figure 2-2) was provided by the laboratory of Joseph Kao. Briefly, 0.9 g of 7-bromomethyl-6-nitrocoumarin (3.1 mmol; obtained by brominating 7-methyl-6-nitrocoumarin with *N*-bromosuccinimide in the presence of benzoyl peroxide), 1.04 g of H-Glu(OtBu)-OtBu·HCl (3.5 mmol), 1.4 mL of triethylamine (10 mmol) and 6 mL of dry DMSO were added. The mixture was stirred at room temperature for 4.5 h. The reaction mixture was dried under high vacuum, and the residue was purified by column chromatography (5-10% CH<sub>3</sub>CN/CH<sub>2</sub>Cl<sub>2</sub>) to yield *N*-[(6-nitrocoumar-7-yl)methyl]-*L*-glutamic acid, di-*t*-butyl ester as a viscous oil (0.83 g, 57% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 8.22 (s, 1H), 7.77 (d, 1H, *J* = 9.5 Hz), 7.72 (s, 1H), 6.54 (d, 1H, *J* = 9.5 Hz), 4.14 (dd, AB type, 2H, *J*<sub>AB</sub> = 16 Hz), 3.15 (m, 1H), 2.38 (m, 2H), 1.98-1.82 (m, 2H), 1.47 (s, 9H), 1.45 (s, 9H). For deprotection, 0.1 g of the di-*t*-butyl ester was dissolved in 1 mL glacial acetic acid; 1 mL conc. HBr was added, and the reaction mixture was maintained in an ice/water bath for 15 min. The reaction mixture was reduced under vacuum, and the residue was dissolved in 1.2 mL water and purified by reverse-phase HPLC (6:4 acetonitrile–water, with 0.1% v/v trifluoroacetic acid). Product fractions were pooled and lyophilized to yield 0.052 g of *N*-[(6-nitrocoumar-7-yl)methyl]-*L*-glutamic acid, or *N*-Ncm-Glu (68% yield). <sup>1</sup>H ((CD<sub>3</sub>)<sub>2</sub>SO) 8.49 (s, 1H), 8.15 (d, 1H, *J* = 9.8 Hz), 7.71 (s, 1H), 6.63 (d, 1H, *J* = 9.5 Hz), 4.05 (dd, AB type, 2H, *J*<sub>AB</sub> = 15.3 Hz), 3.17 (m, 1H), 2.32 - 2.29 (m, 1H), 1.86 – 1.80 (m, 1H), 1.76 – 1.66

(m, 1H). Mass spectrum (HR FAB): calculated for C<sub>15</sub>H<sub>15</sub>N<sub>2</sub>O<sub>8</sub> (MH<sup>+</sup>), 351.0828; found 351.0829.

## **2.5 A DMD-based method for patterned photolysis**

Photolysis of caged glutamate provides the best means for precisely controlling the temporal and spatial pattern of stimulation necessary to test the theory of dendritic hold-and-read. There are two possible strategies for implementing multi-site photostimulation: using either a scanning mirror or an acousto-optical deflector to achieve rapid sequential illumination of the multiple sites with a single intense beam, or simultaneous illumination of all of the target locations using a digital micromirrors device (DMD). The former strategy has the advantage that the technology for rapid scanning has already been developed for confocal microscopy and two-photon microscopy and is commercially available. The DMD strategy has the advantage of being less expensive to build and relatively easy to operate during experimentation. The system built for these experiments followed the latter strategy using the DLP® chip from Texas Instruments. The DLP® chip and its electronic controllers were purchased as the DMD Discovery™ kit from TyRex Services Group (Austin, TX) and ViaLUX GmbH (Chemnitz, Germany).



**B**

Caged glutamate	Molecular structure	$\lambda_{\max}$ (nm)	$\epsilon^a$ ( $M^{-1}cm^{-1}$ )	$Q$	$k_{\text{release}}$ ( $sec^{-1}$ )	$\tau$ ( $\mu sec$ )	Reference
Ncm-Glu		265	1048 (355)	0.047	$>5 \times 10^6$	$<0.2$	Muralidharan and Kao (unpub. observ.); Cai et al. (2004)
Nmoc-Glu		265	505 (355)	0.11	$\leq 2.0 \times 10^3$	$\geq 500$	Rossi et al. (1997)
4-pHP-Glu		282	$<100$ (355) <sup>b</sup>	0.08	$7.0 \times 10^7$	0.014	Givens et al. (1997); Park and Givens (1997); Conrad et al. (2000)
NI-Glu		342	2720 (347)	0.043	$5 \times 10^6$	0.2	Papageorgiou et al. (1999); Morrison et al. (2002)
MINI-Glu		248	4097 (355)	0.085	$2.67 \times 10^3$	375	Papageorgiou et al. (1998, 2000); Canepari et al. (2001)
$\gamma$ -CNB-Glu		262	180 (365)	0.14	$3.47 \times 10^4$	30	Wieboldt et al. (1994); Furuta et al. (1999)
Bhe-Glu		368	17,300 (365)	0.019	N.R. <sup>c</sup>	N.R. <sup>c</sup>	Furuta et al. (1999)

<sup>a</sup>extinction coefficient determined at the wavelength shown in parentheses.

<sup>b</sup>Estimated

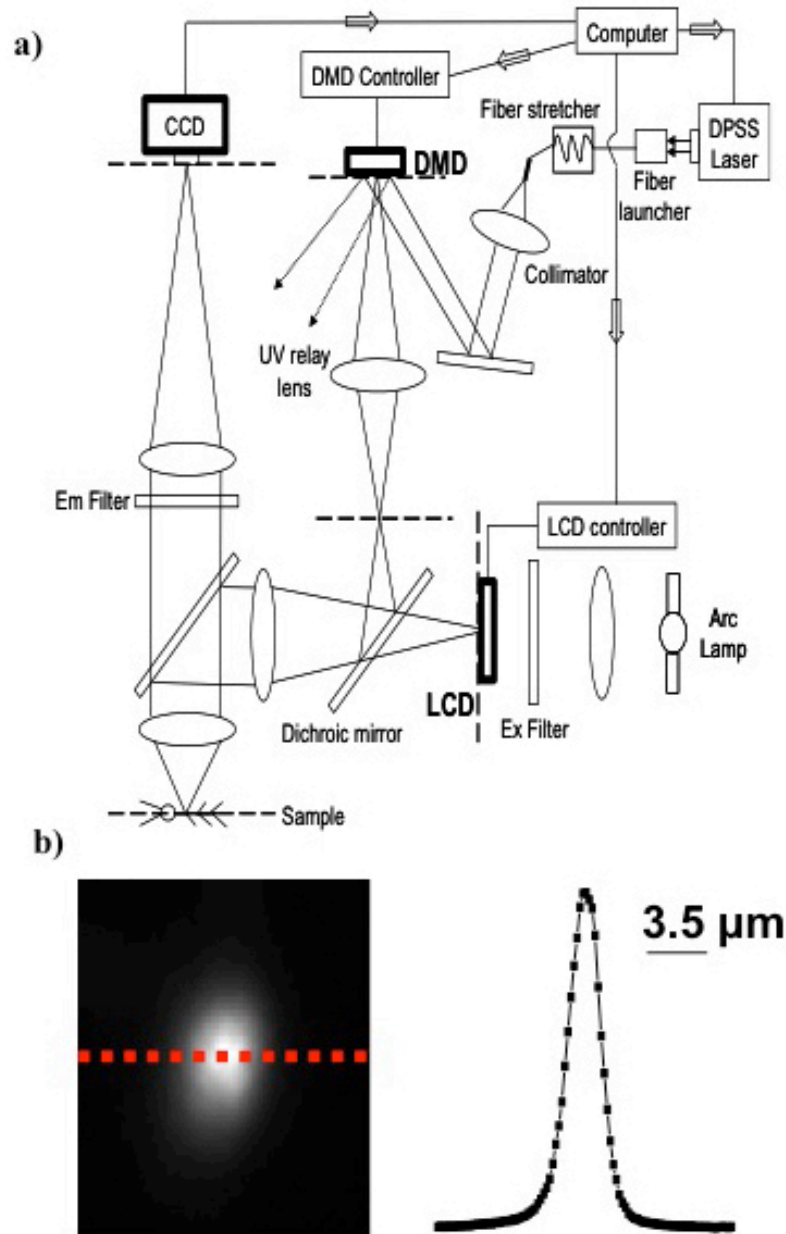
<sup>c</sup>N.R., not reported.

**Figure 2-2. Caged glutamate.** A, Chemical diagram of N-Cm-glu. B, Table of properties of various caged glutamates. Taken from Kao 2006, Figure 6.20.2.

The overall schematic of the photolysis and imaging system is shown in Figure 2-3 (Liang et al. 2010). The energy for single-photon photolysis is provided by a 1-Watt frequency-tripled Nd:VO<sub>4</sub> diode-pumped solid state laser (DPSS Lasers, Santa Clara,

CA) with a wavelength of 355 nm. The UV light needed for photolysis could not be brought through the imaging light path because none of the commercial microscope companies provide a UV-corrected imaging tube lens. Instead, the UV light must be brought in through the epi-illumination light path with a longpass dichroic mirror. A UV relay lens was used to project the spatial pattern generated on the DMD chip onto a conjugate image plane (each such plane is marked by a dashed line in Fig. S1A). The output of the DPSS laser is launched into a 100- $\mu\text{m}$  multimode optical fiber. The fiber and fiber launcher were previously described (1). The fiber is wound around a fiber stretcher (model 915, Canadian Instrumentation & Research, Ltd., Ontario, Canada) operating at  $\sim 40\text{-}50$  kHz. The purpose of the fiber stretcher is to effectively scramble the speckle pattern that would otherwise appear on the DMD chip due to the coherent nature of the laser light source. The output of the fiber is then collimated with a UV microscope objective (Olympus 20X). The collimated light was then steered onto the DMD chip at a  $24^\circ$  angle of incidence in an orientation that is coplanar with the micromirror tilting movement. When a mirror is in the ON state, the light is directed along the optical axis of the microscope. When the mirror is in the OFF state the light is deflected towards a light trap. An in-house graphics user interface (GUI) was written to interface the

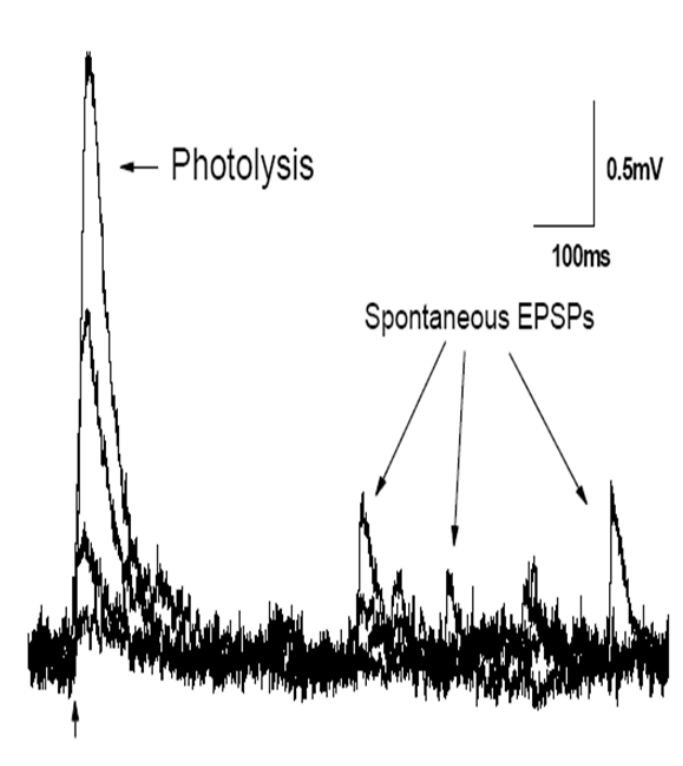
commercial image acquisition software (IPLab), the data acquisition software



**Figure 2-3.** DMD based photolysis system (A) The layout and organization of the photostimulation and imaging system is shown in a schematic. The DMD is placed at a conjugate of the image plane. A graphics user interface is used to co-register the CCD and DMD pixels after which cursor defined regions of interest controlled by the user can direct the corresponding DMD mirrors to illuminate the areas of interest on the dendritic arbor. (B) Optical resolution is measured with a fluorescent target.

(pClamp), and the user. Key tasks of the GUI include: the means to co-register the CCD pixels with the DMD pixels; the means for the user to easily define the target locations in relation to an image of the target dendritic arbor, the means to create a library of spatial patterns for stimulation, and the means to control the timing of the DMD, the laser pulses, the laser shutters, the CCD camera, and the data acquisition software. The GUI was also used to control a LCD placed in the fluorescence illumination path to optimize the effective dynamic range of fluorescence imaging and crop the area for fluorescence illumination. Since this option is not used in these experiments, it will not be described here.

The effective spatial resolution as measured with a fluorescent target was less than theoretically possible, 2  $\mu\text{m}$ . This is probably due to the poor quality of the epillumination tube lens and the UV relay lens, neither of which were designed specifically for imaging. Lenses corrected for near-UV wavelengths would be necessary to optimize the spatial resolution of the system. For the purposes of these experiments such high optical resolution was not required. The effective resolution of photolysis can be tested by measuring responses as the photolysis spot is moved across a dendrite that is  $\sim 2 \mu\text{m}$  in diameter. Finally, the electrical responses elicited by differing photolysis intensities were plotted (Figure 2-4). These fast current responses were obtained under voltage clamp conditions with photolysis on the apical trunk. This suggests that the slower voltage responses obtained under current clamp conditions with stimulation in the middle portions of the thin oblique dendrites may be in part due to the cable properties of the terminal dendrite.



**Figure 2-4. Photolysis-induced excitatory post-synaptic potential (EPSP).** The vertical arrow under the EPSPs indicates when the UV light was sent to the sample via the mirrors on the DMD. Displayed is the result of four trials in which the duration of UV light given was increased step-wise.

## **Chapter III**

### **Dual Gating of NMDA receptor and DHR**

### 3.1 Introduction

The most striking and unique feature of the NMDA receptor is the dual requirement of ligand binding and voltage depolarization for the channel to open and gate ions. This behavior is referred to as “dual gating”. This major discovery was simultaneously described by two independent groups; Dr. Philippe Ascher and Dr. Linda Nowak (Nowak et al. 1984) in Paris and Dr. Mark Mayer and Dr. Gary Westbrook at NIH (Mayer et al. 1984). They showed that the NMDA receptor at resting membrane potential ( $\sim -70$  mV) remain non-conducting even when glutamate is bound to it. But if the post-synaptic membrane were depolarized past  $-50$  mV, inward current will flow. That is, with coincident ligand binding and membrane depolarization the NMDA receptor channel becomes fully conducting for cations. They showed that this voltage dependence could be eliminated if  $Mg^{2+}$  were eliminated from the extracellular solution. Further studies showed that  $Mg^{2+}$  is stabilized within the voltage sensing region of the NMDA channel at polarized membrane potentials, but leaves the channel upon membrane depolarization (Figure 1-4). Another characteristic of the NMDA receptor is its unusually high permeability for  $Ca^{2+}$  (Mayer and Westbrook 1987), which may trigger downstream  $Ca^{2+}$  dependent signaling cascades (for review, Cull-Candy et al. 2001). The coincidence detection property of NMDA receptors combined with its high  $Ca^{2+}$  permeability fit perfectly with the molecular requirements to implement Hebb’s hypothesis for synaptic plasticity. These discoveries generated significant excitement in the field of learning and memory. The focus was almost completely directed to the ability of the NMDA receptor to act as a detector and how modulation of the NMDA receptor could impact learning and memory.

But there is another interesting feature of NMDA receptor that was largely overlooked in the excitement about the NMDA receptor dual gating. NMDA receptors have a very high affinity for glutamate. The unbinding rate for glutamate is in the range of hundreds of milliseconds (Hestrin, et al. 1990; Lester and Jahr 1992). It is particularly relevant for the present study because it allows for a long-lived non-conducting receptor state where glutamate is bound to the receptor but the receptor-channel remains blocked, “glutamate-bound but  $Mg^{2+}$ -blocked” state. Certain investigators such as Dr. Westbrook and Dr. Charles Stevens did consider how this state could implement mnemonic functions without invoking synaptic plasticity (personal communication between Dr. Westbrook with Dr. Tang; Bekkers and Stevens 1990).

This basic concept was developed at a theoretical level in two distinct directions. Lisman and Wang proposed that the slow unbinding of glutamate from the NMDA receptor in conjunction with its voltage dependent  $Mg^{2+}$  block can enable a feedback network to sustain persistent spiking activity after the input to the network has ended (a functional definition of working memory) (Lisman and Wang 1998). In contrast, Bekkers and Stevens proposed that information could be stored by the glutamate-bound but  $Mg^{2+}$ -blocked (non-conducting) state of the NMDA receptors.

Neither of these two groups experimentally demonstrated their theoretical prediction. We realized that these earlier investigators would have been unaware of the ability of the thin terminal dendrites to generate compartmentalized, all-or-none dendritic spikes since it was not reported until 2000 and 2001 (Schiller et al. 2000; Wei et al. 2001). These dendritic spikes provide a plausible means to effectively “read-out” information stored by populations of the NMDA receptor in the bound-but-blocked

state with an output (binary spike) that has high signal-to-noise ratio. With a plausible means for effective information retrieval Dr. Tang's lab reexamined the idea first proposed by Bekkers and Stevens. This led to the novel experimental phenomenon called "dendritic hold and read" (DHR) (Figure 1-5).

In order to better understand DHR, it was essential to test the hypothesis that the mnemonic property of DHR is mediated by the bound-but-blocked state of the NMDA receptor. Alternate mechanisms include activation of mGluRs and the associated downstream second messengers and residual glutamate in the extracellular space from the loading stimulus.

Using a novel DMD-based system to allow for patterned illumination (Liang et al, 2011), different spatial patterns of glutamate photolysis were applied to achieve distinct goals. In order to establish a population of bound-but-blocked NMDA receptors over a length of dendrite the laser pulses were directed to a set of 4-5  $\mu\text{m}$ -diameter spots over a 50-90  $\mu\text{m}$  length of an oblique dendrite of a CA1 pyramidal neuron. The duration of these laser pulses was chosen to be sufficiently brief to photorelease enough glutamate to achieve binding to large numbers of NMDA receptors, while causing only minimal activation of AMPA receptors. This is possible because NMDA receptors have ~1000-fold higher affinity for glutamate than AMPA receptors (Laurie & Seaburg 1994).

DHR involves the application of a pulse of very low concentration of glutamate spread out diffusely over a large area of a single dendritic branch followed hundreds of milliseconds later by a moderately stronger depolarizing stimulus located at a separate location on the dendrite (Figure 3-1a). This "loading" stimulus was designed to mimic

synaptic inputs that arrive on one oblique dendrite and is the information-containing input signal in the schematic for the hold and read memory. “Loading” results in a barely detectable depolarization by itself. In contrast, the “read-out” stimulus was designed to activate a small number of AMPARs at restricted locations in order to create a sufficiently strong depolarization to trigger the read-out of the stored input signal. The gating stimulus comprised simultaneous laser pulses directed at two 4-5  $\mu\text{m}$ -diameter spots on the same dendrite (red circles, Figure 3-1); the pulse duration was set to produce a 2-4 mV depolarization at the soma when the read-out stimulus was given alone (control traces, red arrows, Figure 3-1).

Using this loading and read-out protocol, we observed that the information from the loading pulse was consistently “read-out” as an increase in amplitude of the second pulse (Figure 3-1C). Initially we used durations in the 200-300 ms time scale as this was the most reliable, repeatable inter-stimulus-interval (ISI).

The time course of DHR appeared to be similar to another type of short term plasticity called paired pulse facilitation (PPF). In PPF, two stimuli given in quick succession result in a second response that is greater in magnitude than either response given alone (Debanne et al. 1996; Rozov et al. 2001). PPF is a presynaptic phenomenon (Thomson et al. 1993). In DHR I am controlling the amount of transmitter release by photolytically releasing glutamate on the dendrites, thereby avoiding presynaptic release and its implications. DHR is also novel also in that unlike PPF or other forms of short term plasticity events, the “loading” and “read-out” pulses were spatially distinct. The inter-stimulus-interval (ISI) for DHR aligns with the kinetics glutamate unbinding to the NMDA receptor (Spruston et al. 1995). I propose that DHR

may be one example of information storage that depends on the bound-but-blocked state as originally proposed by Bekkers and Stevens (1990).

Here, I test several predictions of the hypothesis that DHR is mediated by the “glutamate bound” but “Mg<sup>2+</sup> blocked” state of NMDA receptors. I will show that DHR is blocked by NMDA receptor antagonists. I will also demonstrate that by reducing the likelihood of the “bound-but-blocked” state I am unable to readout DHR. Finally, I show that DHR does not require an mGluR-mediated second messenger and that the duration of information storage is consistent with the rate of unbinding of glutamate from the NMDA receptor. Taken together, these results support our hypothesis that a “bound by blocked” state of the NMDA receptor is the key information holding step of DHR.

## **3.2 Methods**

Methods are as described in General Methods (Chapter 2). All recordings are excitatory post-synaptic potentials (EPSPs) in whole cell current clamp mode from the soma of a CA1 pyramidal neuron. Unless otherwise noted, all experiments were performed in acutely prepared hippocampal slice at physiological (32-34°C) temperature.

## **3.3 Results**

### **3.3.1 DHR is sensitive to NMDA receptor antagonists**

If DHR is mediated by the bound-but-blocked state of the NMDA receptor as opposed to other glutamate receptors, then it should show sensitivity to NMDA receptors antagonists but not to an antagonist of mGluRs.

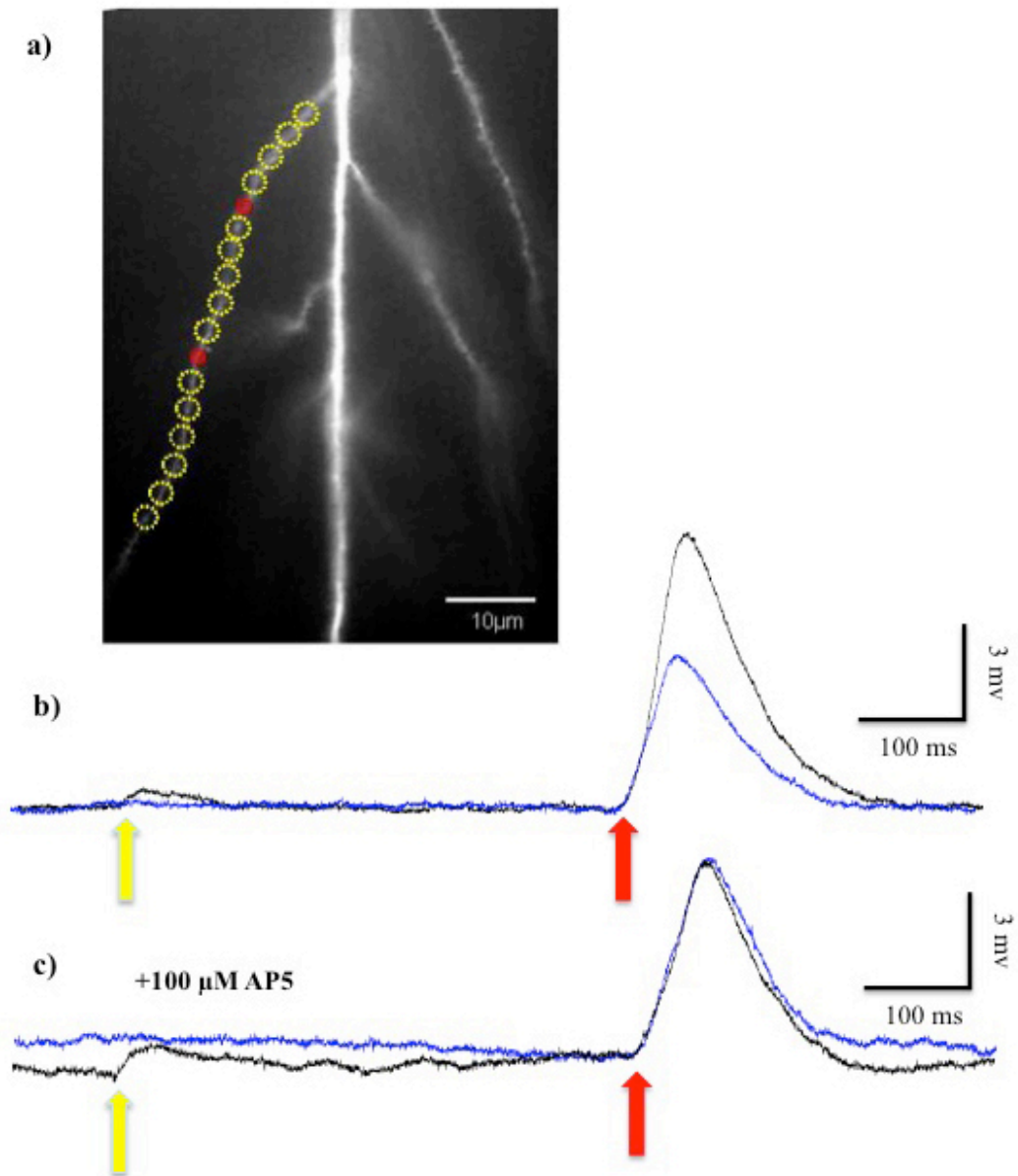
I first target multiple regions along the length of a thin terminal dendrite (Figure 3-1a). Brief flashes of UV light (1-3 ms) were used to uncage glutamate onto specific locations of the dendrite. This “loading” acts to release enough glutamate onto NMDA receptors such that a large population of NMDA receptors bind glutamate, but not enough to activate low-affinity AMPA receptors. This should increase the probability that a large population of NMDA receptors that are in the “bound but blocked” state which can last for 100’s of ms. A second, longer flash of UV light is then targeted to two spots on the dendrite spatially distinct from the loading pulse. This causes a moderate-sized AMPA receptor mediated depolarization that acts to free the  $Mg^{2+}$  block from the bound-but-blocked receptors. As more and more NMDA receptors open, the membrane continues to depolarize until a threshold for regenerative spike is reached. At this point a large amplitude response is observed. This potentiation that relied on the loading of a dendritic compartment and subsequent readout by a large depolarization is DHR.

Upon confirming DHR, I then added 100  $\mu M$  of the NMDA receptor specific antagonist (2*R*)-amino-5-phosphonovaleric acid; (2*R*)-amino-5-phosphonopentanoate) (AP5) (Morris 1989) and repeated the DHR protocol (Figure 3-1c). I observed a complete block of the potentiation of the read-out response in 6 out of 6 cases (group data in Figure 3-4b) while the baseline amplitude of the read-out pulse without a loading pulse was unchanged.

A second group of DHR positive cells was treated with a pharmacologic agent that belongs in the non-competitive class of NMDA antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cycloheptan-5-10-imine maleate (MK801; 100  $\mu$ M) (Coan et al. 1987). MK801 binds with very high affinity inside the NMDA receptor pore. Application of MK801 completely blocked DHR potentiation in 3 out of 3 cells (data not shown). Together these results implicate the necessary involvement of NMDA receptor in DHR.

Two other key types of glutamate receptors on CA1 pyramidal neuron dendrites also need to be considered; AMPA and metabotropic glutamate receptors (mGluRs) (Spruston 2008). The AMPA receptor has about a ~one thousand fold lower affinity for glutamate than the NMDA receptor (Laurie and Seaburg 1994). Activation of AMPA receptors depolarizes the postsynaptic membrane allowing the influx of cations and LTP causes an increase in synaptic AMPA receptor density (Shi et al. 1999). Because I use AMPA receptor depolarization as our read-out stimulus, application of AMPA receptor antagonists would not be compatible with elicitation of DHR. The observation that the loading stimulus does not necessarily elicit any significant depolarization and often no observable depolarization is consistent with this idea.

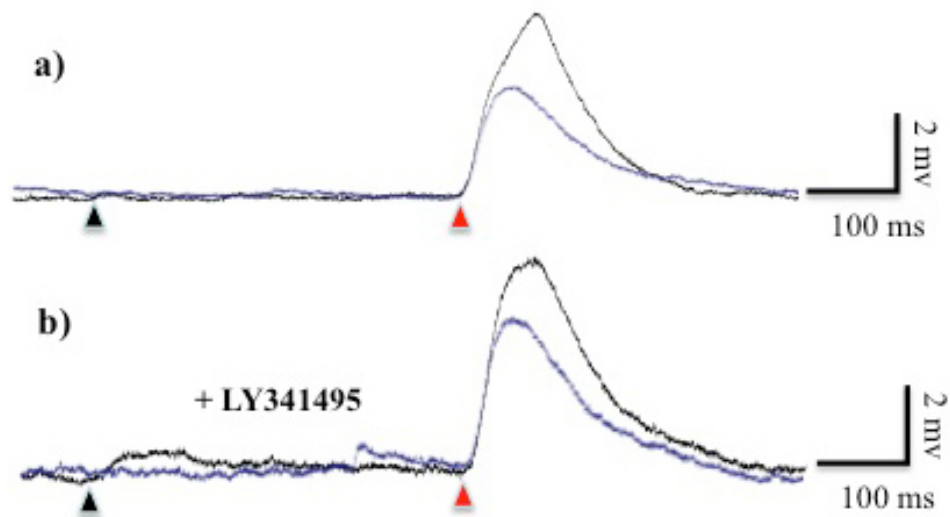
In contrast, activation of mGluRs is a more plausible mechanism. Metabotropic GluRs initiate a variety of G-protein coupled pathways (Coutinho & Knopfel 2002). Group I mGluRs activate the phospholipase C pathway, whereas group II and III mGluRs activate the adenylyl cyclase signaling cascade (Coutinho & Knopfel 2002).



**Figure 3-1. NMDAR required for DHR.** a) Typical distribution of patterned stimulation on a dendrite of a CA1 pyramidal neuron. The yellow circles correspond to the location of the loading pulse (.5 ms exposure). The two red circles correspond to the location of the readout pulse (10 ms exposure). b) Blue trace corresponds to a control in which only the readout (red circles) was given. The black trace corresponds to trial in which the loading pulse (yellow circles) was stimulated 200 ms before the readout pulse. The potentiation was transient in that it was only present for the trial in which the loading pulse was given. c) The same protocol repeated as in b), but with the addition of 100  $\mu$ M AP5.

mGluRs participate in both short- (Edwards et al. 2006) and long-term (Neyman et al. 2008) information storage. Therefore I next asked whether mGluR activation

participates in DHR. LY341495 is a potent, non-selective inhibitor of mGluRs (Fitzjohn et al. 1998). In four cells where DHR was present I added LY341495 (100  $\mu$ M). I observed no effect (Figure 3-2) on the magnitude of potentiation of DHR (167%  $\pm$  14% without vs. 168%  $\pm$  22% with LY341495). This suggests that mGluRs are not strongly linked to DHR.



**Figure 3-2: mGluR Antagonists do not effect DHR.** a) Cell in which DHR was observed, note potentiation in black trace following a brief loading pulse (black arrow). b) Addition of LY341495 (100  $\mu$ M) did not effect DHR. n=4, peak amplitudes of 1.67 vs. 1.77, respectively.

### 3.3.2 The kinetics of information storage in DHR are consistent with the glutamate unbinding rate of NMDA receptors

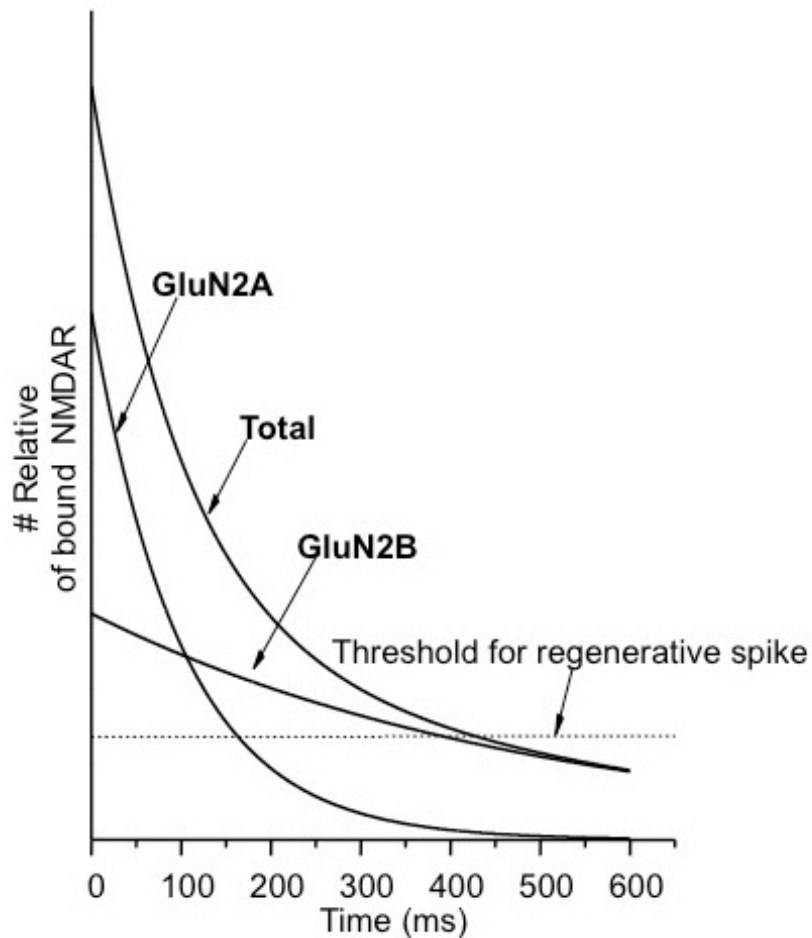
If DHR is mediated by NMDA receptors, then the kinetics of information storage in DHR should be consistent with the glutamate unbinding rate of the NMDA receptors.

Native NMDA receptors are formed from a diverse set of subunits such as GluN1, GluN2-(A-D), and GluN3(A/B) (Dingledine et al. 1999). The affinity and unbinding kinetics of the NMDA receptors is determined by the specific composition of its subunits (Erreger et al. 2005). Of note, NMDA receptors containing the GluN2B subunit have slower glutamate unbinding kinetics than NMDA receptors containing the GluN2A subunit (Yamakura and Shimoji 1999; Figure 3-3). The unbinding kinetics of the GluN2B subunit containing receptors is most consistent with the information holding kinetics observed in DHR. While the glutamate unbinding kinetics for GluN2B subunits is in the 300 to 500 ms range, the unbinding kinetics for GLUN2A subunits is in the 100 ms range (Erreger et al. 2005). Because the information storage time window for DHR is in the 300 to 500 ms range, I predict that DHR should be highly sensitive to subunit specific antagonists against the GluN2B subunit. Two well-documented GluN2B specific antagonists are available, Ifenprodil (Korinek et al. 2011) and Ro25-6981 (Fischer et al. 1997).

I first examined DHR in the absence and presence of the selective GluN2B antagonist Ro 25-6981 (0.5-1  $\mu$ M) (Korinek et al. 2011). Ro 25-6981 did not change the amplitude of the loading or read out pulses alone, but it was effective in reducing the potentiation that was observed when a loading pulse was given prior to the test pulse (Figure 3-4a,  $84 \pm 11\%$ ,  $n = 6$ ). In cells that are positive for DHR, I observed a large reduction in potentiation of the test pulse ( $79 \pm 4\%$ ,  $n = 3$ ).

To confirm our initial findings I used a second set of cells with reliable DHR and repeated this protocol with the GluN2B inhibitor ifenprodil (1-2  $\mu$ M)(Figure 3-4b). Ifenprodil did not change the baseline amplitude of either the control loading or read out

responses (Figure 3-4b). In cells that were positive for DHR, I observed a large reduction in potentiation of the test pulse ( $79 \pm 4\%$ ,  $n = 3$ ). There are no good GluN2A subunit selective antagonists. NVP-AAM077 preferentially blocks the GluN2A subunit at low concentrations (i.e. at  $50 \mu\text{M}$ ) (Frizelle et al. 2006).



**Figure 3-3. Kinetics of NMDA receptors containing GluN2A vs. GluN2B receptors.**

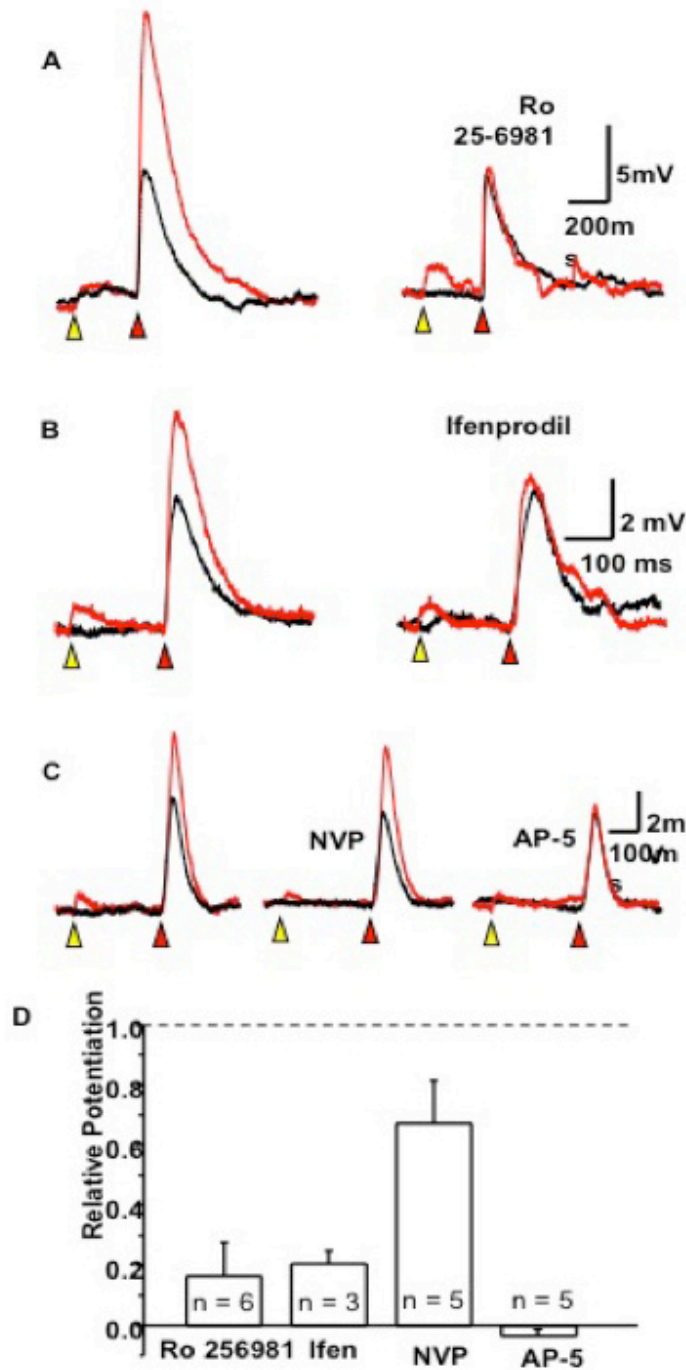
Under the protocol for eliciting DHR I examined DHR in the absence and presence of  $50 \mu\text{M}$  of NVP-AAM077 and found relatively small affect on the DHR potentiation ( $33 \pm 14\%$ ,  $n = 5$ ) (Figure 3-4c). These pharmacologic results suggest that GluN2B subunits which have the glutamate unbinding kinetics consistent with the

observed duration of information storage participate in DHR. The role of GluN2A is less certain because of the absence of highly selective antagonists. But the weak suppression of the DHR potentiation is consistent with the idea that is not an essential requirement.

### **3.3.3 DHR is sensitive to conditions that alter the $Mg^{2+}$ block of the NMDA receptor**

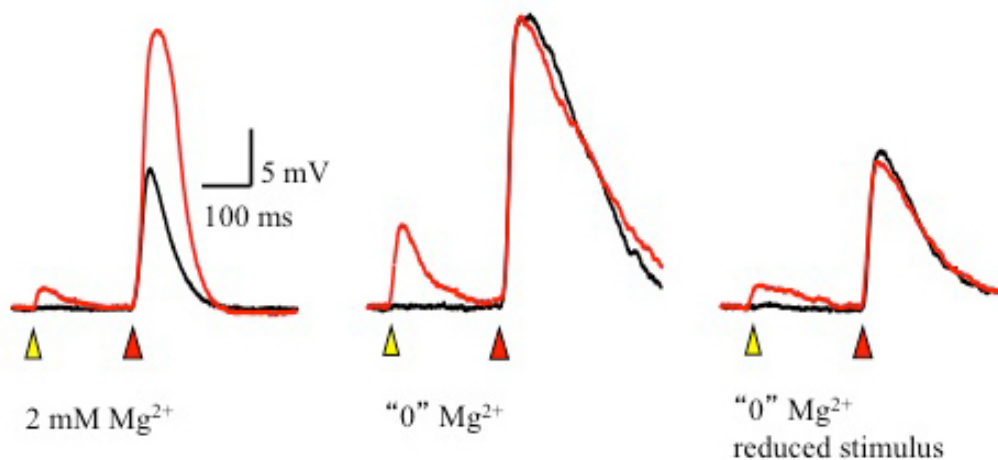
If DHR is mediated by the bound-but-blocked state of the NMDA receptor, DHR would be very sensitive to factors that affect  $Mg^{2+}$  block.

A key unique feature of the NMDA receptor is that at resting membrane potentials ( $\sim -70$  mV) it is blocked by an extracellular  $Mg^{2+}$  ion (Nowak et al. 1984; Westbrook and Mayer, 1984). Membrane depolarization is required to release the  $Mg^{2+}$  ion (Popescu & Auerbach 2004). DHR requires two key steps: 1) glutamate binds NMDA receptors that are resting in a glutamate-unbound and  $Mg^{2+}$  blocked state, 2) a subsequent depolarization releases the  $Mg^{2+}$  of the NMDA receptor therefore reading out dendritic compartments that are “loaded”. A key prediction of this model is that in an environment where the  $Mg^{2+}$  block of the NMDA receptor is reduced, DHR will not function. That is, without a “bound-but-blocked” state, there is no DHR.



**Figure 3-4: Role of NMDA GluN2A vs. GluN2B.** a) Left: Typical DHR experiment. Here, black trace is control trial, red trace corresponds to potentiation following loading pulse. Right: Addition of Ro 25-6981 reduced potentiation. b) Left: DHR control experiment. Right: Addition of GluN2B blocker ifenprodil blocked DHR. c) Left: DHR control. Middle: addition of GluN2A block NVP reduced but did not block potentiation. Right: NMDA receptor antagonist completely blocks DHR. d) Group data. Note that AP5 group data is from 5 cells in which AP5 was added after the normal DHR protocol. These cells were not treated with GluN2A or GluN2B blockers.

I predicted that lowering the extracellular  $Mg^{2+}$  concentration would prevent DHR from occurring. I first obtained read-out under normal conditions and confirmed the presence of DHR. Then, I changed the extracellular  $[Mg^{2+}]$  by washing the chamber with at least a ten-fold volume of nominally  $Mg^{2+}$ -free solution. Not surprisingly, the responses to the loading and read-out stimuli alone became markedly increased because of increased current flow through NMDA receptors in the absence of  $Mg^{2+}$ . But under these conditions the “loading” pulse had no effect on the “read-out” pulse as it did in “normal” (2 mM)  $Mg^{2+}$ . The intensities of the loading and read-out stimuli were then decreased, so that the corresponding depolarization matched those evoked in control saline (Figure 3-5). Nevertheless, potentiation of the test response following loading was still not observed ( $n = 4$ ). The DHR phenomenon thus requires extracellular  $Mg^{2+}$ .



**Figure 3-5:  $Mg^{2+}$  block of NMDAR is required for DHR.**

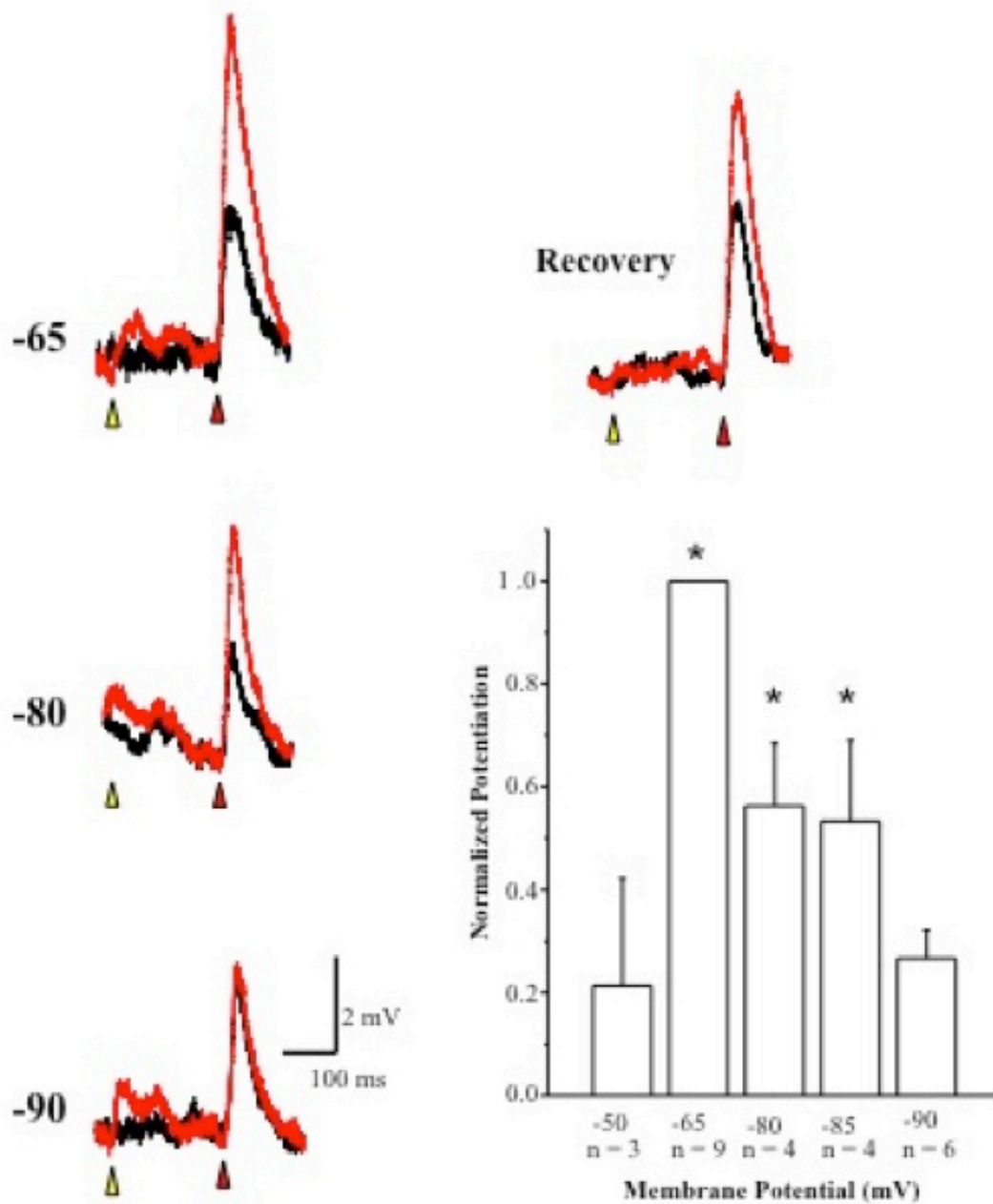
Decreasing the extracellular  $Mg^{2+}$  concentration results in a marked increase in the responses to both the “priming” and the “gating” stimuli (middle panel compared to left panel). Priming is no longer silent and potentiation could no longer be observed. Decreasing the strength of the stimuli to eliminate the possibility of response saturation does not rescue DHR (right panel). Responses to “gating” stimuli alone are shown in black and responses coupled to input stimuli are shown in red.

I next examined the voltage dependence of DHR. If DHR requires the  $Mg^{2+}$  bound-but-blocked state of the NMDA receptor, then DHR would not be possible at depolarized membrane potentials where there was a low likelihood of the bound-but-blocked state (Kampa et al. 2004). I predicted that holding the membrane at a depolarized potential would diminish the block of the NMDA receptor pore by external  $Mg^{2+}$ , and would thereby attenuate or abolish DHR. In addition, if the membrane were hyperpolarized, then it should be harder for the read-out stimulus to depolarize the dendrite sufficiently to activate DHR. Indeed, DHR was found to be maximal at membrane potentials between -65 and -70 mV. The degree of DHR potentiation decreased as the membrane potential was shifted from -65 mV to -55 mV or to -90 mV (Figure 3-6). Because it was not possible to achieve accurate voltage control of the thin oblique dendrites in these electronically complex neurons, these voltage dependence data serve as a semi-quantitative characterization of DHR. The dependence of DHR on extracellular  $Mg^{2+}$  and membrane voltage constitutes biophysical evidence that  $Mg^{2+}$  block of NMDA receptors is critical for DHR. That is, the bound-but-blocked state of the NMDA receptor is key for DHR.

### **3.3.4. DHR is not influenced by the clearance of glutamate**

If the information storage time window of DHR is mediated by the kinetics of glutamate unbinding, then it should follow the glutamate clearance kinetics.

If the dual gating of the NMDA receptor is a key component of DHR, then the time course of DHR would be consistent with the unbinding of glutamate from the

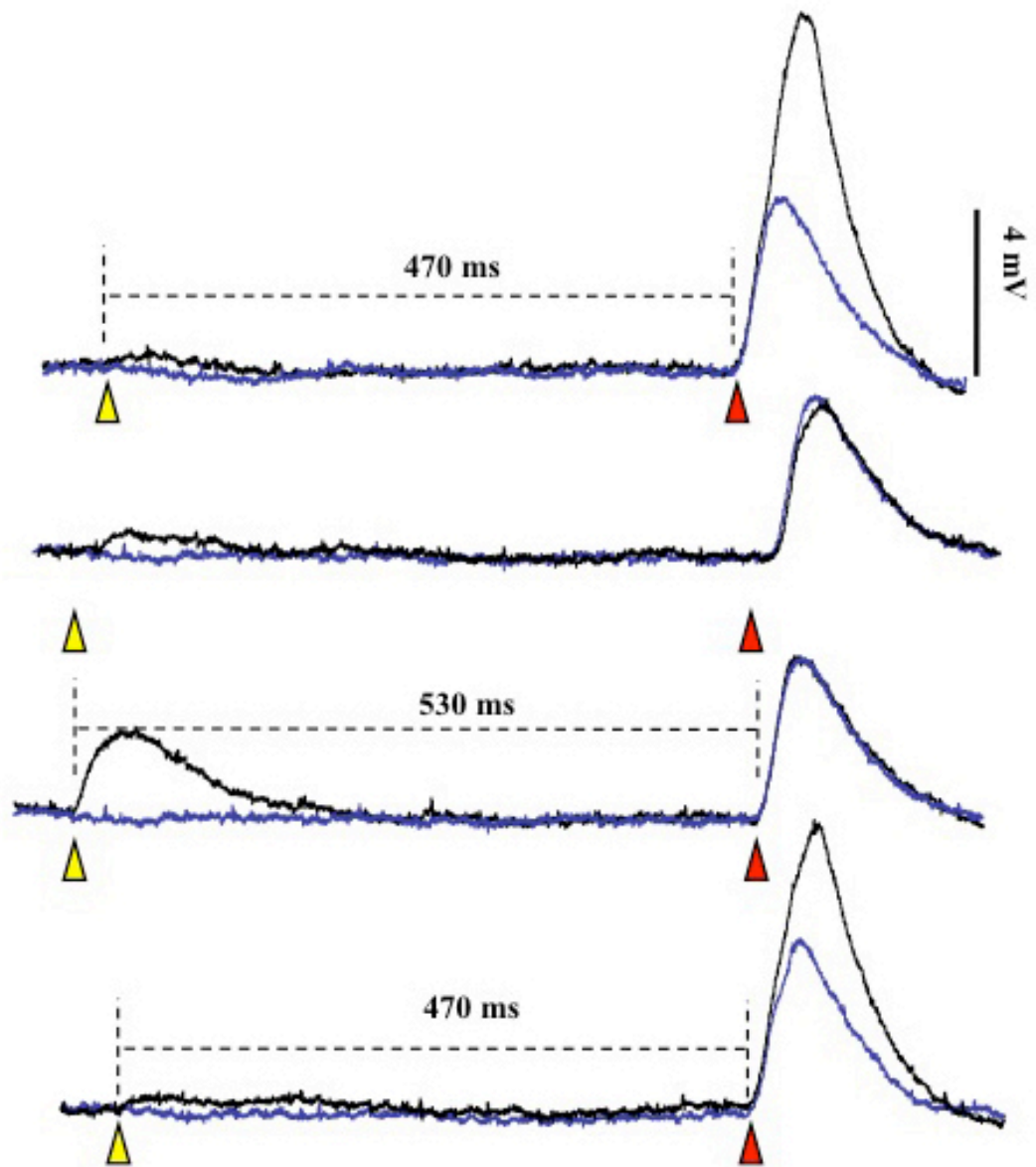


**Figure 3-6. Voltage dependence of DHR.** DHR is voltage dependent. Potentiation is strongest near -65 mV (group data, bottom right panel). At hyperpolarized (-80 mV, -90 mV) potentials DHR is reduced. At -50 mV (depolarized, reduced likelihood of  $Mg^{2+}$  block) DHR is also inhibited (group data, bottom right panel).

NMDA receptor. Does the rate of glutamate clearance have any influence on the duration of the temporal window for DHR? The temporal window for DHR, 400 to 500 ms at room temperature, is much longer than the time required for clearance of synaptically released glutamate (Diamond 2005). The glutamate transporters in the CA1 region were also estimated to maintain a baseline glutamate concentration of only 25 nM (Herman & Jahr 2007). Nevertheless, I specifically tested this hypothesis by varying the amount of glutamate released during the loading pulse. I hypothesized that if glutamate clearance was an important determinant, then the width of the temporal window of potentiation should depend on the amount of glutamate released.

DHR was first evoked using 470-ms ISI between the loading and read-out stimuli (first trace, Figure 3-7). When the ISI was increased by 60 ms to 530 ms, no potentiation of the test pulse was observed. Increasing the photolysis intensity of the loading stimulus by 3-fold led to an increase in the amplitude of the loading response, but did not cause potentiation to occur with the 530-ms ISI (second and third traces, Figure 3-7). Decreasing the ISI back to 470 ms restored the original positive DHR response, indicating that the failure to detect DHR was not due to a deterioration of the cell (Figure 3-7, bottom trace). Similar results were obtained in five other cells.

Another approach I used to determine if glutamate spillover might impact DHR was to ask whether increasing the duration of the “loading” pulse in cells negative for DHR would result in potentiation of the “read out” pulse. I would predict that if DHR is merely a result of excess glutamate, then I would observe DHR with sufficiently large “loading pulse” intensities. To test this, I isolated cells in which DHR was not observed. I then increased the duration of the loading pulse such that a large depolarization (2-5

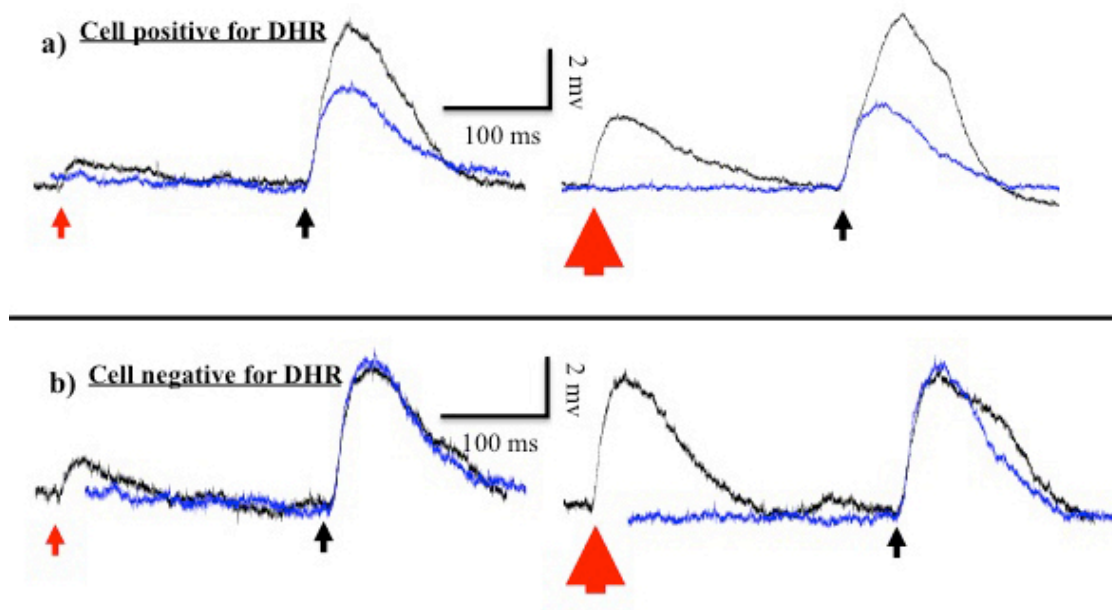


**Figure 3-7. Increasing glutamate released does not rescue DHR.**

Temporal window for DHR is unlikely to be related to glutamate clearance. The cutoff in ISI for positive DHR responses is typically abrupt. Increasing the ISI from 470 to 530 ms led to a complete negative DHR (upper panel vs second panel). Increasing the amount of glutamate released by 3-fold failed to rescue DHR. But decreasing ISI back to 470 ms was able to rescue DHR. The high sensitivity to ISI and poor sensitivity to the amount of glutamate released argues against glutamate clearance as the main determinant of the temporal window for DHR.

mV) was observed. In cells where I did not observe DHR (n=10) using our normal protocol, I could not drive the occurrence of DHR by flooding the synapses with large amounts of glutamate (Figure 3-8).

I conclude that the duration of DHR is determined by the unbinding of glutamate from the primed NMDA receptors rather than by the clearance of glutamate released during the loading pulse.



**Figure 3-8. Increasing glutamate released does not rescue DHR.** a) Left: Typical DHR experiment (1 ms prepulse, 200 ms ISI), blue trace is read-out only. Black trace is pre-pulse + readout and see large potentiation. Right: Increased pre-pulse duration (5ms prepulse, 200 ms ISI) does not effect DHR. b) Left: Cell which was negative for DHR. Right: increasing loading pulse duration is not sufficient to cause DHR.

### 3.4 Discussion

NMDA receptors are unique. They are more permeable to  $\text{Ca}^{2+}$  than to monovalent cations (Mayer and Westbrook 1987), they display slow deactivation kinetics on the order of 100's of ms (Spruston et al. 1995) and they require dual gating

by both a ligand and subsequent depolarization for the channel to conduct cations. NMDA receptors have been shown to play a key role in learning and memory (Bliss and Collingridge 1993), neurodevelopment, behavior, and a variety of neuropathologies (Thompson et al. 1996).

Dual gating of the NMDA receptor suggests that it is a coincidence detector for pre-synaptic glutamate release and post-synaptic membrane depolarization. NMDA receptor activation is involved in mechanisms of information storage including short- and long-term potentiation (Bliss and Collinridge 1993). Bekkers and Stevens (1990) suggested that an undiscovered form of short-term information storage might occur on this time scale. In their model, glutamate “information” was held in the bound-but-blocked of the NMDA receptor. Due to technical limitations of methodologies at the time they and others were unable to test their hypothesis.

Our lab first described a novel form of short-term information storage on dendrites of CA1 pyramidal neurons. The time course of the phenomenon, which I called DHR, was very similar to the deactivation kinetics of the NMDA receptor.

Here, I have rigorously tested predictions that would support the hypothesis that the information holding step in DHR is the glutamate-bound but  $Mg^{2+}$  blocked state of the NMDA receptor. First, I tested whether functional NMDA receptors were required for DHR. If NMDA receptors are not critical to DHR, then I would predict that antagonists of the receptor would have little effect on DHR. On the contrary, I find that DHR will not function in the presence of NMDA receptor antagonists.

Key to our hypothesis is that the “bound-but-blocked” state of the glutamate receptor is the information-holding step in DHR. To test whether the  $Mg^{2+}$  block is

required for DHR, I reduced the extracellular  $Mg^{2+}$  concentrations therefore reducing the probability that NMDA receptors were in the “bound-but-blocked” receptor state. Under these conditions I was unable to reproduce DHR. These findings suggest that DHR requires the  $Mg^{2+}$  bound state of the receptor.

The  $Mg^{2+}$  block of the NMDA receptor is also sensitive to fluctuations in membrane potential (Ascher et al. 1988). Under hyperpolarized conditions, the extent of the  $Mg^{2+}$  block is increased whereas depolarization relieves the  $Mg^{2+}$  block. In our model of DHR, under depolarized membrane potentials I would predict that DHR would be blocked. As with the “zero”  $Mg^{2+}$  study, I observed a block of DHR at depolarized ( $Mg^{2+}$  block-free) membrane potentials. Interestingly, under hyperpolarized membrane potentials I was also unable to reproduce DHR. It is likely that at very hyperpolarized membrane potentials the read-out pulse was insufficient to depolarize the cell sufficiently to free the receptor from the  $Mg^{2+}$  block, therefore preventing DHR. An alternate approach would be to patch the dendrites of CA1 pyramidal neurons to precisely control the resting membrane potential in apical dendrites.

I also considered the influence of other glutamate receptors. mGluRs are known to play a role in other forms of short term and long-term (Bear and Malenka, 1994) memory. I tested the hypothesis that mGluRs contribute to DHR which might indicate that a second messenger signaling pathway is required for DHR. Here I find that a non-specific antagonist to mGluRs did not affect DHR.

An alternate hypothesis for the DHR model is that delayed glutamate clearance from the synapse results in the ISI associated with DHR. The temporal window for

DHR (100's of ms) is significantly longer than known synaptically released glutamate clearance (Diamond 2002; Diamond 2005). I am using a novel glutamate uncaging system and have not measured actual glutamate release but rather rely on electrophysiological responses. To test the hypothesis that glutamate spillover results in DHR I performed two key experiments. First, I show that in a DHR positive cell, I am unable to increase the “loading pulse-read-out pulse” ISI to prolong DHR. I show in a second set of experiments that increasing the intensity of the “loading pulse” does not rescue DHR in a DHR-negative cell. Together, these findings allow us to reject our alternate hypothesis and provide support for the hypothesis that DHR is mediated by a mechanism other than glutamate spillover.

### **3.4.1 Summary:**

Based on this data I can conclude that:

1. The molecular substrate for DHR is the NMDA receptor with specific involvement of the GluN2B subunit. I can't exclude the involvement of other subunits.
2. The biophysical basis of DHR is the transitioning into and out of the bound-but-block state of the NMDA receptor.
3. The time window of information storage is determined by the unbinding rate of glutamate from the NMDA receptor, rather than the clearance of glutamate from the extracellular space.

The NMDA receptor may be modulated by a variety of other agonists or physiological conditions including glycine,  $Zn^{2+}$ , histamine, PH, neurosteroids, and a

variety of enzymes (McBain and Mayer 1994). The role of each of these potential neuromodulators in DHR remains to be examined. For instance, the GluN2B containing NMDA receptors are selectively potentiated by histamine (Williams 1994). I would predict that histamine could rescue cells in which DHR was inhibited by low concentrations of ifenprodil, whereas in cells where DHR was not observed I would see little effect. If histamine does not affect DHR it is likely that a population of NMDA receptors containing the subunit is active during DHR.

These findings suggest that DHR is a novel NMDA-mediated form of short-term information storage on terminal dendrites in area CA1 of the hippocampus. I have provided strong evidence for the non-conducting state of the NMDA receptor (“bound-but-blocked”) as the key information-holding step in DHR. To our knowledge this is the first demonstration of the NMDA receptor dual gating as a mechanism for information storage and provides experimental evidence for a principle first described by Bekkers and Stevens (1990).

**Chapter IV**  
**The physiology of DHR**

## 4.1 Introduction

The ability of a neuron to store information over brief periods of time is essential for signal processing and working memory, as well as long-term synaptic plasticity (Goldman-Rakic 1995; Ganguli & Latham 2009). The basic model of Hebbian plasticity implies that when two cells “fire together” they “wire together” (Hebb 1949). Subsequent work has shown that while Hebb’s original postulate holds true, the nature of the “fire” and “wire” can be rather complex. For instance, a particular form of short term plasticity, STDP, functions on a very fast time scale (0 up to ~50 ms (Markram et al. 1997; for further review see Dan and Poo 2004; Zucker and Regehr 2002).

In STDP pairing of a presynaptic release of neurotransmitter with a postsynaptic depolarization can lead to bidirectional modulation of synaptic efficacy (Markram et al. 1997; Debanne et al. 1998). When a presynaptic event occurs before the depolarization then a potentiation of the synaptic event is seen (Magee and Johnston 1997). If the postsynaptic depolarization precedes the synaptic transmitter release then a depression of synaptic activity is observed (Mu and Poo 2006). The nature of the postsynaptic depolarization is thought to be in the form of a local  $\text{Na}^+$  spike as a consequence of a back-propagating action potential (bAP)(Gasparini et al. 2007), though other studies have implicated dendritic  $\text{Ca}^{2+}$  spikes (Kampa et al. 2007).

A fundamental feature in STDP is that potentiation is linear as compared to inter-stimulus-interval (ISI) duration (Zhang et al. 1998). As the ISI is increased, the

level of potentiation decreases linearly until no potentiation is observed with the pairing (Zhang et al. 1998).

I have described a novel form of short-term information storage with some characteristics that are similar to both PPF and STDP. To this point I have shown that DHR at the molecular and biophysical level is mediated by the bound-but-blocked state of the NMDA receptor and is not a result of glutamate clearance. In our DHR protocol the loading and read-out pulses are spatially distinct, but I do not know if there are spatial limitations to DHR. I also do not know if, like STDP, DHR requires activation of the same synapses, or if the read-out is completely independent of the location/nature of the loading. Further, the question remains as to whether DHR is a graded phenomenon (like STDP) or if it is all-or-none, which could have profound implications for DHR in working memory.

Here I will examine three issues relating to DHR at the cellular and systems level. (1) What is the morphologic substrate for DHR? (2) Should the read-out of DHR be viewed as an analogue signal like a post-synaptic potential or should it be viewed as a discrete signal like a somatic action potential? (3) Is DHR just an artificially produced phenomenon by some artifact of the photorelease of glutamate or is it physiologically relevant?

The synapse is considered by many to be the elementary functional unit for signaling between neurons. The synapse is also considered the primary locus for plasticity involved with learning and memory (Bailey and Kandel 1993). Recently, individual dendritic branches have been introduced as fundamental functional units of excitability in the nervous system (Branco and Hausser 2010). They argued that it is the

basic organizational unit for integrating synaptic input and implementing synaptic and homeostatic plasticity. Our observations of DHR complement this idea. Specifically, I postulate that:

1. The individual terminal dendritic branch serves as the functional unit of the DHR response.
2. DHR is a highly compartmentalized phenomenon and functions in an all-or-none manner
3. DHR is a physiological phenomenon.

I will show that DHR occurs only on individual terminal branches, that DHR is not graded and in fact is “all-or-none” and finally that DHR can be obtained with endogenous glutamate release. Taken together these results support the hypothesis that DHR is a novel, physiologically elicited, form of short term plasticity.

## **4.2 Methods**

### **4.2.1 Hippocampal slice preparation and electrophysiological recording.**

Methods as described in General Methods. All data acquired with acute slices at physiological (32-34 degrees) temperature unless otherwise noted.

### **4.2.2 Synaptic stimulation of stratum Radiatum.**

For two sets of experiments I introduced electrical stimulation of the S. Collateral pathway in area CA3/CA1 of the hippocampus. I placed a bipolar concentric electrode in s. radiatum to stimulate the SC pathway in either area CA3 (Figure 4-6) or near the dendrite of interest in area CA1 (Figure 4-7). Current was injected into the S.C. pathway until a small AMPA receptor mediated EPSP was observed. In both sets of experiments, I used the output of the cell as recorded by an EPSP to standardize to the pEPSPs observed in previous experiments (Chapter 3).

### **4.3 Results**

#### **4.3.1 - Individual terminal branch serves as a functional unit for DHR.**

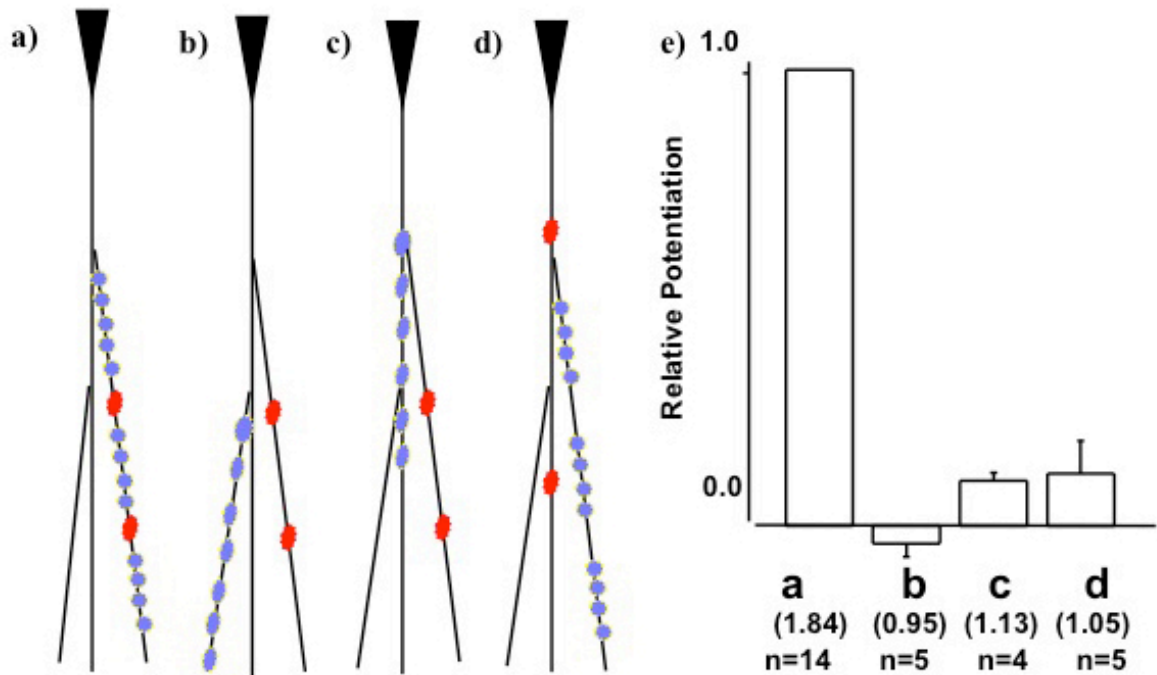
I predict that positive DHR response can be produced if the loading and read-out signal arrive on the same terminal branch, but not if they are on different branches.

Dendritic compartmentalization is important for the summation of incoming information (Wei et al. 2001). The question remains as to whether DHR is a branch specific phenomenon, or whether it is possible across branches, on the soma or apical trunk. To address the issue of the spatial characteristics of DHR, I first confirmed that a particular cell was positive for DHR (Fig 4-1a) by releasing a brief pulse of glutamate over the length of a dendrite to load NMDA receptors and then delivered a second, larger pulse of glutamate to depolarize the dendrite and effectively read-out the original information. I first confirmed DHR on a specific terminal dendrite (Figure 4-1a). I then moved the spot of the pre-pulse to a neighboring dendrite (Figure 4-1b) or the main

apical trunk (Figure 4-1c). In both cases, no potentiation was observed relative to the initial potentiation seen in Figure 4-1a (see group data in Fig 4-1e). These results suggest that the source of glutamate for loading a dendrite for readout must be very near or on the dendrite in which the read-out occurs. Finally, I asked whether glutamate that was released onto an oblique dendrite could be read-out by a depolarization to the main apical trunk in the form of glutamate release on the trunk. In this set of experiments, again, DHR was not observed (Fig 4-1d). I conclude from this data that DHR is a branch-specific phenomenon that requires loading and read-out to occur over the same dendritic compartment.

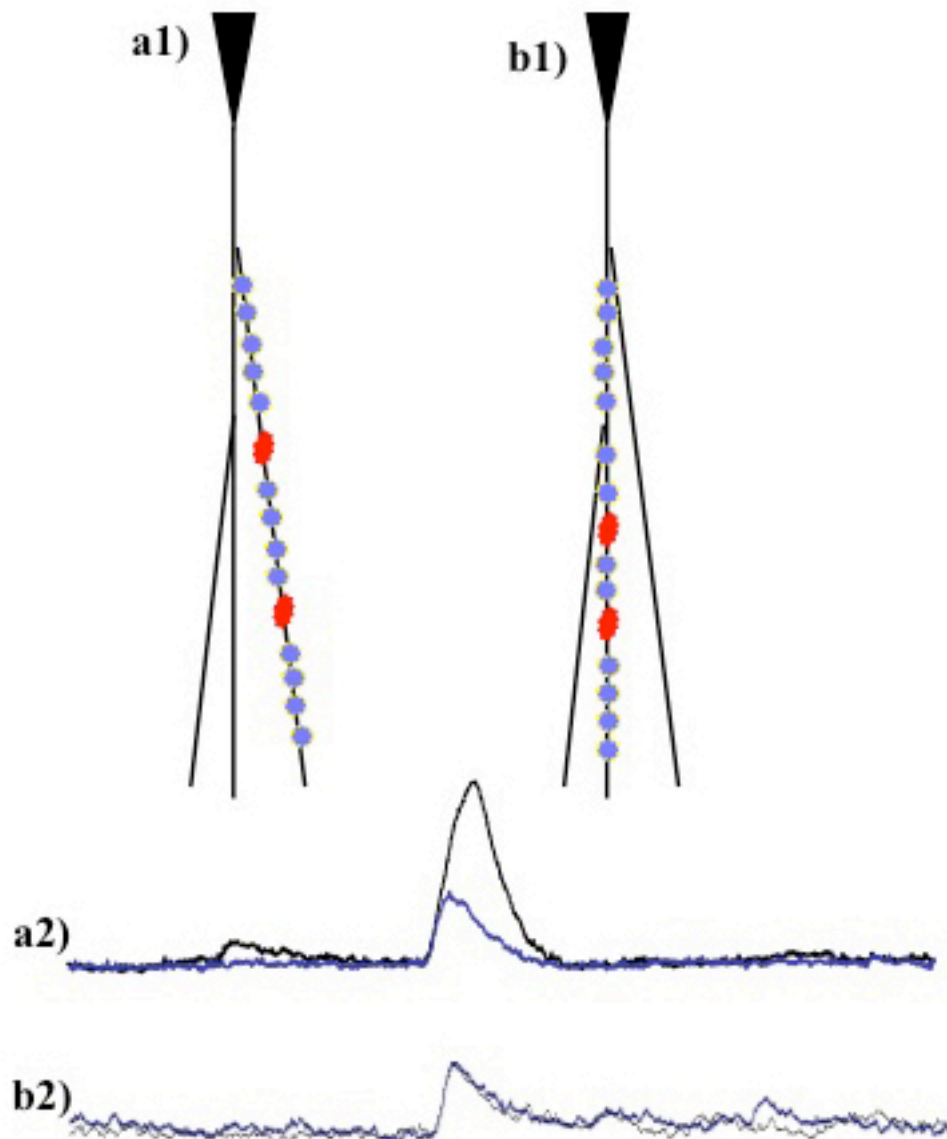
All previous experiments were done on thin, oblique dendrites of CA1 pyramidal neurons. While these contain the highest concentration of NMDARs on the pyramidal neuron, there are NMDARs on the apical trunk and soma (Rao and Craig 1997). If DHR only requires the bound-but-blocked state of the NMDRA, then it should be possible to observe a DHR-like phenomenon on both the apical trunk and the soma.

To test this hypothesis, I began by placing both the loading and the test pulse on the main apical trunk. I found that in cells positive for DHR on the oblique dendrites, DHR was not seen on the main apical trunk (Figure 4-2). One reason for this may be that the apical trunk lacks the high density of NMDA receptors as well as the characteristics of an electrical compartment that is seen on thin terminal dendrites. An alternate approach would be to obtain a patch clamp recording in the dendrite to better control local dendritic voltage.



**Figure 4-1. Positive DHR responses occur only within individual dendritic electrical compartments.** a) Typical spatial pattern for DHR loading (blue) and readout (red) on a single apical dendrite shows robust potentiation. (b-d) failed to show potentiation with comparable stimulation intensities in the same cell when b) loading is moved to a nearby dendrite, c) loading is on the apical trunk, d) read-out is on the apical trunk. e) group data showing that DHR requires a single dendrite compartment.

I propose that DHR is distinct from STDP in that the loading and readout in DHR do not have to occur on the same synapses. To test this hypothesis, I placed the loading pulse ROIs  $> 25\mu\text{m}$  from the read out pulses and asked whether DHR could be initiated. I observed that under these conditions I were still able to see potentiation of the read-out response when loading had occurred on the same branch. That is, DHR does not require loading and readout to occur near each other as long as they occur on the same terminal branch (Figure 4-3). This particular experiment was expanded upon by a post-doctoral researcher in the lab and has been confirmed in 5 cells with a spatial separation between loading and readout of  $>50\ \mu\text{m}$  (Santos et al. 2012).

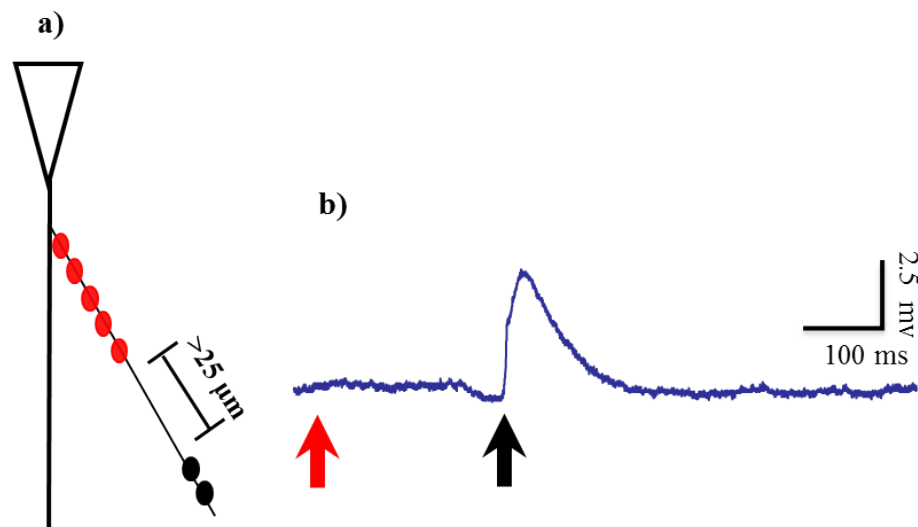


**Figure 4-2. No DHR on apical trunk.** a1) Typical spatial pattern for DHR loading (blue) and readout (red) on a single apical dendrite shows robust potentiation (a2). b1) Loading and read-out on the apical trunk fails to elicit DHR (b2). This was true in 2 cells in which it was tested.

**4.3.2 The read-out of information in DHR is fundamentally an all-or-none process.**

I predict that the amplitude of the DHR response would remain largely all-or-none as the input intensity and the ISI are gradually changed.

Dendritic integration is generally considered to be an analogue process, whereas the generation of the somatic action potential is considered a digital or all-or-none process. Digital transmission optimizes the signal-to-noise ratio of network output because it is easier to distinguish a well-defined signal against the background noise. Is there reason to believe DHR is a digital signaling process on the dendritic arbor where signaling proceeds mainly in a graded manner?



**Figure 4-3. DHR does not require repeated stimulation of same synapses.** a) Cartoon drawing of placement of the loading pulse on the dendrite at a location at least  $25 \mu\text{m}$  away from the read-out pulse. b) Black trace shows the read-out pulse alone. Red trace shows a prepulse (subthreshold) on  $\frac{1}{2}$  of the length of the dendrite followed by the same read-out pulse (200 ms ITI). Note that the potentiation that we call DHR is still present ( $n=2$ ).

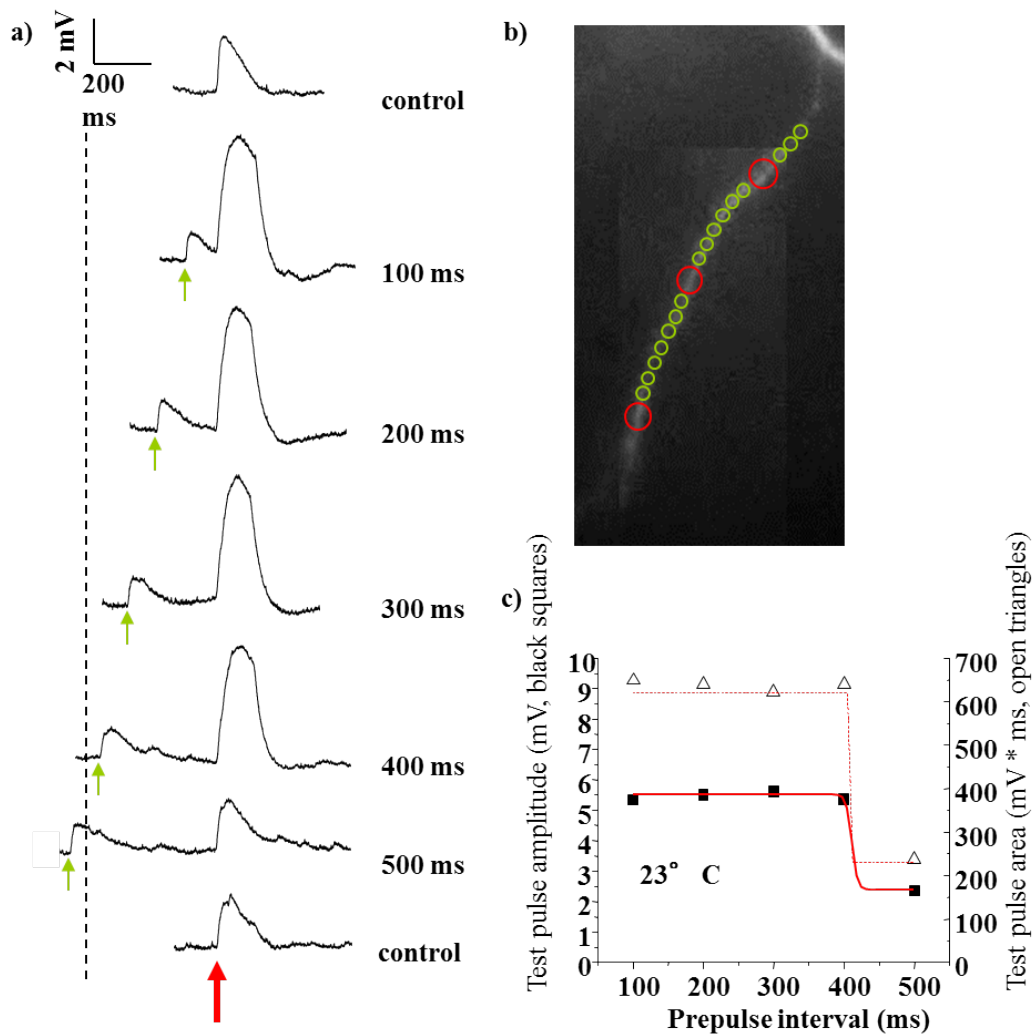
A key difference between DHR and PPF is on the basis of time course. Whereas PPF generally occurs in the 10's of ms range (Dan and Poo 2004) I generally used an interval of 200 ms for DHR (Chapter 3). Glutamate unbinding from the NMDAR depends on receptor subtype composition (Dzubay and Jahr 1996).

NMDA receptors open slowly compared to other ionotropic receptors and stay open long after (50-500 ms) glutamate has cleared the synaptic cleft (Clements et al. 1992). If DHR is an all-or-none phenomenon, then plotting a time course of magnitude of DHR ISI would reveal a supralinear component.

I first asked whether the history of glutamate binding to NMDARs as represented by the loading input signal could be retrieved at a later time by a separate gating signal. Our original work on this topic utilized organotypic slice culture (General Methods). Indeed, as long as the glutamatergic input was applied within 500 ms of the gating signal, I observed an unequivocal potentiation of the “read-out” response to the gating stimulus (black traces vs. gray control traces, Figure 4-4). When the loading input and the gating signal were separated by  $> 500$  ms, the read-out response showed no detectable change from the control responses. These results were consistent in dendrites on acute slices at room temperature (22°C)(Figure 4-5).

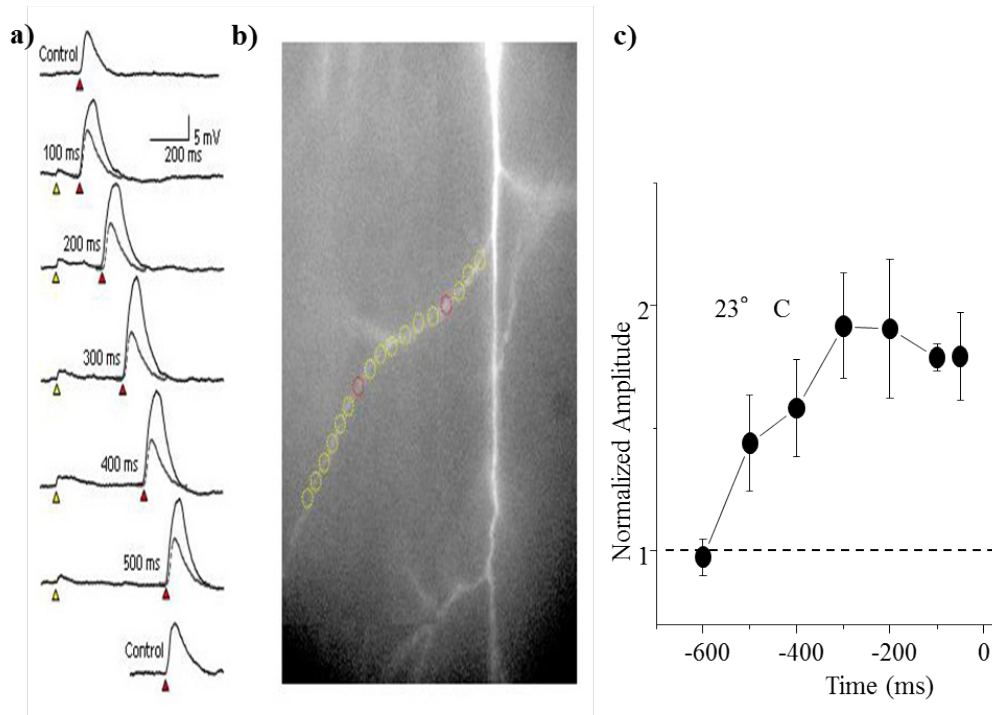
The majority of our data was acquired under physiological conditions (32-35°C) in acute slice. In Figure 4-6a I show that under these conditions the ISI window that was optimal for DHR was between 10-400ms. The responses of a single dendrite at varying ISIs is illustrated in Figure 4-6c (red triangles) and are plotted together with the group data (black circles). Potentiation could be observed with ISIs as brief as 10 ms and as long as 500 ms at 22°C. The responses from 42 dendrites are plotted in Figure 4-

6c. It is important to note that the maximal ISI at which there was still potentiation differed between dendrites. This may be due to variability in the number of GluN2A vs. GluN2B subunits (Figure 3-5 shows the relative contribution of each NMDA receptor subunit).



**Figure 4-4. DHR in organotypic slice culture at room temperature.** a) Example of the role of varying ISI in DHR output. b) The information containing input is experimentally simulated using weak photolysis of caged glutamate (yellow circles) to create spatial distributions of “bound but blocked” NMDARs over a dendritic branch. Representative location of the stronger photolysis induced AMPA-mediated ‘gating’ stimuli is shown in red. c) The amplitudes of ‘read-out’ responses as a function of input intensity show a step-like behavior, consistent with behavior of regenerative spikes. Black squares show test pulse amplitude, open triangles show test pulse area.

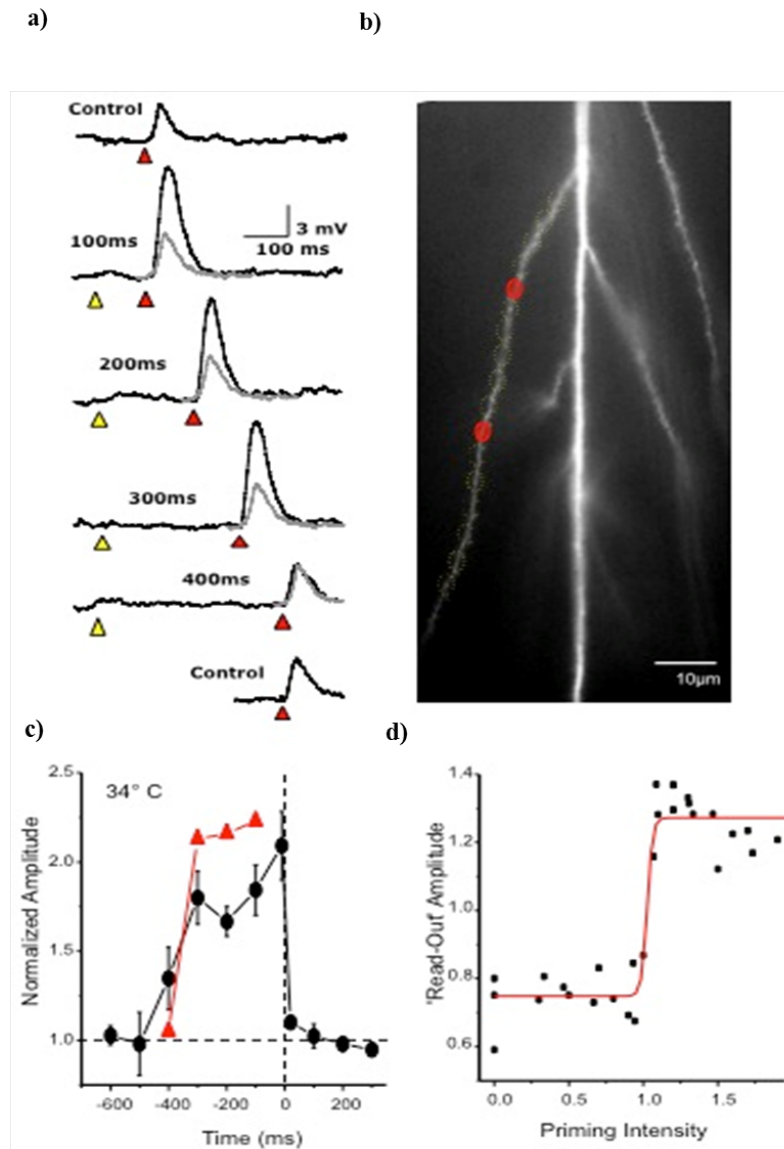
Most forms of short-term plasticity, such as paired-pulse facilitation (PPF), are expressed in a graded manner, typically decaying exponentially as a function of increasing ISI (Zhang et al. 1998).



**Figure 4-5. DHR in acute slice, room temperature.** a) DHR in acute slice at room temperature robust at ISI's up to 500ms. b) The information containing input is experimentally simulated using weak photolysis of caged glutamate (yellow circles) for loading and intense photolysis of glutamate (red circles) for read-out. c) Group data (n=9)

In DHR, the magnitude of the potentiation of the test pulse shows a non-exponential dependence on the interval between the loading and test stimuli: the amount of potentiation remains constant (Figures 4-4c, 4-5c, 4-6d.) What advantage might the all-or-none behavior benefit DHR? It would make sense if one considers DHR as a short-term memory mechanism. One of the fundamental problems in neuroscience is distinguishing signal from noise. The same problem exists for memory recall at the behavioral level. A regenerative dendritic spike amplifies the signal and clearly sets it

apart from noise in both amplitude and duration.



**Figure 4-6. All or none nature of DHR under physiological conditions.** a) Example of the role of varying ISI in DHR in acute hippocampal slice at 32-35°C . b) The information containing input is experimentally simulated using weak photolysis of caged glutamate (yellow circles) to create spatial distributions of “bound but blocked” NMDARs over a dendritic branch. Representative location of the stronger photolysis induced AMPA-mediated ‘gating’ stimuli is shown in red. c) Super-threshold levels of primed NMDARs can be conditional “read-out” in the form of a potentiated response (black vs gray traces) to the gating signal if the input and gating signals were separated by <300 ms. d) Group data of potentiation as a function ISI from 42 dendrites at 32-35°C is shown in black. Responses at different ISIs of a single dendrite are shown in red. e) The amplitudes of ‘read-out’ responses as a function of input intensity show a step-like behavior, consistent with behavior of regenerative spikes.

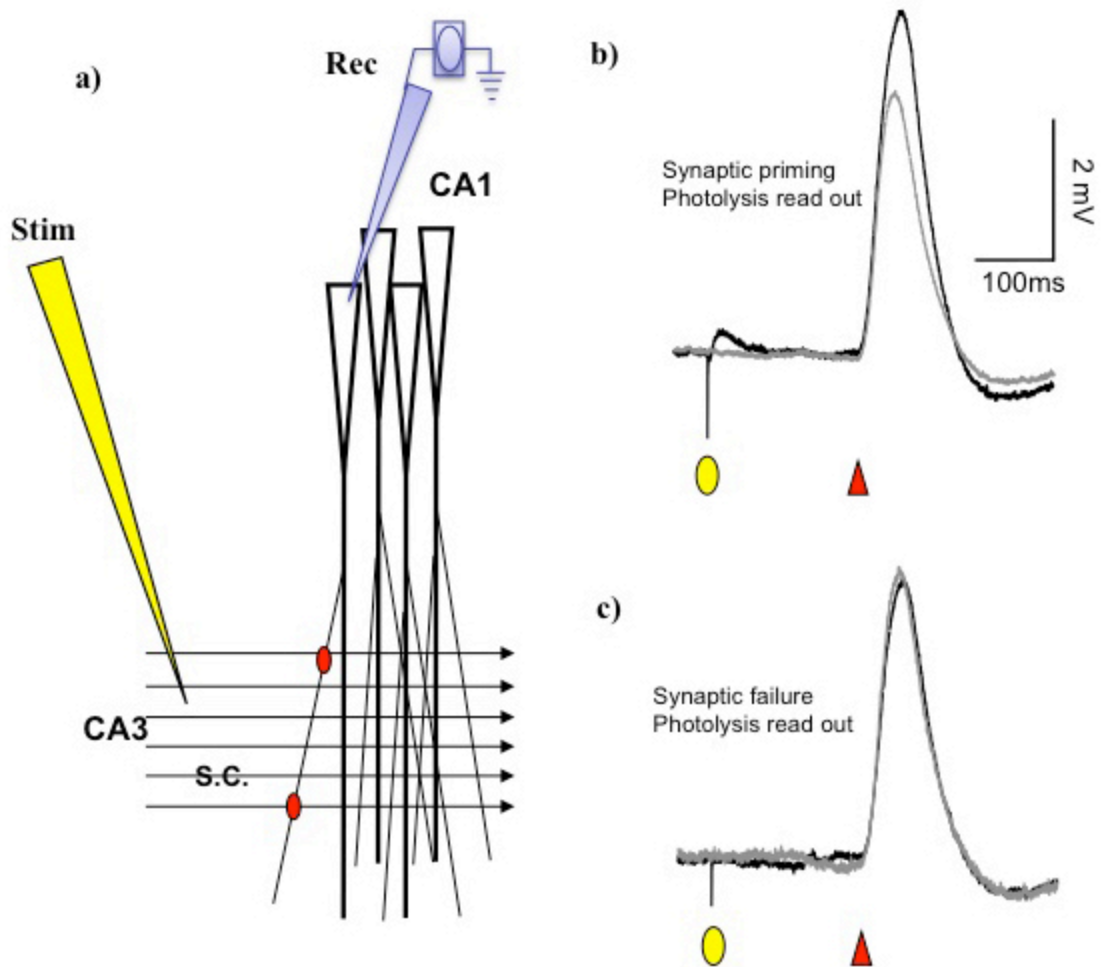
### 4.3.3 DHR is a physiological phenomenon

I predict that DHR can be produced with synaptic stimulation.

Based on the previous finding that hippocampal dendrites could be loaded with glutamate released photolytically, I asked whether endogenous glutamate release would be sufficient to load the dendrites. Stimulation of the SC in area CA3 of the hippocampus activates a network of excitatory axons in area CA1. If DHR has a physiological correlate, then glutamate released endogenously could be stored and read-out in the same manner. I therefore asked whether electrical stimulation of the SC pathway in area CA3 would be sufficient to load a cell for read-out in a DHR dependent manner.

For this first set of experiments I placed a bipolar concentric stimulating electrode within the SC pathway in area CA3 (Figure 4-7a). To prime target dendrites using synaptic stimulation, the extracellular stimulating electrode in the Schaffer collateral was moved farther away from the target dendrite and the stimulus intensity was increased so that more synapses on the targeted branch would be activated. In Figure 4-7b (black trace), the timing of the electrical stimulation is marked by the yellow circle. Focal photolysis was used for the read-out depolarization in this configuration (red arrow, 4-7c). Responses of the same dendrite to ten consecutive pairings of the synaptic loading and photolysis induced read-out were averaged (black trace, Figure 4-7c). Synaptically evoked loading resulted in a  $30 \pm 14\%$  ( $n = 5$ ) potentiation of the read-out response. When the electrical stimulation failed to elicit any detectable EPSPs, no potentiation was observed (lower panel, Figure 4-7c). Our

observation of the DHR phenomenon using electrically evoked synaptic potentials as either the loading or the read-out stimulus allows us to conclude that DHR is not simply an artifact of photostimulation.



**Figure 4-7. Synaptically released glutamate loading for DHR.** a) The extracellular stimulating electrode (yellow) is placed far away when simulating a diffuse input signal. b) Example of DHR using field stimulation as the ‘input’ signal. Priming of NMDARs by synaptically released glutamate via polysynaptic pathways can not be excluded under these experimental conditions. Upper panel illustrates the average of 10 individual responses. Lower panel illustrates the average of 2 responses without evidence of electrically evoked EPSPs and is used as internal controls.

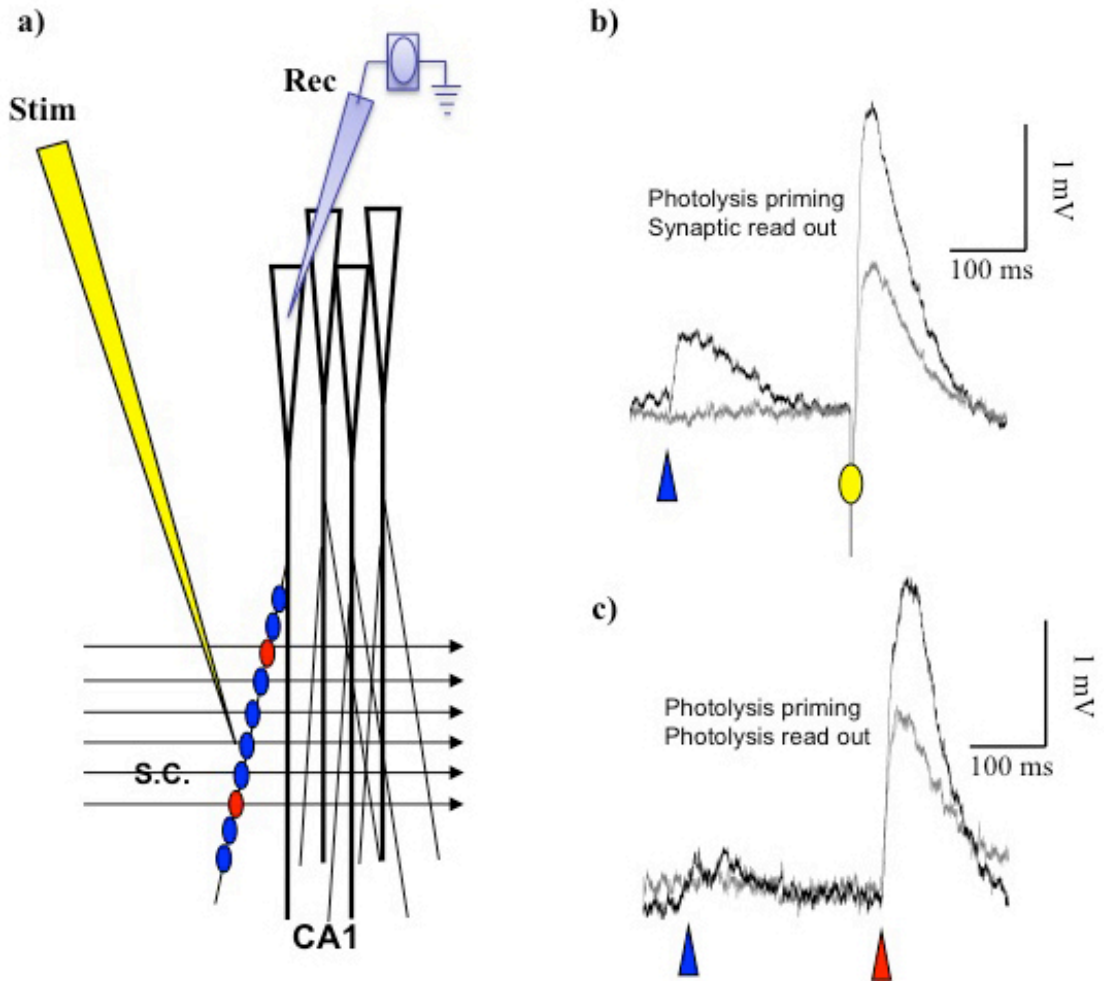
In a second set of experiments I asked whether synaptic loading of a dendrite with endogenous glutamate could be read out by photolysis of glutamate. To load a

dendrite with synaptically released glutamate I placed an extracellular stimulating electrode positioned within Stratum radiatum in close proximity (10 – 20  $\mu\text{m}$ ) to an oblique dendrite of the recorded CA1 neuron (Figure 4-8a). The stimulus intensity was adjusted to evoke a depolarization comparable to that evoked by photolysis (1-4 mV) (grey trace, yellow circle, top panel of Figure 4-8b). Photolysis of caged glutamate was used to prime the length of the targeted dendrite (black trace, blue arrow). Synaptically evoked read-out response was increased by  $40 \pm 17\%$  ( $n = 5$ ) if evoked 200 ms following the loading stimulus. The control and test traces in the top panel of Figure 4-8b were the average of 6 consecutive responses. Comparable potentiation was observed when photolysis was used as the read-out stimulus in the same dendrite (lower panel, Figure 4-8c).

#### **4.4 Discussion**

Paired-pulse facilitation, or PPF, occurs when a presynaptic neuron receives a small depolarization that acts to increase the amount of calcium in the presynaptic terminal (Katz and Miledi 1968; Zucker 1989). This calcium helps recruit a significant amount of bound vesicles on the presynaptic terminal thereby increasing the number of quanta of glutamate available for release (Schulz et al. 1995). When a second presynaptic depolarization occurs within 10's of milliseconds, the probability of release of neurotransmitter is significantly increased and the subsequent depolarization (which is of the same amplitude as the first depolarization) results in a much larger EPSP as

recorded in the post synaptic neuron. PPF is therefore generally considered a presynaptic phenomenon.



**Figure 4-8. Endogenous glutamate as a trigger for readout.** a) The configuration for synaptic stimulation. The extracellular stimulating electrode (yellow) is placed in close proximity to the dendrite when simulating the “gating” signal b) An example of DHR elicited from synaptically-evoked ‘gating’ stimuli and photolysis-evoked input (averages of 6 control and test traces). Responses of the same dendrite to photolysis-evoked gating and input stimuli are shown in the lower panel.

#### **4.4.1 The individual terminal dendritic branch serves as the functional unit of the DHR response**

A key difference between DHR and PPF is the morphologic substrate for their elementary unit of excitability. For PPF it is individual synapses. For DHR it is functional electrical compartments on the dendritic arbor. Electrical compartmentalization is determined by a combination of factors such as dendritic morphology, active intrinsic conductances, and the pattern of synaptic inputs. Assuming a diffuse pattern of synaptic excitation, the input impedance mismatch at the junction of the thin terminal dendrites to the thick apical trunk would suggest that the compartments are likely to comprise individual terminal dendrites (Wei et al. 2001). I have shown here that DHR is only observable when the loading and read-out occur on the same terminal branch.

#### **4.4.2 DHR is “all-or-none” in nature**

DHR is superficially similar to PPF in some respects, but unlike classical PPF, which reflects changes in the presynaptic terminal, DHR is exclusively a postsynaptic phenomenon. One characteristic that can distinguish between PPF and DHR is whether their respective potentiation is a graded or an all-or-none phenomenon. PPF has been consistently shown to be a graded phenomenon that is a function of both the ISI between the two stimulus pulses and the relative strength of the pulses (Zucker and Regehr 2002). Another form of short term plasticity, STDP, has also been shown to be a graded phenomenon (Dan and Poo 2004). As noted earlier, the read-out response accompanying DHR is an all-or-none NMDA spike. The DHR hypothesis predicts that

the potentiation observed in response to the DHR experimental paradigm will be a binary event. Here I have demonstrated in three conditions that DHR is an all-or-none process and is unique from both PPF and STDP.

#### **4.4.3 DHR is a physiological phenomenon**

DHR was first observed, and subsequently examined, using glutamate photolysis. While this technique is very well documented and accepted in the field (Tang 2006) I questioned whether some artifact of uncaging may explain DHR. I found that DHR could be achieved by two separate protocols that utilize release of endogenous glutamate. Specifically I showed that both loading, and read-out, were achievable with focal electrical stimulation. Even though electrically evoked synaptic stimulation is more physiological, the precise location of the electrically evoked EPSPs on the dendritic arbor cannot be reliably controlled. It is more difficult to demonstrate DHR with synaptic stimulation and the magnitude of the resulting DHR is likely underestimated, because of the poorer selectivity in co-localizing the loading and read-out stimuli on the same dendritic branch and the less-than-optimal synaptic loading of the dendrite.

#### **4.5 Summary**

These experiments demonstrate that synaptic activity, as simulated with glutamate photorelease or elicited with electrical stimulation, can leave a transient “memory trace” encoded on the dendritic arbor as spatially distributed population of bound, but blocked NMDARs. This memory trace can persist up to hundreds of

milliseconds. The information it holds is partitioned within individual dendritic branches, and can be conditionally and selectively retrieved by a moderate targeted depolarization. Read-out of the information is in the form of all-or-none local regenerative spikes. This dendritic hold-and-read mechanism is distinct from known forms of short-term plasticity and may be configured to efficiently support short-term and working memory.

## **Chapter V**

### **Conclusions**

## **5.1 Dendritic Hold and Read**

Bekkers and Stevens (1990) first suggested that dual-gating of the NMDA receptor could be an information holding state, but until recently the techniques necessary to test this hypothesis were not available. Our lab previously demonstrated that individual dendrites could store information in the form of a glutamate pulse for hundreds of milliseconds. Based on the original finding, I proposed that an ideal mechanism by which DHR might function is the dual gating property of the NMDA receptor. Using a novel DMD-based illumination system that allowed for very precise targeting of glutamate photoactivation, I was able to further investigate DHR, addressing four key hypotheses related to the mechanisms underlying DHR and how DHR may be the first experimental representation of the original ideas of Bekkers and Stevens.

## **5.2 The information-holding step in DHR is mediated by the “glutamate bound” but “Mg<sup>2+</sup>-blocked” state of NMDA receptors.**

DHR is blocked by NMDA receptor antagonists.

The NMDA receptor is unique in its dual gating requirement. Pharmacologic tools exist that allow us to specifically and completely block populations of NMDA receptors including specific blockers for particular NMDA receptor subunits. Using NMDA receptor antagonists, I have demonstrated that the NMDA receptor is an absolute requirement for DHR. More specifically I have shown that the GluN2B containing NMDA receptors appear to play a major role in DHR. I also showed that the Mg<sup>2+</sup> bound-but-blocked state of NMDA receptors was required by presenting evidence

that in “zero”  $Mg^{2+}$ , or at membrane potentials (depolarized or very hyperpolarized) where  $Mg^{2+}$  was less likely to be bound, DHR was less likely. Finally I showed that DHR could not be explained simply by increasing the amount of glutamate in the area of the dendrite. The data from these experiments support the hypothesis that DHR is mediated by the bound-but-blocked state of the NMDA receptor.

Potential limitations of the data recorded in “0”  $Mg^{2+}$  exist. While I did remove the  $Mg^{2+}$  from the extracellular solution, the experiment was not repeated in the presence of a EGTA (ethylene glycol tetraacetic acid) which would chelate free  $Mg^{2+}$  and give a better indication of how DHR would behave in true zero  $Mg^{2+}$ . In a true zero  $Mg^{2+}$  environment the initial loading pulse would create a sustained NMDA receptor current since the receptors would remain completely unblocked and would open and close based on the unbinding of glutamate as well as desensitization of the channel. I would still predict that under these conditions DHR would not occur because the essential “bound but blocked” state is unlikely.

Another important, related consideration is that in “0”  $Mg^{2+}$ , I saw a small increase in the loading and readout pulse amplitudes. This suggests that some NMDA receptors are still contributing to the EPSP as if it were purely an AMPA receptor mediated EPSP no change would have been seen. One possible explanation for why I am able to observe NMDA receptor mediated changes in EPSP, but not DHR, is that by reducing the  $Mg^{2+}$  I have reduced number of NMDA receptors available to maintain the bound-but-blocked state. In doing so, I have decreased the density of DHR-ready NMDA receptors on the dendrite and am unable to drive the existing population of NMDA receptors to the threshold needed for a regenerative spike and therefore DHR.

### **5.3 Individual terminal branch serves as the functional unit for a DHR response.**

Positive DHR response could be produced if the loading and read-out signal arrive on the same terminal branch, but not if they are on different branches.

Our lab (Wei et al. 2001; Liang et al. 2011) and others (Branco and Hausser 2010) have shown that dendritic compartmentalization is required for a dendritic spike. If DHR is mediated by a dendritic spike, then the compartmentalization that is required for DHR is a single dendritic branch. I tested several manipulations of spatial patterns for loading and read-out and found that DHR was only observed in conditions where the two stimuli occurred on the same terminal branch.

One likely explanation for the requirement of a dendritic compartment for DHR is that DHR is a threshold-based phenomenon. While the basic “hold and read” requirement of the DHR phenomenon is well established to exist at the NMDA receptor level (NMDA receptors bind glutamate and can hold that information for 100’s of ms), the DHR phenomenon as I have described it requires a regenerative depolarization in the form of a high density of NMDA receptors. In this model, a single “bound-but-blocked” NMDA receptor may be depolarized by a depolarizing readout pulse, allowing an influx of  $\text{Ca}^{2+}$  and  $\text{Na}^+$  that creates a very small local depolarization. As subsequent bound-but-blocked NMDA receptors are depolarized, the membrane depolarizes further until a certain threshold is achieved at which point all receptors that have bound glutamate open and a large, regenerative, all-or-none spike is recorded. I can not rule out the contribution of other voltage-gated ion channels ( $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ) in activating this spike and additional research is required to tease out the relative contributions of

VDCC's,  $\text{Na}^+$  as well as a myriad of  $\text{K}^+$  channels that may act to modulate the intrinsic excitability of a thin dendrite thereby affecting the baseline threshold for DHR. Preliminary data with the  $\text{K}^+$  channel blockers 4-aminopyridine (4-AP),  $\text{Cs}^+$  and, as well as 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride (ZD 7288), a potent blocker of  $I_h$ , were inconclusive (unpublished data).

There is a concern that glutamate photoreleased during the loading and gating stimuli might have reached a shared set of receptors on a given dendritic branch via lateral diffusion, in which case what appeared to be DHR, would actually be a variant of post-synaptic PPF (Polksy et al. 2009). If that were the case, then potentiation would not be observed if the two stimuli were separated widely in space. Importantly, DHR did not require the loading and read-out to be in the same region of a terminal dendrite, suggesting that DHR is quite distinct from other forms of short-term information storage such as PPF.

#### **5.4 DHR is mediated by a dendritic spike.**

The amplitude of the DHR response would remain largely all-or-none as the input intensity and the ISI are gradually changed.

I propose that DHR is an all-or-none phenomenon that is threshold dependent based on the ability of a thin terminal dendrite to generate a spike. If DHR is all-or-none, then I would see a step-like relationship between the priming intensity and the amplitude of the gated read-out response. I noted in three separate conditions that DHR has a distinct step-like function and that DHR amplitude does not show a graded relationship to ISI. It appears from these observations that read-out takes the form of an

all-or-none regenerative dendritic spike, as described previously (Schiller et al. 2000; Wei et al. 2001).

These observations suggest the following model for DHR. 1. The loading stimulus (the information-containing input signal) causes glutamate to bind to a population of NMDARs with slow unbinding kinetics. 2. These receptors remain blocked by  $Mg^{2+}$  ions, that is, the loading stimulus is designed to mimic dispersed inputs which do not cause significant dendritic depolarization thus creating a population of bound-but-blocked NMDARs. 3. A gating signal triggers a depolarization mediated by the unblocking of the NMDARs and the activation of voltage-dependent calcium and sodium channels. 4. With a sufficient depolarization and depending on the density of bound-but-blocked NMDA receptor as well as the threshold for a regenerative spike (determined by the intrinsic excitability of the dendritic compartment), sufficient numbers of NMDA receptors will open leading to a spike.

### **5.5 DHR is physiological**

DHR can be produced with synaptic stimulation protocols.

I have described in detail a phenomenon based solely on the photolytic uncaging of glutamate. The question remained whether our stimulation protocol may produce some artifact that could account for the potentiation I see in DHR. I hypothesized that DHR would be produced with synaptic stimulation. If loading and readout could be accomplished using a purely synaptic stimulation protocol, then DHR has a physiological correlate. Synapses on the CA1 pyramidal neuron are primarily glutamatergic, so a field stimulation protocol would allow us to release glutamate

near/on the dendrite from which I was recording. I show in two different variations, one with synaptic stimulation for loading and one for read-out, that I can elicit a DHR response. I conclude that DHR is accessible using a physiologically relevant stimulus.

## **5.6 Other considerations**

The work presented within this thesis did not address one key question previously unmentioned but that requires consideration. DHR was only observed in ~35% of the dendrites tested in this work. In attempting to address this issue a variety of approaches were considered without success.

I first asked whether the ISI for DHR was very dependent on potential contributions of GluN2A versus GluN2B receptors. I saw no difference in DHR ISI sensitivity down to 50 ms intervals. A better-designed study might look at very short ISIs, such as 10ms ISIs up to 100ms total to tease apart differences that may have been overlooked by the protocol I used (100's of ms).

A second consideration is the technology used for glutamate photolysis and therefore readout. A newer technology is currently being used in the Tang lab (halographic imaging, see methods, Santos et al. 2012) that allows for very fast uncaging to achieve read-out (1-2 ms versus 20-40ms). It is likely that the longer duration read-out pulse is not pushing populations of bound-but-blocked NMDA receptors to threshold quickly enough to overcome opposing mechanisms (discussed below). Supporting this hypothesis is the data that Dr. Yang has shown (unpublished) that he is able to observe DHR in >90% of dendrites in which he performs the protocol. He has further confirmed a high success rate of DHR using two-photon uncaging, which

is very fast and allows for even more precise spatio-temporal control over the stimulus compared to single photon excitation.

### **5.7 The roles of voltage gated calcium channels and potassium channels on the read-out of DHR**

A second likely explanation for why DHR was not observed in a majority of dendrites is the potential contribution of voltage gated potassium channels. An all-or-none spike phenomenon typically requires a threshold. For sodium spikes the threshold is strongly influenced by the hyperpolarizing actions of voltage gated potassium channels. The shallow negative slope conductance of the NMDA receptor channel may reduce the spike threshold thereby not requiring much of a hyperpolarizing conductance to reduce the probability of a voltage-gated regenerative spike. This would be consistent with the conclusions of Wei et al. (2001). In contrast, the SK channel is not voltage dependent and does not get involved with the regulation of the NMDA spike except for terminating the spike.

SK channels are distributed in the thin distal dendrites and have been shown to have a role in terminating plateau potentials (Cai et al. 2004). The SK channel most likely does not play a significant role in regulating the threshold for DHR except if the dendrite maintains increased concentrations of intracellular calcium. This can occur if the priming stimulus is too strong or if there a recent dendritic spike has just occurred. In this sense SK channels may regulate the refractory period of DHR.

There are also BK channels to consider, but their proximity to the soma suggest that these may not have played a role in the experiments presented here as most of the dendrites were more distal ( $>150\ \mu\text{m}$  from the soma) (Johnston et al. 2000).

Perhaps the most important player in setting the threshold for DHR is the hyperpolarization activated, mixed cation channel  $I_h$  which is expressed in high levels in distal dendrites (Magee 1999). Activation of this channel depolarizes the membrane, but potentially important for DHR it also decreases the input resistance of the dendrite. The DHR model requires a compartment with a high density of NMDA receptors as well as high input resistance to overcome a threshold for a regenerative spike. I did test the well-known  $I_h$  channel blocker ZD-7288 in 3 cells that were negative for DHR, but no effect on DHR was observed (data not shown). Future work should focus on the potential influence of these, as well as other, potassium channels on the intrinsic excitability of the dendrite and how that may affect the ability of populations of NMDA receptors, as well as other voltage-dependent cation channels to participate in DHR.

## **5.8 Functional significance of DHR**

The experiments described here show that DHR is a robust and repeatable experimental phenomenon. They also establish the fundamental biophysical and physiological characteristics of DHR. A reasonable question is whether DHR is just an experimental curiosity or does it serve some functional role in the nervous system. While it is beyond the scope of this thesis to definitively answer this question, it may be

useful to discuss some plausible functional roles. It may also be useful to discuss how disturbances of DHR may underlie important disease affecting the brain.

Processing of time-encoded information requires memory (Van Rullen and Thorpe 2001). Without some form of short-term memory buffer to hold together a temporal sequence, the individual bits of information that are received at any moment in time cannot be properly interpreted. For example, in order to predict the trajectory of a flying ball, it is necessary to hold in mind a temporal sequence of recent positions of the ball. Similarly, the meaning of a sentence can be dramatically different depending on the order of the words and the presence or absence of a single word. The cellular mechanism for such a short-term memory buffer is poorly understood (Gupta and MacWhinney 1997). Two contrasting theories have been proposed to explain the mechanistic basis of short-term memory. One theory posits that short-term memory is represented by persistent neural activity supported by reverberating feedback networks (Goldman-Rakic 1987). In another words short term memory is an “echo” within a resonant network. An alternate, more recent theory posits that short-term memory would be better supported by feed-forward networks (Lim and Goldman 2012). They envision short-term memory does not have an echo within a resonant network but as exists as the forward propagation of the information. I propose that DHR is uniquely suited to implement the elementary building block of such feed-forward network.

Feedback memory involves the repeated activation of the same synapse or circuit. In contrast, feed-forward memory requires only a single input and for that readout to spread down a daisy-chain of identical neurons (i.e. longitudinally connect CA1 neurons of the hippocampus). An analogy for feed-forward memory is simply by

dropping a pebble into a pool of water. Once the pebble hits the water a small wave is formed that propagates at a certain amplitude and speed away from the original point. At some future time the spatial and temporal information from the original input can be determined based on its active position, that is, the forward movement of the wave holds the original information without the need to have any resonant walls. In the DHR model, chains of neurons transform the temporal pattern of a signal into a spatial pattern within that chain.

The nature of DHR as a binary event that requires only a single pairing of NMDA receptor loading paired with a subsequent depolarization makes DHR an ideal candidate for feed-forward memory. The all-or-none spike I observe in DHR may trigger a somatic action potential (unpublished data) that may then allow for a single pyramidal neuron to feed information forward to neighboring CA1 neurons. In this model, populations of CA1 pyramidal neurons firing in patterns based on the output of dendrites that have been read-out in DHR could then be a spatial substrate for storing time encoded (by nature of their temporal patterning) information for 100's of ms. DHR therefore may in fact be a primary mechanism by which individual dendrites have the ability to time-stamp incoming information and allow it to be readout if it is pertinent information.

While I have shown considerable evidence in support of DHR as a memory mechanism on individual dendrites, many major questions regarding the mechanism and readout still exist. What is the nature of the 'read-out' or 'test' pulse in the brain? I have shown data that suggests that any recurrent depolarization would be sufficient to read out the bound-but-blocked NMDA receptor. If future studies wish to prove that

DHR is a physiological process, the most significant unanswered question would be the physiological correlate of the readout signal. Dr. Tang speculates that it would be the theta rhythm, which operates at the proper frequency for DHR. Where and how the theta rhythm interacts with the dendrite is unclear. Dr. Tang further speculates that the most logical site for the gating signal to trigger DHR is at the tips of the basal dendrites. The tips of the basal dendrites are located in the medial region of the alveus where large presynaptic terminal can be observed with synaptophysin stains (Dr. Yang, unpublished data).

## **5.9 Future Questions**

If memory buffers are so critical, where are they located in the brain? I speculate they are located in all cortical structures. The hippocampus happens to be the simplest to study. Such a network would need to comprise of identical neurons connected sparsely to each other in a daisy chain fashion. Dr. Sunggu Yang is actively addressing this issue in Dr. Tang's lab. His preliminary results suggest that DHR can be observed along the longitudinal axis of the hippocampus traveling in the temporal to septal direction (personal communication). CA1 neurons are remarkably similar to each other (Spruston 2008). So a daisy chain of CA1 neurons oriented along the longitudinal axis could in theory form a feed-forward network. There is no reason that such a chain could not exist in the neocortex, but due to the current limitations of studying network behavior in neocortical preparations this hypothesis is quite difficult to test.

In this thesis project I only examined DHR on oblique dendrites of CA1 neurons. Dr. Yang found that the basal dendrites can support DHR better than the oblique

dendrites (unpublished data) Furthermore, morphological studies suggest that the longitudinally oriented CA1 axons make synaptic contacts on the basal dendrites of downstream CA1 dendrites.

### **5.10 DHR and neurological disorders**

While it purely speculative at this time, it still may be important to start to think about the relation between disturbances to DHR and human disease. The human neurological disorder with the greatest impact on society is Alzheimer's disease. The earliest manifestation of Alzheimer's disease is short-term memory. If it is correct that DHR is the building block for short-term memory, it behooves neuroscientists to examine the linkages between Alzheimer's disease and DHR.

In psychiatry one of the diseases with the highest impact for society is schizophrenia (Park and Holzman 1992). The pathogenesis of this condition is poorly understood, but the linkages between DHR and schizophrenia are compelling. The best way to mimic schizophrenia is to create a hypoNMDA state (Strous et al. 1995). Entrance into and exit from the bound-but-blocked state of the NMDA receptor is the molecular basis of DHR. While learning and long-term memory is not affected in schizophrenia, there is ample evidence of disturbances in working memory (Lee and Park 2005). This disturbance can well explain the "flight of ideas" that characterizes the thought disorder in schizophrenia (Aleman et al. 2000). And interestingly, sequence memory is particularly disturbed in patients with schizophrenia (Keefe et al. 2012). One of the most widely accepted objective abnormalities linked to schizophrenia is "prepulse inhibition" (Braff et al. 2001, Carroll et al. 2008). Interestingly, the procedure

is remarkably similar to how DHR is elicited. If a good transgenic animal model for schizophrenia is ever developed, it would be interesting to examine DHR in such an animal.

### **5.11 Conclusion**

I have presented the data that provides the foundation for an understanding of DHR, a novel form of short-term information storage and read-out on CA1 pyramidal neuron dendrites. My contributions to the DHR hypothesis are the basic mechanisms that mediate DHR as well as the conditions in which DHR will function. Future work must first focus on a better understanding of the gating of DHR, and then how DHR may be involved in complex physiological networks.

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