Mechanisms involved in the norepinephrine-mediated protection of synapses from depotentiation.

by

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Abstract

Norepinephrine (NE) is a key neuromodulator that controls the longevity of synaptic plasticity such as long term potentiation (LTP). Activation of beta-adrenergic receptors (β-ARs) is known to boost persistence of LTP in area CA1 of mouse hippocampal slices. Activity-induced weakening (depotentiation, DPT) via 5Hz stimulation depresses the strength of synaptic transmission at synapses that have recently undergone LTP. I tested the hypothesis that NE primes synapses for subsequent long-lasting LTP, serving to protect potentiated synapses from depotentiation. Using population excitatory postsynaptic potential (EPSP) recordings from CA1 of mouse hippocampal slices, I show that NE (10uM) applied well before weak tetanic stimulation (1 x 100-Hz, 1s) protects synapses from depotentiation. This protection is no longer present when PKA and ERK (downstream protein kinases of the β-AR pathway) have been pharmacologically blocked. I also present evidence suggesting that, in addition to these kinases, NMDA receptors may also play a role in initiating the intracellular mechanisms that protect, or immunize, potentiated synapses from activity-induced weakening.

Acknowledgments

I would like to thank my supervisor, Prof. Peter Nguyen. I have been extremely lucky to have a supervisor who takes great interest not only in my research but also my wellbeing. He trusted me enough to give me autonomy in the lab, but always steered me in the right direction whenever he thought I needed it. This achievement would not have been possible without his guidance.

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Table of Contents

List of Figures

Chapter 1 – Introduction

1.1 Learning and Memory

The ability to remember names, places and even something trivial as where you placed your keys may seem indifferent to us in everyday life, but it is one of the most significant abilities that has shaped human society. The ability to retain and to utilize acquired information or knowledge to alter future behavior is a feat not possible without this facility. Organisms can acquire knowledge, a process called learning. They can also retain this knowledge over a period of time, a process called memory. The ability to recall old memories to modify current and future actions acts as an indicator of learning, but also provides substantial survival benefits.

Without learning and memory, organisms are limited only to simple reflexes and stereotyped behaviors. Thus, learning and memory are the most intensively studied processes in neuroscience. Various approaches have been used to understand the mechanisms underlying learning and memory.

1.2 Multiple memory systems

As much as our memory systems assist us, we do not learn everything in the same manner, nor do we store memories in equal capacity. We store and use different types of information appropriate for each specific decision or situation that we encounter. For example, we store significant information that might have a lasting impact on our lives, within long term storage. In contrast, less significant information relevant only to the current situation can be quickly forgotten.

Memory is classified into different types. Memories concerning events and facts are available to our consciousness, and are called declarative memories. Another type of memory, called procedural memory, is not available to consciousness. This type is associated with learning motor skills and habits, which can be improved through practice (Squire and Zola-Morgan 1991). Evidence for multiple forms of memory was highlighted by studies conducted on the amnesic patient H.M.

H.M. suffered severe epileptic seizures that resisted treatment. In an effort to ease the severity and frequency of the seizures, tissue from the medial temporal lobe (MTL) was surgically removed. As a result, H.M. developed severe anterograde amnesia. He was now unable to form new memories (about places, facts or people), although his intellect remained intact (Milner, Corkin et al. 1968, Corkin, Amaral et al. 1997, Scoville and Milner 2000). His ability to retain new information was nonexistent; however, H.M.'s ability to learn new sensorimotor skills remained intact. The sensorimotor skill was tested by 'mirror drawing' (a test where a subject has to trace a line drawing, but they are only allowed to view the drawing and their hands in a mirror). H.M. demonstrated significant improvement during the training trials and was able to retain the memory for this skill across trials, despite not remembering that he had previously performed the task. The pattern of intact capacities and deficits in patient H.M. shows that there exists at least two types of memory, declarative memory and procedural memory (Milner, Corkin et al. 1968).

Studies of memory function in other amnesic patients with specific brain lesions have also provided insights into the neural bases of learning and memory. The results have shown that there exist different types of memory that are mediated by distinct brain structures (Warrington and Weiskrantz 1968, Cohen and Squire 1980, Tulving and Schacter 1990, Squire and McKee

1993). Unfortunately, determining which specific functions belong to which region of the brain cannot be elucidated because often lesions/damage in the human brain are not isolated to a single structure, but rather cover multiple structures/regions. For example, in the case of H.M., removal of the medial temporal lobe included multiple brain regions (hippocampus, amygdala, subiculum, entorhinal, perirhinal and parahippocampal cortices (Corkin, Amaral et al. 1997)). Thus, animal models were developed to try to mitigate the confounds of human case studies. Research conducted on animal models allowed better control of experimental conditions, such as controlled lesions or ablation of specific regions in the brain, and it also has the added benefit of overcoming inherent ethical issues associated with human research.

1.3 The Hippocampus

Studies of memory in animal models have provided insight into the nature and interactions of memory systems in the brain. Research on rodents has specifically shed light on the functions of the hippocampus in learning and memory.

One function of the hippocampus is to form “cognitive maps” that represent spatial information about the surroundings. This is supported by the discovery of place cells (O'Keefe 1976). These neurons in the hippocampus fire when the rodent occupies a specific location within its environment. The place cell fires when the rodent is within a specific area; combining all of the place cells forms a cognitive “map” (O'Keefe and Dostrovsky 1971, O'Keefe and Burgess 1996). Rodents with hippocampal damage showed impairment in multiple forms of spatial learning and memory. (O’keefe and Conway 1980, Burgess, Maguire et al. 2002).

One test used to assess spatial memory is the Morris water maze (Morris 1984). In this experimental setup, the task involves a rodent finding an escape platform which is hidden within a large pool of opaque fluid. After multiple training trials, the rodent learns to use visual cues on the walls around the room to locate the hidden platform. Eventually the time it takes for the rodent to locate the hidden platform decreases. To assess the role of the hippocampus in spatial memory, the hippocampus was ablated prior to training (Morris, Garrud et al. 1982) or a glutamatergic receptor antagonist was infused to block synaptic transmission in the hippocampus (Davis, Butcher et al. 1992). These manipulations resulted in much longer escape times (time to find the hidden platform).

The hippocampal system is also critical for encoding short term memory to long term memory. This process is called memory consolidation during which new memories are transformed from an unstable state to a more persistent form (Squire, Cohen et al. 1984). Humans with damage to medial temporal lobe typically have impaired consolidation, such as the case of patient H.M. (Scoville and Milner 1957).

The hippocampus has been shown to be significant in learning and memory. However, one role it does not fulfill is memory storage. Once the hippocampus has consolidated a memory, the memory may be stored in other brain regions (Squire, Cohen et al. 1984, Squire and Alvarez 1995).

1.4 Synaptic transmission

The brain receives information and sends out commands via neuron to neuron communication. The synapse is the site of communication between neurons via chemical signals. It is believed that cognitive processes ultimately originate from chemical synaptic transmission. This means that modifying synaptic transmission within different regions of the brain can cause behavioral changes. Thus, it is reasonable that an understanding of the synaptic properties of individual neurons is critical for elucidating the cellular bases of behavior (Kandel, 1976).

Communication between neurons is initiated when an action potential arrives at the presynaptic terminal. The depolarization causes an influx of Ca^{2+} in the presynaptic terminal and results in the release of a chemical neurotransmitter into the synaptic cleft. The neurotransmitter then binds to its respective receptor on a postsynaptic cell. This results in a transient change in ion conductance of the postsynaptic cell, creating a change in membrane potential. There are two forms of potentials, called excitatory and inhibitory postsynaptic potentials (EPSP and IPSP respectively). When summation of EPSPs occur and the synaptic potential reaches or exceeds a particular threshold, an action potential is initiated.

The strength of a synapse can often can be modified; it can be either enhanced or depressed. These modifications are thought to be the cause of some critical behaviors. For example, temporary modification of synaptic transmission has been associated with sensory adaptation and receptive field alteration (O'shea and Rowell 1976, Furukawa, Kuno et al. 1982).

1.5 Synaptic Plasticity

How do you learn? This is a tougher question than it seems. You might know that you learn by studying, for example, but what changes occur in your brain when you read a chapter for the first time or when you test yourself on that knowledge? Your brain does not grow new cells to store your memories, otherwise our heads would just continually grow larger. What actually happens is a phenomenon called synaptic plasticity. Synaptic plasticity is the activity-dependent modification of synaptic strength (reviewed by Martin, Grimwood et al. 2000).

It was Donald Hebb, who first formulated the concept that cellular activity underlies memory storage. As he eloquently states:

“Let us assume that the persistence or repetition of a reverberatory activity (or "trace") tends to induce lasting cellular changes that add to its stability.… When an axon of cell A is near enough to excite a cell B and 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)

To summarize, his idea proposed that the co-activation could lead to synaptic changes that would outlast the initial signal. To shorten it even more, the idea can be condensed to "Cells that fire together, wire together", a phrase coined by Siegrid Löwel (Lowel and Singer 1992).

Theoretical models suggested that the experience-dependent alteration of neural connections could mediate information storage in the nervous system. However, experimental evidence of such a phenomenon in mammals was not obtained until the 1970s, when Bliss and Lomo discovered long-term potentiation (LTP) of synaptic strength (Bliss and Gardner-Medwin 1973, Bliss and Lomo 1973). In their experiment, high-frequency hippocampal stimulation

(tetanization) was given to anesthetized rabbits. Results showed that tetanization of the entorhinal perforant pathway led to a long-lasting increase in synaptic transmission and that a single pulse after the tetanus elicited an excitatory synaptic potential with significantly a steeper slope (greater strength of synaptic transmission). This enhancement was shown to be persistent long after the tetanus (Bliss and Lomo 1973). Due to the fact that LTP induction and expression can be modulated by numerous signaling paths and neurotransmitters, it has attracted significant amounts of attention from neuroscientists.

1.6 Hippocampal LTP and Learning and Memory

Modern neuroscience has made significant advances in our comprehension of LTP and its role in memory. As mentioned earlier, an integral part of Hebb's rule is co-activation of connected cells. Indeed, the hippocampus displays Hebbian forms of LTP that are associative in nature (Levy and Steward 1979, Barrionuevo and Brown 1983). This associativity is characterized by the observation that when a synapse is activated by presynaptic activity and there is substantial postsynaptic depolarization, the synapse will be strengthened. (Lynch, Larson et al. 1983, Malinow and Miller 1986, Wigstrom, Gustafsson et al. 1986).

Hippocampal LTP has other features which seem to reinforce the idea that it is a strong candidate mechanism for learning and memory. Hippocampal LTP has shown to be persistent over time (Abraham 2003). LTP induced in brain slices can persist for many hours (Bliss and Gardner-Medwin 1973, McNaughton, Barnes et al. 1986) and up to a year in intact animals (Abraham, Logan et al. 2002). Solidifying the link between LTP and memory, a study performed by Barnes in rats showed that the amount of synaptic enhancement was correlated with the rat's ability to

perform spatial memory task (Barnes 1979). This idea was confirmed in later studies (Barnes and McNaughton 1985).

In addition to modulation of synaptic efficacy, there are other properties that reinforce the link between LTP and memory. One of the most compelling aspects of the hypothesis that hippocampal LTP is involved in memory formation is the idea of synaptic specificity. Specificity refers to the changes that will occur when LTP occurs, but the changes will be restricted to those synapses that are activated during the induction of LTP. This specificity is proposed to reflect an independent memory store, which would be preferentially active during recall, a critical feature of memory (Andersen, Sundberg et al. 1977, Clark and Kandel 1993). This implies that each synapse can store specific information, thus, synapse specificity may importantly contribute to the large storage capacity of the brain (Shors and Matzel 1997).

1.7 Other synaptic plasticity

Historically, research in mechanisms of learning and memory favored LTP more than other types of synaptic plasticity. This allowed research on LTP to progress at a faster rate than other forms of plasticity. However, LTP is not the only form of synaptic plasticity to have a link to learning and memory.

A) Long term depression (LTD)

Long term depression (LTD) is a phenomenon often associated as being the 'opposite' of LTP. During LTD, synaptic connections are persistently weakened. It is proposed that LTD acts as a counter balance to LTP and that it prevents synapses from saturating. Studies have shown that

LTD can be elicited if a prolonged low-frequency stimulation is given (this stimulation would be too weak to induce LTP). It is proposed that in vivo this low-frequency activity would be mimicking a situation where a presynaptic cell consistently failed to activate the postsynaptic cell, and LTD would reduce the efficacy of the connection between these cells (Stent 1973).

B) Metaplasticity

Metaplasticity, to put it simply, is the plasticity of synaptic plasticity. It is the ability of synapses to change their propensity for future plasticity through prior activity or experience (Abraham and Bear 1996). This prior activity or experience can be viewed as 'priming' which can consist of either electrical stimuli or pharmacological modulation **[Figure 1]** (Abraham 2008). Previous studies have shown that behavioral stress can be considered a priming stimulus for metaplasticity (Kim and Yoon 1998).

This control of plasticity is hypothesized to serve two roles: 1) a role in homeostasis, preventing saturation of synapses from LTP and LTD (Abraham and Tate 1997), 2) greatly enhancing the ability of a synapse to summate synaptic potentials (Abraham, Mason-Parker et al. 2001, Hulme, Jones et al. 2014).

C) Depotentiation (DPT)

There has been much discussion on the identity of depotentiation (DPT). DPT is frequently viewed as an "analog" of LTD. This is because both processes share some mechanistic features with each other. For instance, studies performed in young animals have shown that both LTD and DPT are produced by long periods of low-frequency stimulation and are blocked by NMDA receptor antagonists. However, differences between the two processes appear when the same experiments are performed in adult animals. In the mature animal, LTD is absent but DPT is still

present. This suggests that the two processes are unique or perhaps a distinct variation of a common process (Fujii, Saito et al. 1991, O'Dell and Kandel 1994). Another difference between the two is that DPT is an activity-dependent reduction in synaptic strength at *previously potentiated* synapses, whereas LTD is depression of a *naive synapse* that had *not been previously strengthened* **[Figure 2]** (Wagner and Alger 1996). Currently, depotentiation is viewed as a mechanism for resetting synaptic strength, preventing the physiological saturation of synapses.

1.8 LTP mechanisms

The best studied form of LTP involves the activation of NMDA receptors (Collingridge, Kehl et al. 1983). LTP induction begins when action potential arrives at the axon terminal of the presynaptic neuron. The depolarization of the terminal opens the voltage gated calcium channel. The influx of Ca^{2+} causes vesicles containing glutamate to be released into the synaptic cleft. The glutamate diffuses across the synaptic cleft and binds to the glutamate receptors on the post-synaptic neuron. The glutamate binds to one type of ionotropic receptor, the alpha-amino-3-hydroy-6-methyl-4-isoxazolepropionate receptor (AMPAR), and causes depolarization of the post-synaptic cells by allowing sodium ions influx. The depolarization of the post-synaptic cell via AMPAR allows the N-methyl-D-aspartate receptor (NMDAR) to activate. The NMDAR opens because the previous depolarization by AMPAR allows Mg^{2+} ion to dissociate from blocking the NMDAR, opening up the channel pore. The open NMDAR allows Ca^{2+} and Na^{+} to enter the postsynaptic cell and it is this influx of Ca^{2+} that is critical for LTP induction (Durand, Kovalchuk et al. 1996). The increase in Ca^{2+} level within the postsynaptic cell is required to activate kinases. These kinases phosphorylate specific sites of the AMPA and NMDA receptors,

increasing channel conductance and allowing LTP to be maintained (Benke, Luthi et al. 1998, Derkach, Barria et al. 1999). Studies have been conducted where LTP has been blocked by applying NMDAR antagonists (Collingridge, Kehl et al. 1983, Teyler and DiScenna 1987) and by lowering intracellular Ca2+ concentration (Lynch, Larson et al. 1983). In contrast, increasing intracellular Ca2+ mimicked LTP (Malenka, Kauer et al. 1988, Neveu and Zucker 1996, Yang, Tang et al. 1999).

As mentioned earlier, hippocampal LTP is a leading candidate mechanism for learning and memory. One of the characteristics which seems to solidify the idea that LTP is a mechanism for learning and memory is Donald Hebb's notion of cooperativity and associativity. In essence, Hebb's concept was realized by the discovery of LTP by Bliss and Lomo. If the presynaptic signal was too weak, there would be not be enough glutamate release to sufficiently activate enough AMPARs in the postsynaptic neuron. This means that the postsynaptic neuron will not be depolarized sufficiently and that NMDARs would remain inactive due to the Mg^{2+} blocking the channel. This means there would be no LTP. However, if a number of presynaptic fibers were activated (with the same weak stimulation) at a close time interval or spatial proximity, there would be a higher chance of eliciting LTP via summation (allowing the weak signals to stack on top of each other). This summed signal can now depolarize the postsynaptic cell sufficiently, allowing NMDAR activation and LTP induction. This is *cooperativity*.

Suppose there are two pathways. Pathway 1 is activated by a strong presynaptic stimulus that would have no difficulty in depolarizing postsynaptic neurons and inducing LTP. Pathway 2 has a weak stimulus and does not produce LTP on its own. However, if both pathways synapse onto the same neuron and fire at the same time, both synapses (pathway 1 and 2) are strengthened. This is *associativity* **[Figure 3].**

1.9 Early/Late LTP

LTP in the mammalian brain is commonly divided into two types, early LTP (E-LTP) and late LTP (L-LTP). These are distinguished by their temporal durations and mechanisms of expression. E-LTP lasts anywhere from 30-60 minutes (Huang, Nguyen et al. 1996) and only requires modification of pre-existing proteins (Huang and Kandel 1994). On the other hand, L-LTP can last for hours in vitro (Andersen, Sundberg et al. 1977, Huang, Li et al. 1994, Abel, Nguyen et al. 1997). Unlike E-LTP, new mRNA and protein synthesis are required to express L-LTP in the hippocampus (Frey, Krug et al. 1988). This form of LTP requires multiple 100Hz tetani, whereas E-LTP can be triggered by a single 100Hz train (Bliss and Collingridge 1993).

Studies have shown that the difference between the two forms of LTP can be traced back to specific protein kinases. It has been shown that triggering LTP also activates protein kinase-A (PKA); however, blocking of PKA by pharmacological means had little effect on E-LTP (Abel, Nguyen et al. 1997, Blitzer, Connor et al. 1998, Duffy and Nguyen 2003).By contrast, L-LTP requires PKA activation. The application of a PKA inhibitor, KT5720, blocks L-LTP in hippocampal slices that have been stimulated multiple times to elicit L-LTP (Huang, Li et al. 1994) (Huang and Kandel 1994).

Protein synthesis dependent L-LTP can not only be expressed with multiple trains of high frequency stimulus (HFS), but it can also be expressed with the help of neuromodulators (Sara 2009). In later parts of this chapter, I will further explain the role of PKA in L-LTP mediated by a neurotransmitter.

1.10 DPT mechanism

Depotentiation is induced when a low frequency stimulation is given within a time period after a synapse has been potentiated. Similar to LTP, depotentiation is triggered through the activation of NMDARs. However, unlike LTP, depotentiation is triggered when NMDARs allow a low level of Ca^{2+} influx into the postsynaptic cell. The change in postsynaptic calcium concentration (raised, but not high enough to trigger LTP) activates a complex protein phosphatase cascade. Calcium enters via the NMDAR and binds to calmodulin (CaM) which in turn activates calcineurin (PP2B). PP2B then dephosphorylates inhibitor-1 (I1). The dephosphorylated I1 can no longer inhibit protein phosphatase-1 (PP1). The active PP1 then goes on to dephosphorylate numerous targets and contributes to the generation of LTD **[Figure 4]**.

One potential target of PP1 can be Ca2+/CaM dependent kinase II (CaMKII), a protein kinase required for LTP induction. The cascade could be activating specific proteins via dephosphorylation to express DPT, but it could also be suppressing LTP (Wagner and Alger 1996).

1.11 Norepinephrine

Many psychological states are mediated by neuromodulators. Unlike fast neurotransmitters, which mediate synaptic transmission between two neurons, neuromodulators are allowed to diffuse over a more widespread region in the brain, affecting populations of neurons. As mentioned before, neuromodulators are not involved in triggering EPSPs or IPSP, but instead, they mediate their effects via secondary messenger cascades, which produce a slower, longer lasting modulation of transmission. These long-lasting effects can alter attention, perception, and

our ability to retrieve, and to make, new memories. The major neuromodulator in the central nervous system that has a key role in all of these activities is norepinephrine, which originates from the locus coeruleus (LC) (Sara 2009).

The majority of noradrenergic cells in the brain are concentrated in the LC. The LC projects widely, allowing norepinephrine access to the entire CNS **[Figure 5]** (Moore and Bloom 1979, Sara 2009). There is especially dense innervation in the thalamus, amygdala and the hippocampus (Morrison and Foote 1986, Simpson, Waterhouse et al. 2006).

1.12 Norepinephrine's effects

Pharmacological studies have revealed that norepinephrine (NE) is responsible for modulating several brain functions such as attention, arousal, sleep, learning, and memory (Arnsten and Li 2005, Robbins and Roberts 2007, Sara 2009, O'Dell, Connor et al. 2015). This makes NE a crucial neuromodulator in our brain.

In the past sections, I have explained why LTP is a good model for learning and memory. The LC heavily innervates the hippocampus and hippocampal neurons can respond, thanks to the presence of hippocampal beta-adrenergic receptors (β-ARs). This is probably why NE is able to modulate hippocampal learning and memory. Evidence gathered from numerous animal studies suggest that the activation of β-ARs by emotionally-charged events tends to enhance memories possibly by boosting LTP (McGaugh 1989, McGaugh 2000, Gelinas and Nguyen 2005). NE when paired with a single 100Hz train elicits LTP that has been shown to mimic protein synthesis-dependent L-LTP (O'Dell, Connor et al. 2015). This NE-LTP also occurs when NE is

applied in a metaplastic protocol, where NE is applied well before a single 100Hz tetanus is given (Maity, Rah et al. 2015).

Studies have been done with human subjects to further explore the effects of β-AR activation on memory. In one study, subjects were either given propranolol (a β-AR antagonist) or placebo 1 hour before being exposed to an emotionally-charged story or an emotionally neutral story. The results showed that propranolol impaired the recall of emotionally charged stories, but had no effect on the neutral story (Cahill, Prins et al. 1994).

Taken together, the evidence could be summarized as 'emotionally charged memories are encoded better due to norepinephrine activation of β-AR'. Because β-AR activation by NE leads to significantly increased memory formation and retention, I have chosen it to be the focus of my thesis.

1.13 Beta-AR mediated LTP mechanism

The β-AR is a G-protein coupled receptor and is activated when norepinephrine binds to it. The binding of norepinephrine has a strengthening effect on LTP. Normally, a single 100Hz train can only elicit E-LTP through the NMDA receptor and only temporarily potentiates the synapse. However, with the activation of β-ARs, the lone 100Hz stimulus can invoke the protein synthesis-dependent LTP (L-LTP) lasting several hours in slices (Gelinas and Nguyen 2005, Gelinas, Banko et al. 2007, O'Dell, Connor et al. 2015).

But how does β-AR activation elevate a single 100Hz stimulus from expressing E-LTP to L-LTP? At the molecular level, β-AR activation triggers multiple protein kinases which facilitate

L-LTP. This includes modification of channels via phosphorylation, and protein synthesis, as emphasized earlier (O'Dell, Connor et al. 2015).

A) Protein Kinase A (PKA)

When β-ARs are bound to norepinephrine, the subunit Gs-α activates adenylyl cyclase (AC) to start producing the secondary messenger cAMP. cAMP then binds to the regulatory subunits of PKA, releasing the catalytic subunit to phosphorylate its targets within the neuron to mediate LTP **[Figure 6].**

In the hippocampus, all NMDARs carry phosphorylation sites for PKA (Leonard and Hell 1997, Tingley, Ehlers et al. 1997, Murphy, Stein et al. 2014, O'Dell, Connor et al. 2015). The phosphorylation of the NMDAR increases its conductance (Skeberdis, Chevaleyre et al. 2006). Taken together, the evidence suggests that PKA-modified NMDARs play a crucial role in expressing β-AR-mediated L-LTP.

AMPA receptors have a PKA phosphorylation site. Studies have found that PKA phosphorylation of AMPARs causes the incorporation of AMPA receptors into the synapse (Esteban, Shi et al. 2003, Granger, Shi et al. 2013, Chater and Goda 2014). This means that β-AR activation increases the number of AMPARs after the initial stimulus, allowing for more AMPARs to be active (Henley and Wilkinson 2013).

B) ERK

Mitogen-activated protein kinases (MAPK), also known as extracellular signal-regulated kinases (ERK), is a crucial pathway for LTP expression. In most cells it is responsible for expression of

genes involved in proliferation, differentiation and survival. In neurons, ERK is crucial for regulating protein synthesis, which contributes to LTP expression and maintenance.

The ERK pathway is susceptible to crosstalk, where components of one signal pathway affect another pathway's activities. The cAMP generated from β-AR activation has the capacity to regulate the ERK cascade (Stork and Schmitt 2002). cAMP activates a protein called Rap1, part of the RAS family of proteins. Rap1 when activated interacts with a protein called B-Raf, which is a protein kinase. Their interaction leads to ERK activation (Stork and Schmitt 2002). The actual cascade occurs when active B-Raf phosphorylates and activates MEK 1 and MEK 2, which in turn phosphorylate and activate ERK 1 and ERK 2; this kinase cascade ends up phosphorylating proteins regulating both translation and transcription **[Figure 7]** (Gutkind 1998)

As mentioned previously, protein synthesis is critical for tetanus-induced L-LTP. Gelinas et al., have linked activation of β-ARs to translational machinery. The key kinase involved is ERK (Gelinas, Banko et al. 2007). Inhibiting ERK prevented the maintenance of beta-AR-induced LTP (Gelinas Banko et al. 2007). In addition, they observed an increase in phosphorylation of eukaryotic initiation factor complexes when one train of 100Hz was paired with an application of a β-AR agonist, isoproterenol. This phosphorylation was weakened when ERK was inhibited.

1.14 Hypothesis

Previous research has already explored the mechanisms behind NE-mediated LTP within the hippocampal slice. However, the roles of NE and downstream signaling pathways in synaptic protection from depotentiation has not been explored.

Depotentiation (DPT) is viewed as a mechanism for resetting synaptic strength, preventing the physiological saturation of synapses. The existence of DPT means that there must exist a mechanism for *protecting synapses from DPT,* which may allow for long-term retention of information stored in the brain. Since β-AR activation leads to significantly increased memory formation and retention (reviewed by Sara 2009), it is likely that β-ARs also have the potential to provide synaptic protection from DPT. Knowledge of the specific mechanisms and signaling cascades underlying a β-AR-activated protection from DPT (more specifically, *NE-activated protection*) might provide novel strategies for protecting weaker memories or removing harmful memories, such as those recalled during post-traumatic stress disorder (PTSD).

The main objective of my thesis is to determine whether NE provides protection from synaptic depotentiation and to identify the mechanisms by which activation of the β-AR (by NE) can provide this protection. The questions to be addressed in my thesis are:

Does prior activation of β-ARs by NE protect LTP from depotentiation?

Is PKA activation required for protection?

Is ERK activation required for protection?

Is NMDAR activation required for protection?

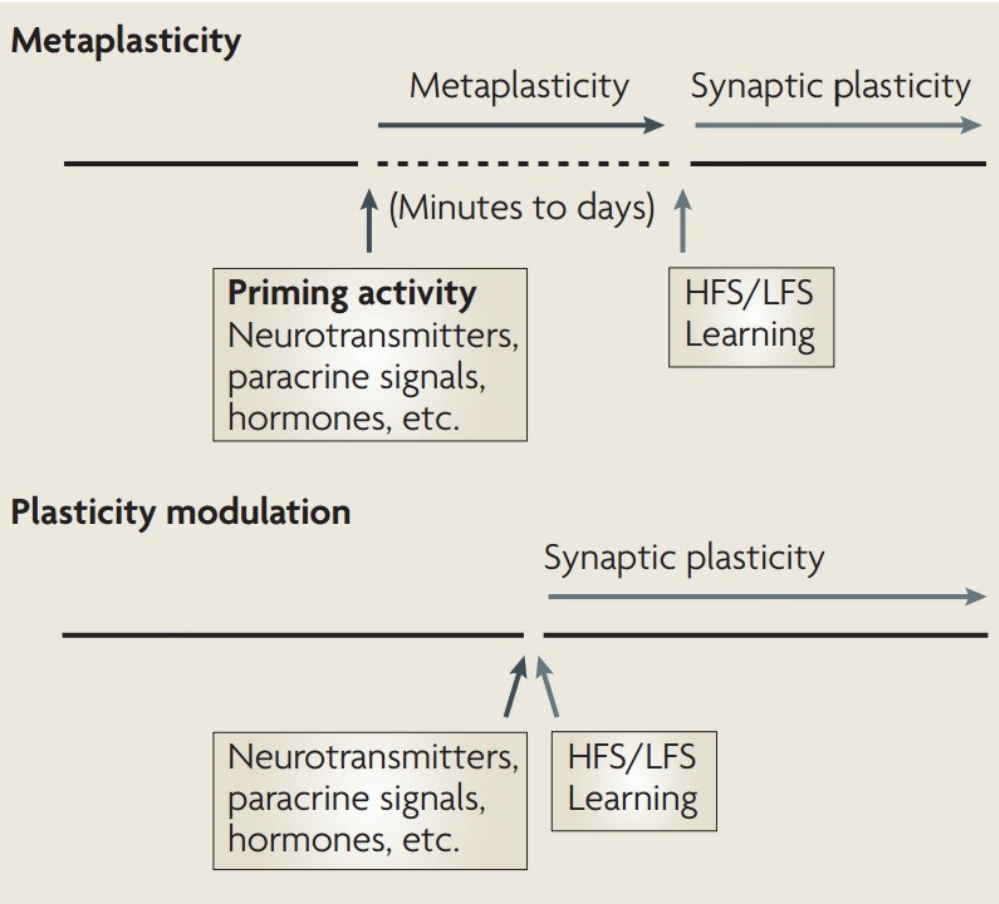

Figure 1. Metaplasticity vs. Plasticity. Unlike regular plasticity, during metaplasticity the priming activity gives rise to long lasting changes, which are present even after the priming activity is gone and can affect subsequent synaptic plasticity in the same neuron. (Figure taken from Abraham, 2008)

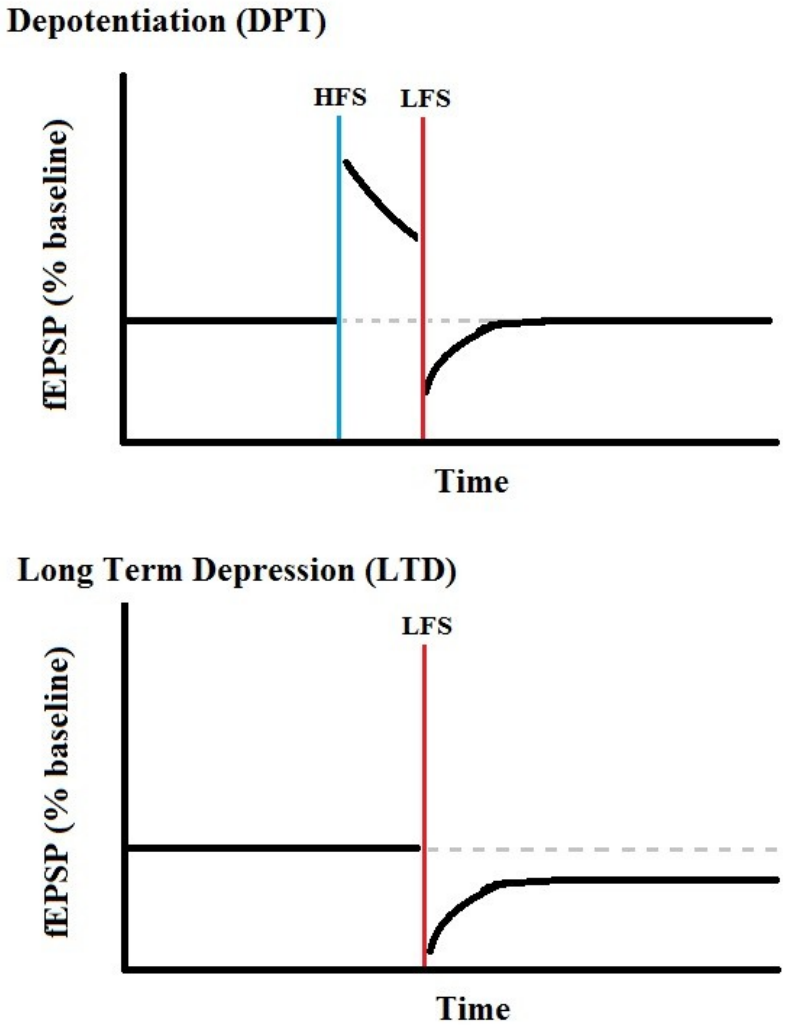

Figure 2 DPT vs. LTD. Depotentiation is when low frequency stimulus (LFS) (5Hz for 3min) is given to a slice after a high frequency stimulus (HFS) (100Hz 1 second) which induces LTP. Depotentiation brings fEPSPs back to baseline values. In LTD, the LFS is given in the absence of HFS, which leads to a sustained depression of the synapse.

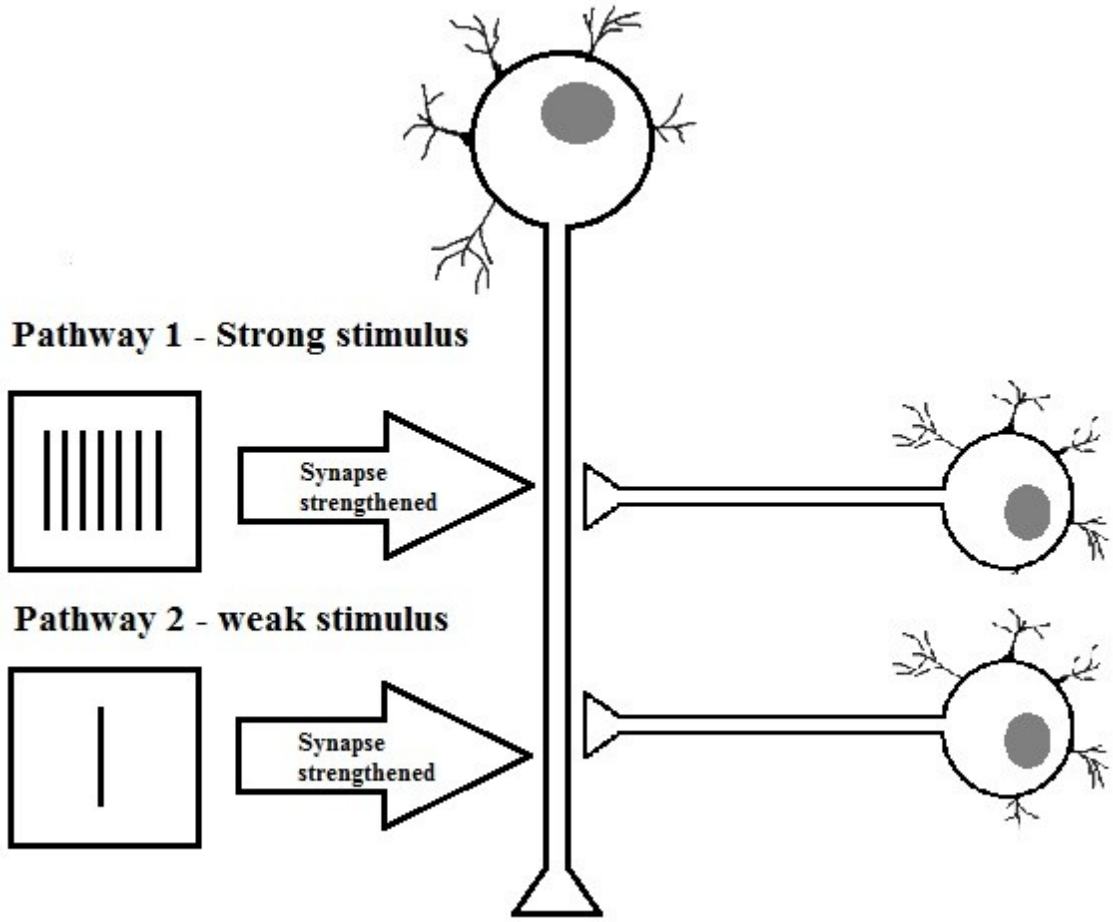

Figure 3. Associative property of LTP. Pathway 2 cannot induce LTP alone and cannot strengthen its synapse (single line indicates weak stimulus). However, if stimulated together with pathway 2 which has a strong stimulus, both pathways will display LTP.

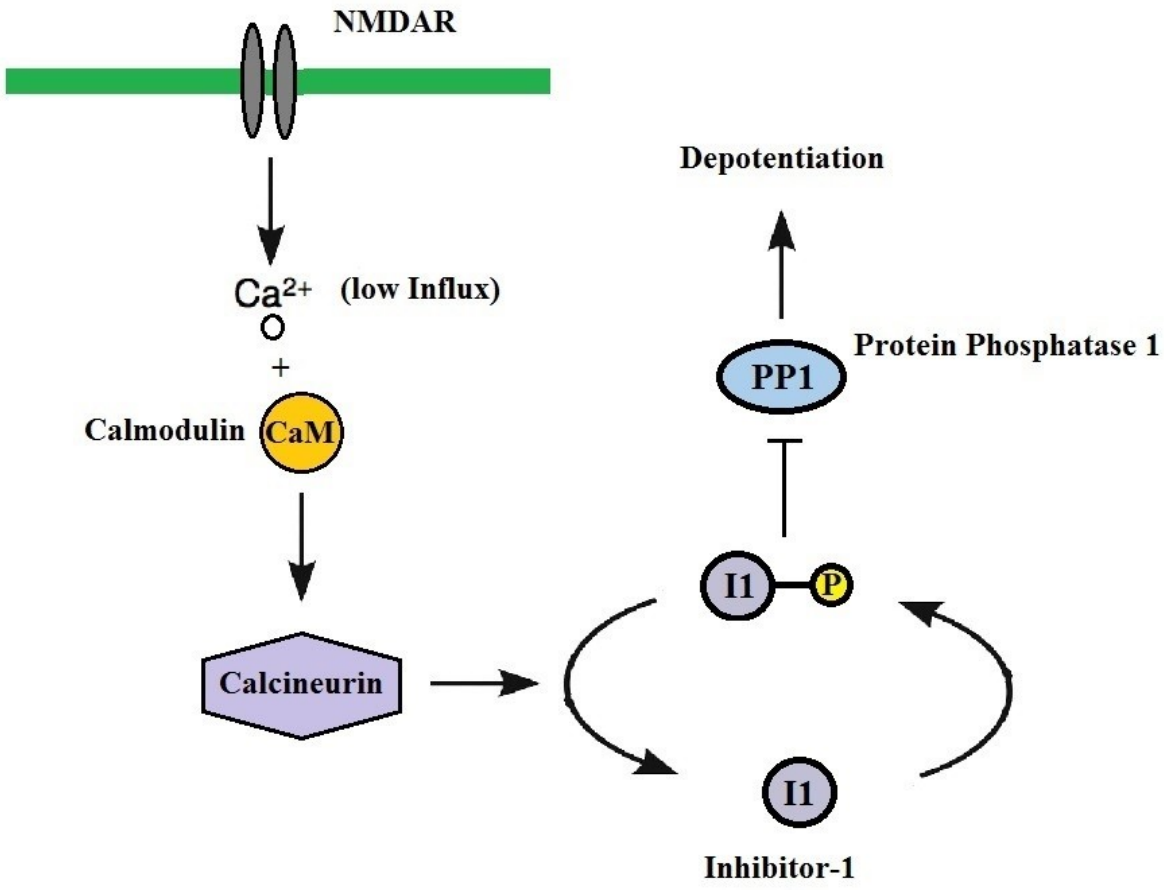

Figure 4. DPT mechanism. Proteins potentially involved in mediating depotentiation.

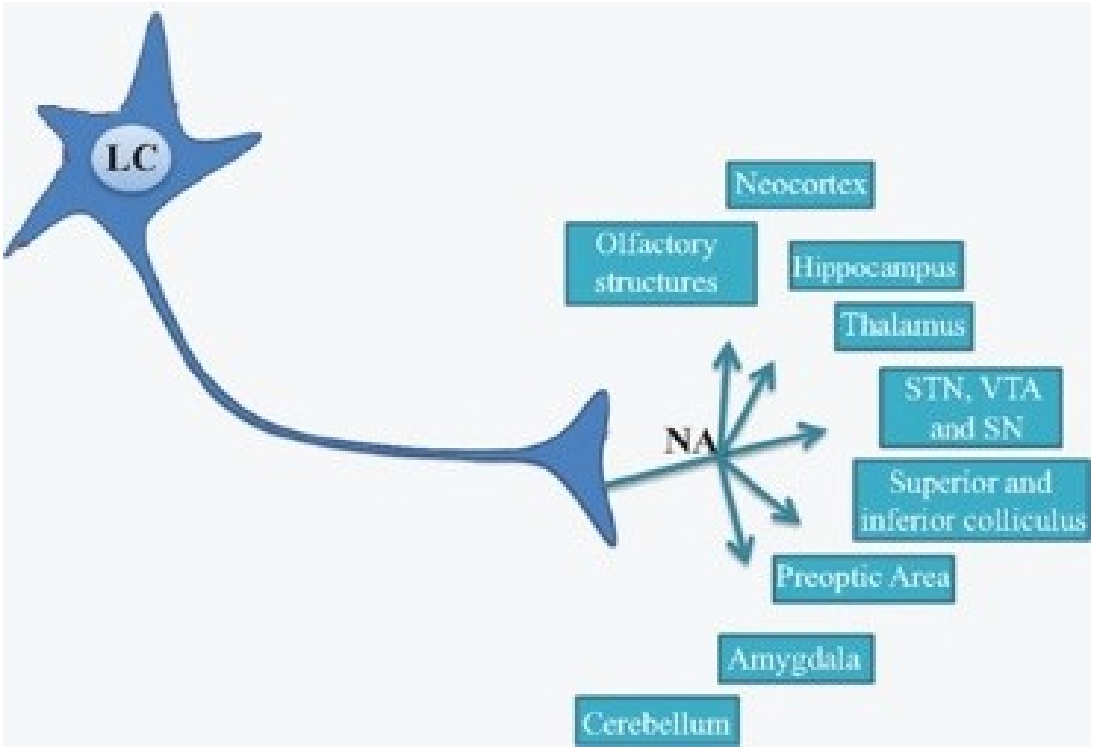

Figure 5. Locus coeruleus projections. Efferent projections of the LC bring norepinephrine to vast regions of the CNS (substantia Nigra (SN), subthalamic nucleus (STN), ventral tegmental area (VTN)) (Modified figure from Delaville 2011).

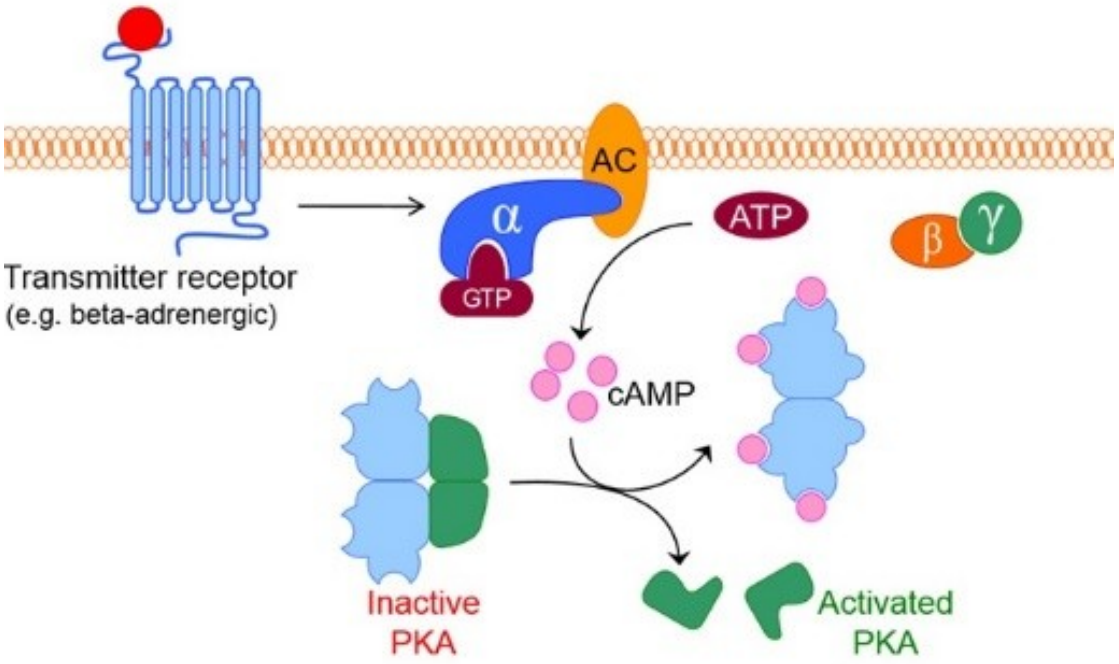

Figure 6. PKA pathway. The subunit alpha from Gs activates adenylyl cyclase (AC) which consequently produces cAMP. Two cAMP molecules (pink) bind each of the two regulatory subunits light blue). This causes the regulatory subunits to dissociate and allows the catalytic subunit of PKA (green) to be activated (Modified from Nguyen and Woo, (2003) Prog. Neurobiol. 71: 404-437).

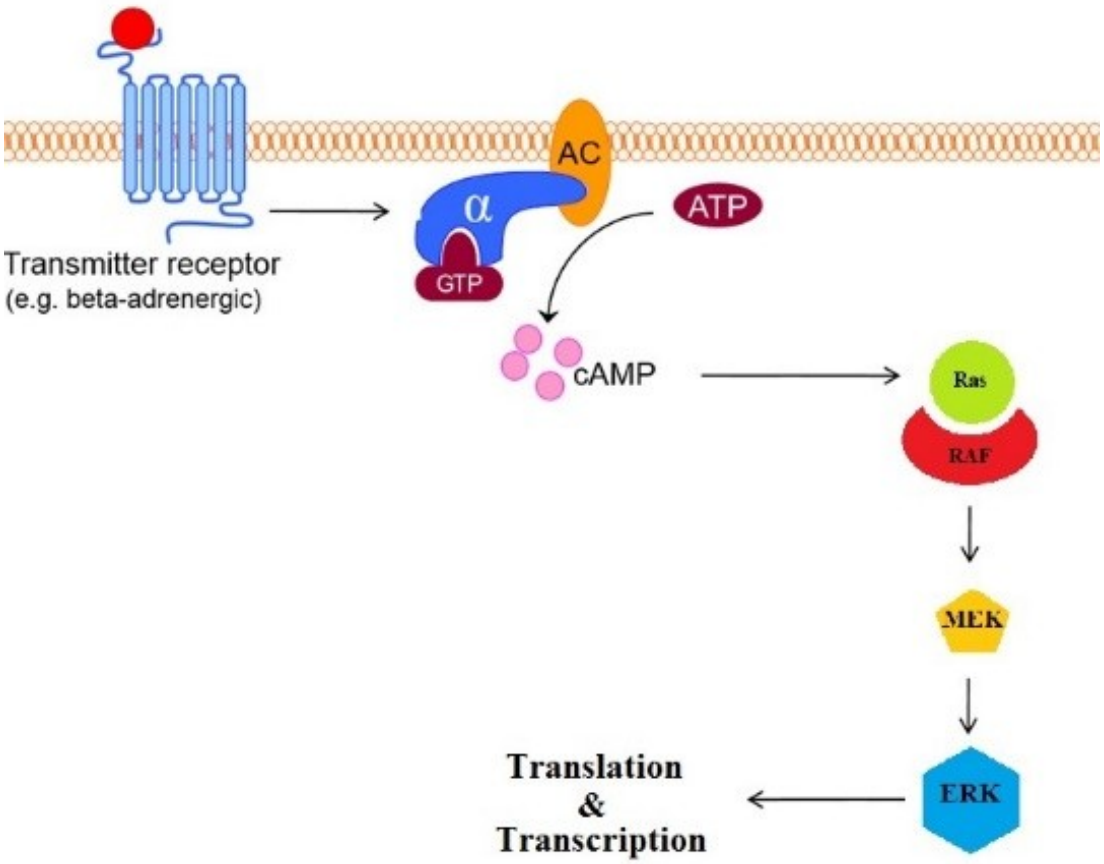

Figure 7. Activation of ERK pathway via β-AR. cAMP can initiate crosstalk between the β-AR pathway and the ERK pathway via Ras [Adapted from Stork and Schmitt 2002]

Chapter 2 - Materials and Methods

2.1 Animals

Male mice (Charles River Canada, C57BL/6, and aged 7 – 12 weeks) were used for all experiments. Animals were kept on a 12h light-dark cycle with no environmental enrichment in cages. All experiments were carried out during the light duration of the cycle. The animals were housed at the University of Alberta's animal facility centre.

2.2 Slice Preparation

Following rapid cervical dislocation and decapitation, the intact brain was removed quickly and placed in a beaker of ice-cold artificial cerebrospinal fluid (aCSF) composed of (in mM): 124 NaCl, 4.4 KCl, 1.3 $MgSO_4$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, and 10 glucose, aerated with carbogen (95% O_2 and 5% CO_2). The brain was then divided into 2 hemispheres, and the hippocampus was extracted from each hemisphere. The hippocampus was then placed on a tissue chopper (Stoelting Co.). The ends of the hippocampus were removed and transverse hippocampal slices (400-μm thick) were cut. The slices were then placed in an interface recording chamber (Nguyen 2006) maintained at 30°C. The slices were continuously perfused with aCSF (1 mL/min). Electrophysiological recordings of extracellular field excitatory postsynaptic potentials (fEPSPs) began following a 90 min recovery period.

2.3 Electrophysiology

A glass microelectrode (2 – 3 MΩ resistance) filled with aCSF was positioned in stratum radiatum of area CA1 for recording fEPSPs. For the PD98059 (an ERK pathway inhibitor) experiment, the Schaeffer collateral fibers were stimulated at two separate sets of inputs (S1 and S2) converging onto the same postsynaptic population of neurons using two bipolar nickel-chromium electrodes (diameter 130 μm; AM Systems). One set of inputs was used for monitoring fEPSPs during basal stimulation in the presence of the ERK inhibitor.

Test stimulus intensity was set to elicit baseline fEPSP sizes that were 40% of maximal amplitude (all experiments required a minimal amplitude of 3mV). Subsequent fEPSPs were obtained at a "basal" stimulation rate of once per minute at this test intensity, with S2 stimulation following S1 stimulation by 200 ms. To confirm independent pathways, I used inter-pathway paired pulse facilitation (PPF) elicited by successive stimulation through the two electrodes (S1 and S2) at 50, 100, 150, and 200-ms inter-pulse intervals. Pathways were considered independent when no PPF was observed. After establishing a 20-30 min baseline, LTP was induced at S1 alone, through application of one train of high frequency stimulation (HFS; 100-Hz, 1-s duration) 30 min after application of NE (10 μM for 15 min). A low-frequency stimulation (LFS; 5-Hz, 3 min duration) was applied 10 min after HFS to induce depotentiation (DPT). All fEPSPs were measured by an amplifier and were low-pass filtered at 2 kHz. Responses were digitized at a rate of 20 kHz by a Digidata 1200 system and recordings were analyzed offline with pClamp-10 software (Axon Instrument Inc.).

2.4 Drugs

Norepinephrine (NE): Fresh stock solutions of NE were made daily to minimize oxidation. NE (L-(2)-norepinephrine (+)-bitartrate salt monohydrate; Sigma) was prepared in aCSF as 1 mM stock solution and diluted to a final concentration of 10 mM for bath application. An NMDA receptor antagonist, DL-2-amino-5-phosphonopentanoic acid (APV) stock solution was created in aCSF at a concentration of 50 mM, which was later diluted to 50 μM for experimental use. Propranolol, a β-adrenergic receptor antagonist, was also prepared in aCSF at a stock solution of 10 mM, and was diluted to 50 μM for experiments. KT5720: Dimethylsulfoxide (DMSO) was used to dissolve this PKA inhibitor at a stock concentration of 1 mM. It was diluted to a final concentration of 1 μM for bath application. A mitogen-activated protein kinase (MEK) inhibitor, PD98059, was dissolved in DMSO at a stock concentration of 10 mM. This was diluted to 50 μM for bath application to slices. Both working solutions of KT5720 and PD98059 had 0.1% DMSO, which has no effect on basal fEPSPs in area CA1 of mouse slices (Woo and Nguyen 2002). PKI was prepared in aCSF as 1 mM stock solution and diluted to a final concentration of 5 μM for bath application.

2.5 Data Analysis

The initial slope of fEPSP was measured as an index of synaptic strength. Baseline fEPSP slopes were measured during the first 20 min of recording. Subsequent fEPSP slopes were compared with baseline slope values and were plotted as a percentage of baseline. fEPSP slopes measured 60 min after LFS (5-Hz) were used for comparisons of LTP.

One-way ANOVA and Tukey-Kramer post hoc tests (Graphpad Instat Software) were done for comparison of more than two groups to determine which groups were significantly different from the others. Data are reported as means +/- SEM, with n = number of slices.

Chapter 3 - Results

3.1 Norepinephrine provides protection from depotentiation

Depotentiation is an activity-dependent reversal of previously-induced LTP that can occur for a short duration after LTP induction (Fujii, Saito et al. 1991, Huang, Liang et al. 1999). It has been established that low-frequency stimulation (LFS; 3 min of 5-Hz stimulation) can induce depotentiation (O'Dell and Kandel 1994). Although NE can "prime" synapses to express persistent LTP (i.e. NE-mediated metaplasticity of LTP) that requires translation (Maity et al. 2015), and translation functions to protect CA1 synapses from depotentiation (Woo and Nguyen 2002), it is unclear whether exposure to NE can elicit protection from depotentiation. Thus, I wanted to determine if NE-mediated metaplasticity could protect the synapse from depotentiation.

HFS alone elicits short-lasting potentiation that decays to baseline within 90 minutes **[Figure 8]** and LFS alone does not elicit depression **[Figure 9]**. In the present study, LFS applied 10 min. after HFS in the absence of prior NE elicited successful depotentiation of LTP **[Figure 10A]**. Thirty minutes after NE was applied **[Figure 10B]**, a high frequency stimulus (HFS; 100-Hz for 1 s) was given to elicit LTP. Ten minutes afterwards, LFS was given **[Figure 10B]**. I observed that with the presence of NE, fEPSPs returned to potentiated levels observed prior to the LFS **[Figure 10B]** (mean fEPSP slope was potentiated to 143 +/- 10% at 60 min after LFS; $p < 0.01$ compared with controls).

The modulation of the synapse via NE has been shown to be more dependent on beta-adrenergic receptors (β-ARs) than on alpha-adrenergic receptors (Katsuki, Izumi et al. 1997). A β-AR

antagonist, propranolol, was used in the present thesis to test whether β-AR was critical for NE-mediated protection. My results show that propranolol applied prior to, during, and after NE blocked recovery from LFS **[Figure 10C]**. There were no signs of recovery to potentiated levels prior to the LFS **[Figure 15]** (mean fEPSP slope was 106 +/- 4% 60 min after depotentiation stimuli; $p < 0.05$ compared with controls). Thus, my results establish that NE can prime synapses to be protected from activity-induced depotentiation of previously induced LTP. Also, this protection required activation of β-ARs.

Previous research has shown that protein synthesis-dependent forms of LTP are immune to depotentiation (Woo and Nguyen 2002). One form of protein synthesis-dependent LTP is β-AR mediated LTP, which was shown to require protein synthesis to *maintain* LTP (Gelinas, Banko et al. 2007). Which signaling pathways distal to β-AR activation are critical for protection?

3.2 PKA activation is required for protection from depotentiation

β-ARs can recruit PKA activation (Thomas, Moody et al. 1996). PKA itself is crucial for expressing metaplasticity of LTP when β-ARs are activated (Tenorio, Connor et al. 2010). However, it is unknown whether PKA activation is required for protection from depotentiation. I therefore hypothesized that PKA is required for protection from depotentiation.

A PKA antagonist, KT5720, was applied after HFS. My data show that activation of PKA is necessary for protection. KT5720 alone, in the absence of prior NE, elicited no modification of LTP (Figure 11A; mean fEPSP slope was 93 +/- 5% of baseline, 60 min after LFS; $p < 0.01$ compared to NE). When NE was applied prior to HFS, with KT5720 post-HFS, there was no evidence of protection. The fEPSPs returned to baseline after LFS, without recovering to

potentiated levels (Figure 11B, mean fEPSP slope was 105 +/- 9 % of baseline, 60 min after LFS; $p < 0.05$ compared to NE).

A more specific PKA inhibitor, PKI, was used to confirm previous result. PKI was applied after HFS for 30min. Results show that when NE was applied prior to HFS, with PKI post-HFS, there was no protection. The fEPSPs returned to baseline after LFS, without recovering to potentiated levels (Figure 12; mean fEPSP slope was 101+/- 4% of baseline, 60min after LFS; $p<0.05$ compared to NE).

Thus my experiments here demonstrated a critical requirement for PKA activation in protection from depotentiation following NE-induced priming of LTP. As PKA indirectly engages the ERK pathway, which in turn recruits translation initiation during β-AR-dependent LTP (Gelinas et al. 2007), I next asked whether ERK activation is required for protection from depotentiation following NE-induced metaplasticity.

3.3 ERK activation is required for protecting synapses from depotentiation

Another protein kinase downstream of PKA which has been strongly implicated in LTP is the extracellular signal-regulated kinase (ERK). ERK is activated during LTP induced by conjoint HFS and β-AR activation (Gelinas et al. 2007). To determine if ERK activation is required in metaplasticity, I applied PD98059, a mitogen-activated protein kinase kinase (MEK) inhibitor. MEK needs to be active to phosphorylate ERK to activate it; thus, inhibiting MEK ultimately inhibits ERK activation. Slices treated with PD98059 showed no recovery to potentiated levels post-LFS (Figure 13, mean fEPSP slope was 101 +/- 13 % of baseline 60 min after LFS; $p < 0.05$ compared to NE), while a second pathway showed that PD98059 did not affect baseline fEPSPs

(Figure 13, black curve). These results suggest that ERK activation is required for NE-mediated protection from depotentiation. They are consistent with previous findings that ERK initiates protein synthesis (during β-adrenergic LTP: Gelinas et al. 2007) that in turn, can protect previously induced translation-dependent LTP (elicited by HFS alone) from depotentiation (Woo and Nguyen 2002).

3.4 Role of NMDARs in protection from depotentiation

Activation of N-methyl-D-aspartate (NMDA) receptors is a key trigger for LTP (Collingridge, Kehl et al. 1983, Morris, Davis et al. 1990, Nicoll and Malenka 1999). The NMDA receptor is a glutamatergic receptor, which when activated allows postsynaptic influx of calcium that triggers calcium-dependent pathways critical for eliciting LTP (O'Dell and Kandel 1994, Lisman, Schulman et al. 2002). NMDARs have also been shown to modulate metaplasticity, as their prior activation can *block* the future induction of LTP (Collingridge, Isaac et al. 2004, Andersen, Morris et al. 2006).

To determine if NMDAR activation is required for protection from depotentiation provided by NE-mediated metaplasticity, I applied 2-amino-5-phosphonovaleric acid, APV (50 μM for 10 min), an NMDAR antagonist, after LTP induction by HFS. My results indicate that APV alone does not alter LTP **[Figure 14A]**. The mean fEPSP slope was 118 +/- 9% of baseline, 60 min after LFS; $p > 0.1$ compared with NE **[Figure 14]**. When NE was applied, I observed that subsequent addition of APV blocked the protection provided by the NE seen previously (Figure 14B, mean fEPSP slope was 102 +/- 5% of baseline levels, 60 min after LFS; $p < 0.05$ compared

with NE in Figure 10B) . Taken together, these results show that activation of NMDARs is necessary for NE-mediated protection from depotentiation.

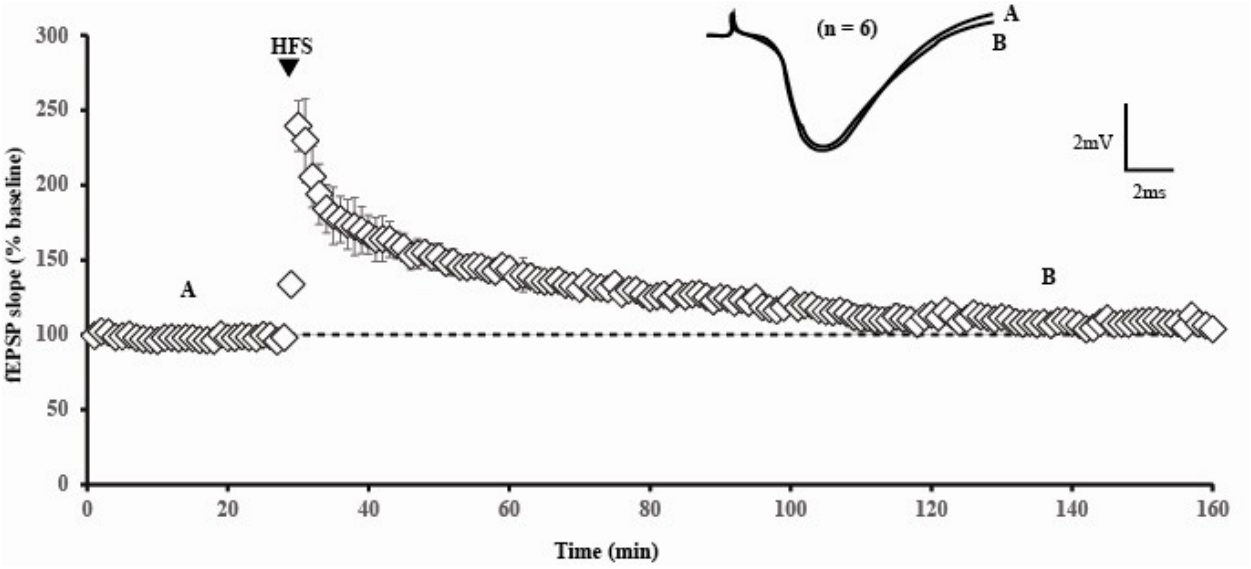

Figure 8. HFS stimulation elicits short-term potentiation. Returns to baseline within 90min.

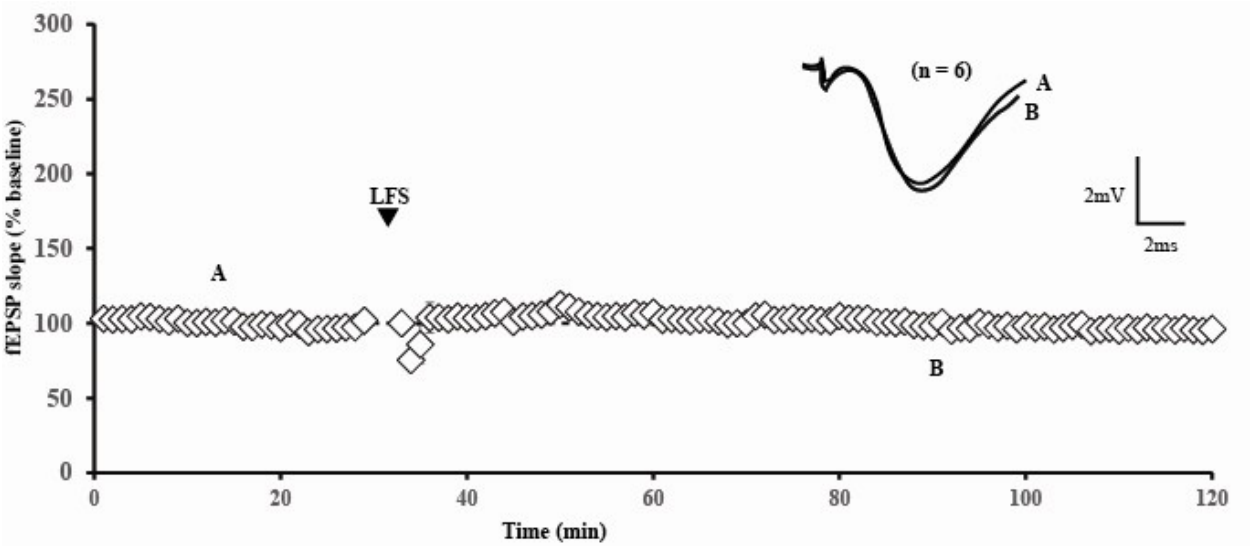

Figure 9. LFS alone does not elicit depression of the slice.

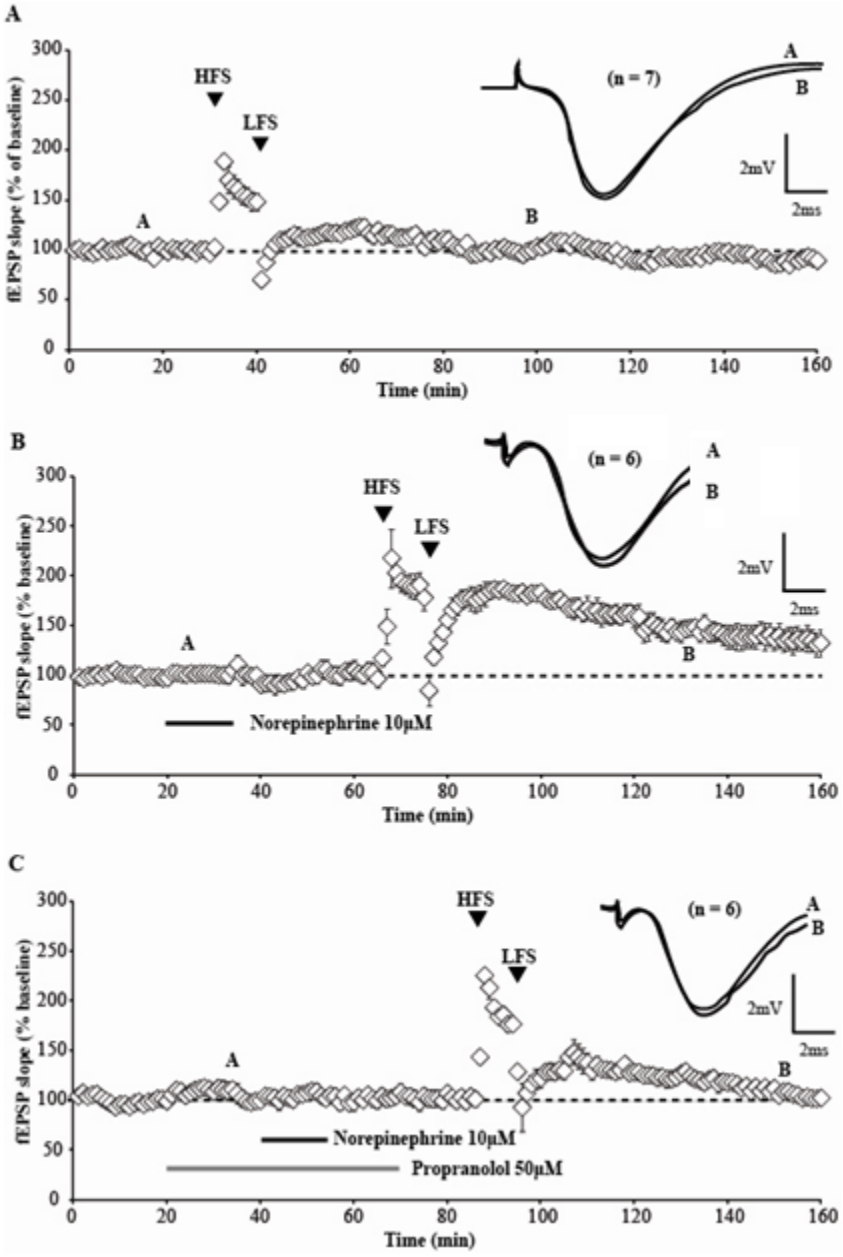

Figure 10. NE-induced metaplasticity provides protection against depotentiation. (A) HFS alone (open diamonds) elicits LTP. After LFS, fEPSPs return to baseline pre-HFS levels. (B) Applying HFS 30 min after NE produces LTP that is resistant to LFS-induced depotentiation. Note gradual recovery ("creep-up") of fEPSPs to potentiated levels, after LFS. (C) When a β-AR antagonist, propranolol was applied for 45 min overlapping with NE, it removed NE-mediated protection. Note fEPSPs now decay back to pre-HFS levels at point B. Sample traces were taken at points A and B on graphs.

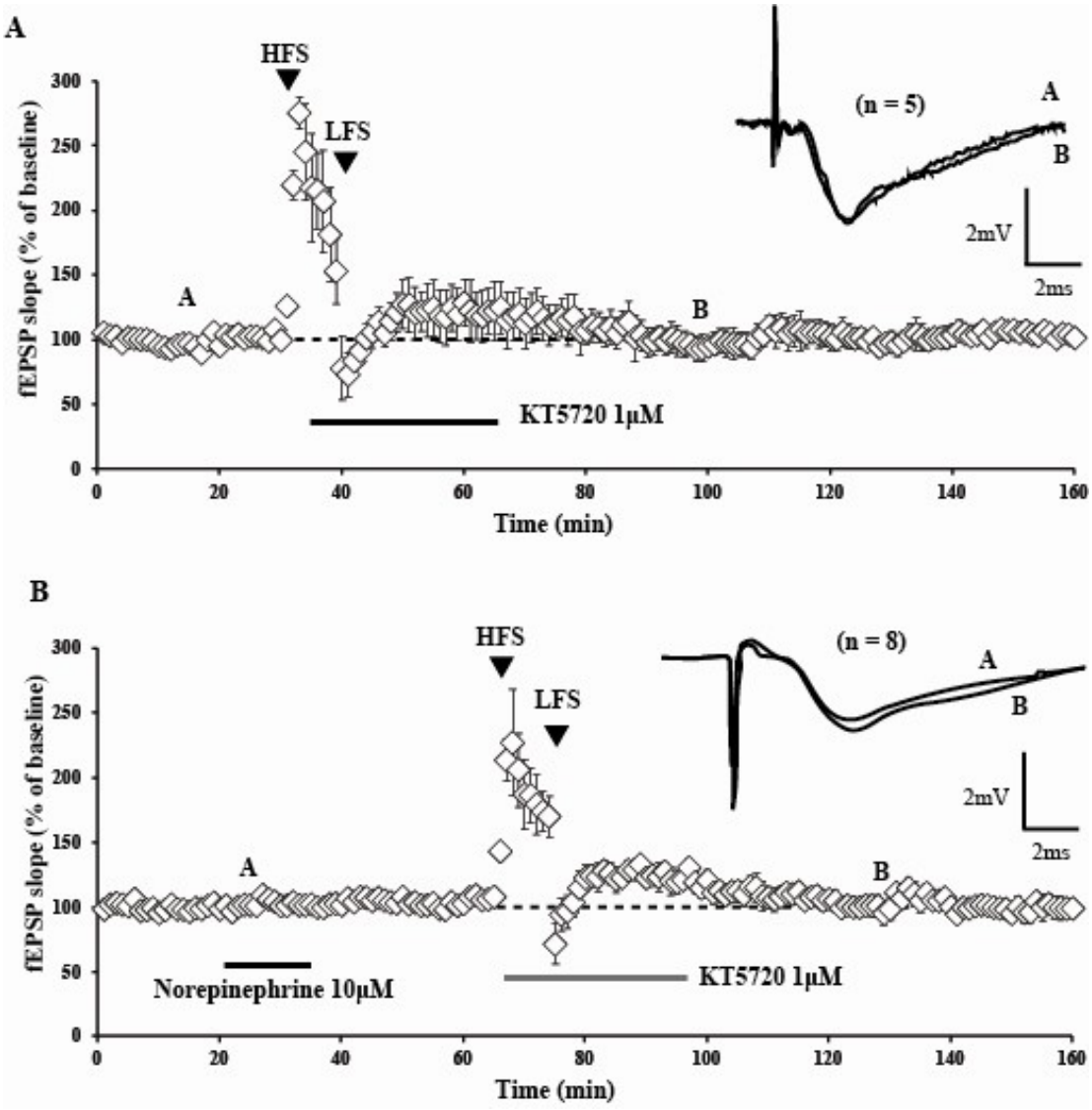

Figure 11. NE-mediated depotentiation protection requires PKA activity. (A) Application of KT5720 alone did not affect successful depotentiation. (B) Thirty minutes of KT5720 post-HFS prevented the NE-mediated protection. fEPSPs failed to recover to previously potentiated levels, instead decaying to baseline pre-HFS levels. Sample traces taken at points A and B on graphs.

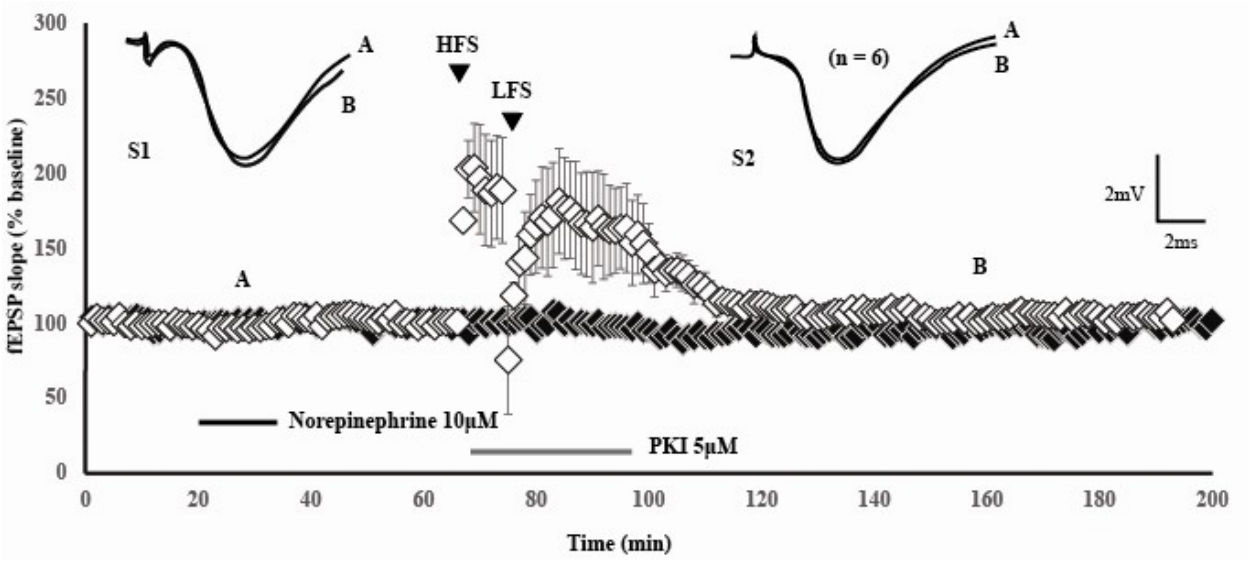

Figure 12. Figure 11 continued. Application of PKI for 30 min after HFS prevented NE-mediated protection (open diamonds). PKI application did not alter basal synaptic transmission in a second independent pathway receiving only basal stimulation at once per minute (black diamonds). Sample traces were taken at points A and B on graphs.

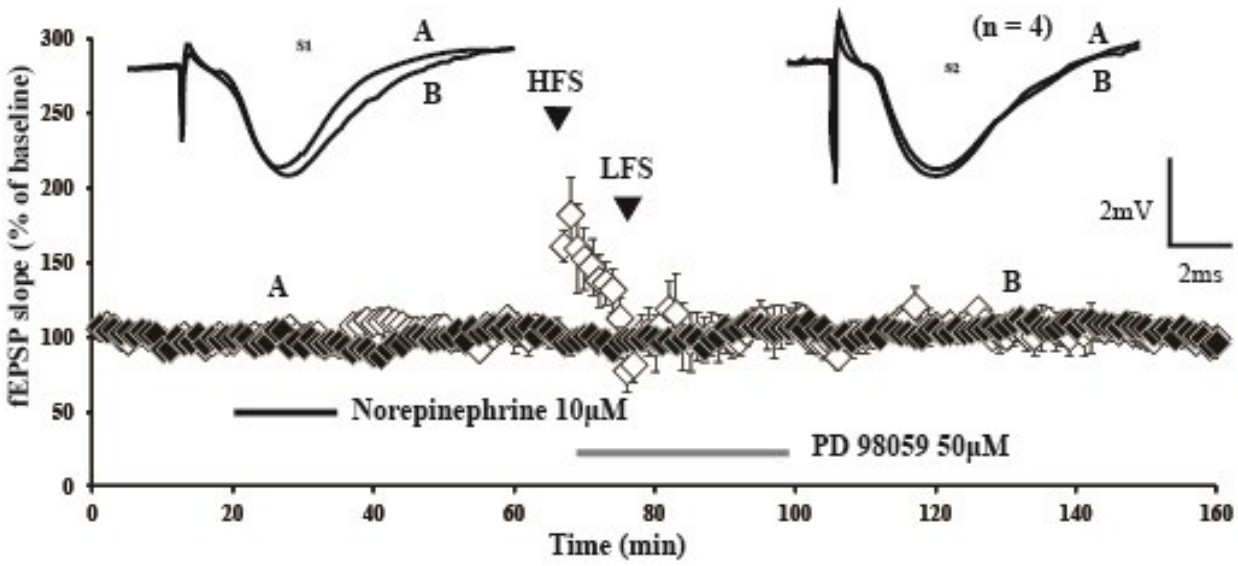

Figure 13. NE-mediated depotentiation protection requires ERK activity. Application of PD 98059 for 30 min after HFS prevented NE-mediated protection (open diamonds). PD 98059 application did not alter basal synaptic transmission in a second independent pathway receiving only basal stimulation at once per minute (black diamonds). Sample traces were taken at points A and B on graphs.

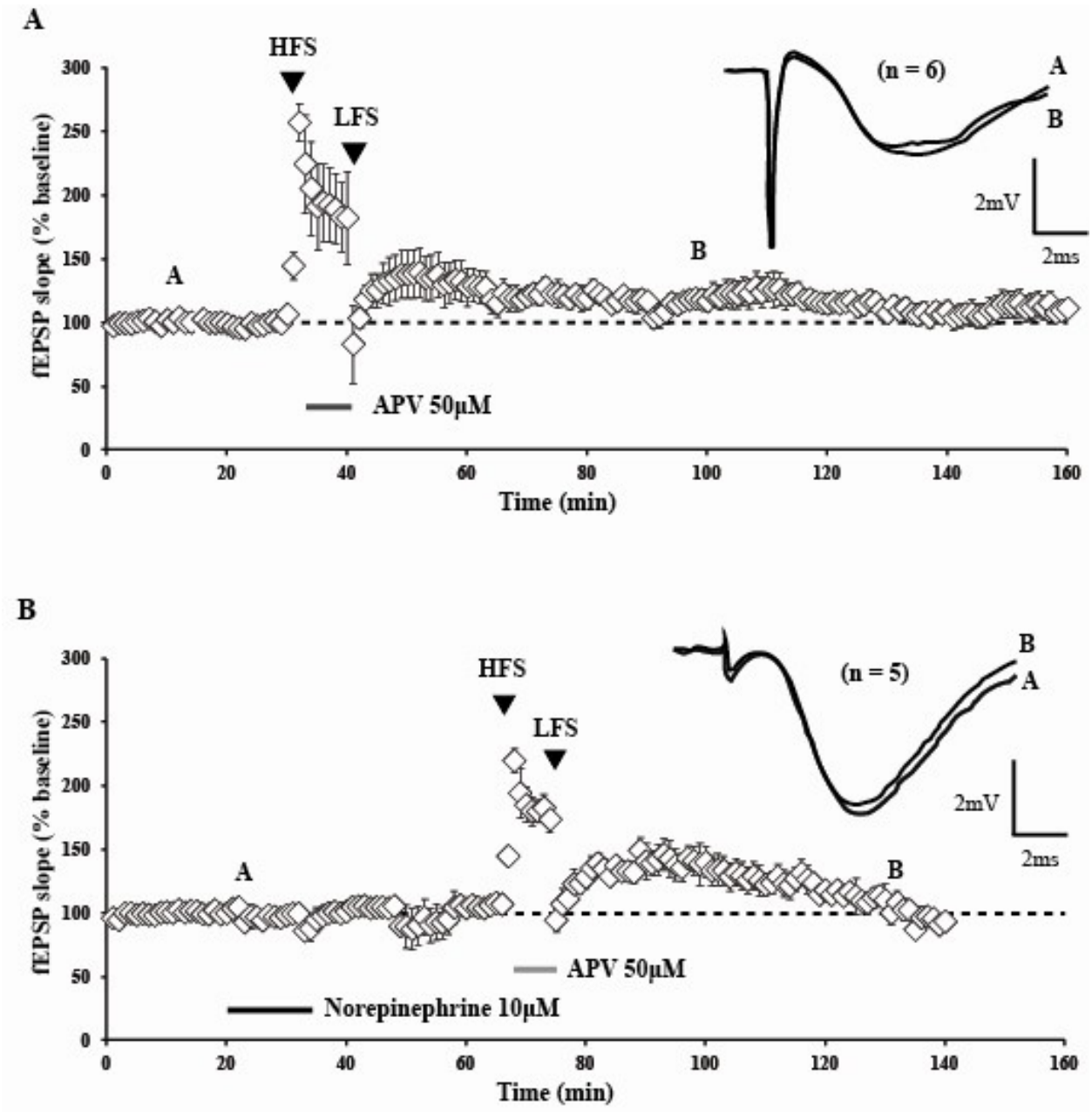

Figure 14. NMDA receptors are required for NE-mediated protection. (A) APV alone did not alter successful depotentiation after LFS (B) APV applied for 13 minutes after HFS prevents the slice from recovering to potentiated post-HFS levels of fEPSP slopes. fEPSPs decayed instead to baseline pre-HFS levels. Sample traces taken at points A and B on graph.

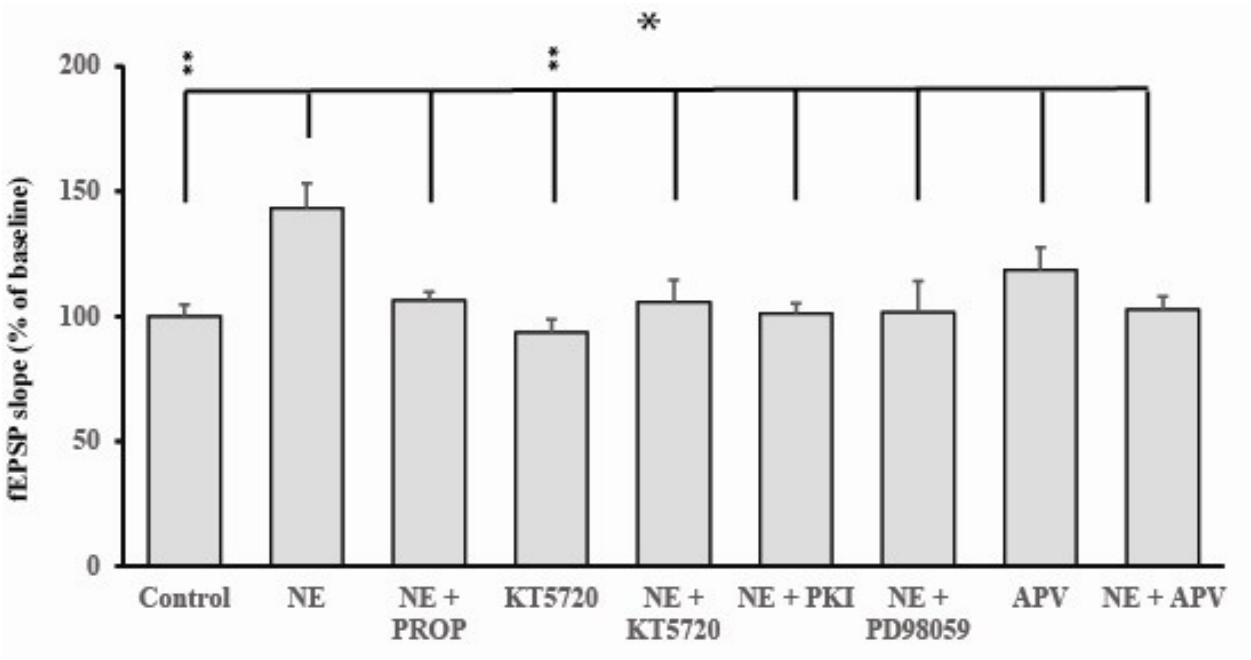

Figure 15. Summary histograms of fEPSP slopes obtained 60 min after LFS. Comparisons are to NE. Results represent means ± SEM. (*) $p < 0.05$ (**) $p < 0.01$, see text for group comparison data.

Chapter 4 - Discussion

The synaptic strength in the CA1 region of the hippocampus can be increased and decreased depending on the pattern of stimulation that activates NMDA receptors. (Malenka and Bear 2004). Low frequency stimulation, at 5-Hz, triggers a signal cascade through NMDARs, causing LTD or depotentiation (O'Dell and Kandel 1994). Others have shown that immunity to depotentiation is dependent on the amount of synaptic stimulation used to induce LTP, that it is input specific, and it is prevented by inhibitors of protein synthesis (Woo and Nguyen 2002). While Woo and Nguyen (2002) used specific stimulation (4 x 100Hz) to induce LTP that engaged protein synthesis, there are other ways of obtaining translation-dependent LTP. Gelinas et al. (2007) have shown that activation of β-ARs via isoproterenol (ISO) and pairing ISO with 1 x 100Hz, can elicit LTP lasting for several hours in a protein synthesis-dependent manner.

Taking a look at the previous studies, if 4 x 100Hz resulted in a protein synthesis-dependent LTP that was capable of protecting itself from depotentiation, then it would make sense that β-AR-activated LTP could be protected as well. For my experiments, I chose to use norepinephrine as my β-AR agonist rather than ISO, since NE is found naturally within the mammalian brain. One train of 100Hz is not enough to trigger a protein synthesis-dependent LTP, and thus it susceptible to depotentiation. When NE was added, LFS did not depress the potentiated synapse; this is synaptic immunity to depotentiation. However, when I added propranolol (Prop), a beta receptor blocker, it prevented NE from protecting synapses from depotentiation.

β-AR activation is known to recruit PKA (Thomas, Moody et al. 1996, Esteban, Shi et al. 2003). In my experiments, I showed that KT5720, a PKA inhibitor, prevented NE from protecting the synapse from depotentiation. The mechanism that allows NE to provide synaptic immunity to

depotentiation likely involves something downstream of PKA. As shown by Gelinas et al. (2007), β-AR-mediated LTP maintains its longevity by recruiting protein synthesis. This suggests that ERK may be important for immunity to depotentiation. ERK is critical for many forms of synaptic plasticity (Thomas and Huganir 2004), including β-AR mediated LTP. When ERK is phosphorylated, its activation eventually phosphorylates the eukaryotic initiation factor eIF4E, which will increase translation (Kelleher, Govindarajan et al. 2004).

To assess ERK involvement in the protection mechanism, I applied PD 98059, a selective MEK inhibitor. My results showed that the inhibition of MEK also prevented the protection mechanism. Taken together, my results suggest that PKA-ERK signaling is key to the mechanism behind NE-mediated protection of the synapse from depotentiation.

So far, all of my results have advanced previous findings and revealed a novel function for NE in regulation of synaptic plasticity. However, my work with APV has shown some divergence. O'Dell and Kandel (1994) showed that the depression of potentiated synapses by 5-Hz stimulation can be blocked by APV. My result showed that when NE was applied 30 min prior to APV, there was depression of synaptic transmission. While my result seems to confirm my hypothesis regarding NMDAR involvement in NE-mediated protection, it contradicts O'Dell's result. From O'Dell's experiments, we know that NMDARs are critical for depotentiation and that application of APV blocked any depression from occurring. Yet my results showed depression in the presence of APV.

My initial thought was that there had to be a link between NMDAR and β-AR that was altering the NMDAR function to diverge my results from previous studies. One potential link between the two pathways are the Src family kinases (SFKs). This family of kinases act as a crucial

intermediary in multiple signaling pathways that upregulate NMDAR function (Kalia, Gingrich et al. 2004), including the G-protein coupled receptor activated by NE. In this case, β-AR activation may be upregulating NMDAR activity via SFK to a point where APV is no longer having the desired effect. If my theory was correct, that would mean NE would prevent NMDAR from being inhibited by APV. Unfortunately, this theory further complicates my interpretation. This is because it would mean that NE has no protective function while NMDAR is still active, which is not true since my experiments have already shown NEs ability to protect [Figure 10]. Therefore, an alternative explanation for the NMDAR results is required.

Previous studies have shown that NMDA receptor activity is required for β-ARs to mediate LTP (Tenorio, Connor et al. 2010). So another explanation could be that β-AR-mediated LTP requires NMDA receptor activation post-HFS to maintain potentiation. This would mean my APV application after the 100-Hz could have blocked potentiation prior to the LFS. Consequently, after the LFS was applied, rather than returning to the potentiated level, the slice returned to baseline.

My NMDAR experiments have not produced a clear conclusion to NMDAR function in protection. Future prospects in this field should target proteins downstream of NMDARs to better elucidate the role of NMDARs in NE-mediated protection. It is known that depotentiation is mediated through the NMDA receptor. The activation of the receptor eventually activates calcineurin (PP2B), which dephosphorylates inhibitor-1(I1) at a PKA-regulated site. This allows protein phosphatase-1 (PP1) to be active and to dephosphorylate postsynaptic proteins to mediate depotentiation (O'Dell and Kandel 1994, Bear 2009). To show if NMDAR activation is required for NE-mediated protection, NMDARs first must be allowed to function intact, since it was proven to be required for β-AR-mediated potentiation (Tenorio, Connor et al. 2010). Therefore,

to specifically target depotentiation, future experiments should examine these protein phosphatases [Figure 16].

In summary, while the NMDAR experiments proved to be inconclusive, my PKA and ERK data shed light on potential mechanisms required for depotentiation protection. As I mentioned, depotentiation requires PP1 activation (O'Dell and Kandel, 1994), thus it likely requires I1 activation as well. I1 can be inactivated by being phosphorylated by PKA, thus preventing depotentiation (O'Dell and Kandel 1994, Bear 2009, Sanderson 2012). PKA also seems to phosphorylate AMPA receptor subunits to increase receptor insertion into the synapses (Esteban, Shi et al. 2003). Such insertion would assist in maintaining potentiation of the EPSPs over time. Taken together, it seems PKA plays a crucial role in both mediating potentiation and providing protection from depotentiation, as evident from the recovery of EPSPs to previously potentiated levels seen after HFS.

Previous research has shown, via polysome profiling, that NE and 100-Hz are capable of recruiting translation of GluA1/2 subunit mRNAs (Maity, Rah et al. 2015). This means that the enhancement of LTP by NE is being maintained by the upregulation of newly made AMPARs. But how exactly is protection from depotentiation being conferred by synthesizing new protein? Although no study has been done to answer this specific question, my hypothesis would be that the additional AMPAR subunits produced by ERK-activated translation work in conjunction with the PKA mechanism described above. I speculate that the addition of new AMPAR subunits into the synapse may potentially "outnumber" the de-phosphorylation of pre-existing AMPARs via PP1.

Norepinephrine-facilitated β-AR activation leads to significantly increased memory formation and retention. I have shown that β-AR activation by NE also provides synaptic protection from depotentiation. Although I have characterized some of the mechanisms, more research is needed to fully understand the microphysiology of NE-mediated protection.

In the context of the human mind, what does my research really mean? Enormous amounts of information pass through our brains. I hypothesize that LTP-like processes (perhaps "equal" to memories) are elicited in the hippocampus, along with sporadic occurrences of depotentiation (resetting of synaptic strength), meaning that some of these LTPs are being reduced by DPT. This might explain the phenomenon of forgetting. NE, which is released during emotional arousal/stress, primes hippocampal neurons, allowing even a modest stimulus to trigger L-LTP (long term) rather than E-LTP (short term). This priming could mean that emotional events prime our brain to remember with much more ease. My research could also imply that emotional arousal prevents LTP from being erased within the hippocampus. From an evolutionary point of view, this could have benefits. A strong emotional event can help the organism remember important information with extra clarity (without repetition), which can potentially alter the organisms' behavior in the future.

Emotionally-charged memories of some events (e.g. birthdays) can be harmless. Unfortunately, they can be also detrimental to a person's health (e.g. post-traumatic stress disorder (PTSD). Thus, clarification of this mechanism by which DPT protects LTP from erasure is critical, as it might bring insights on the nature of memory strengthening, or it may yield potential treatments for suppressing "negative" memories during PTSD.

4.2 Future work

In addition to my work, there is another alternative explanation for the NMDAR results. There exists a theory called the BCM theory, named after the three who proposed the idea: Bienenstock, Cooper, and Munro. Generally this theory proposes a sliding threshold for long term potentiation or depression induction. In this case, the prior application of NE may modify baseline glutamatergic neurotransmission. Therefore, low frequency stimulation may not induce DPT due to increased NMDAR function, depressing NMDAR with APV reduces this enhanced function and allows DPT to occur afterwards. To confirm this idea, I could do an experiment where the parameters before the HFS can be the same as my experiments (baseline, NE for 15min, 30min washout) but instead of HFS, I would only apply the LFS and potentially observe a change in baseline transmission.

If the experiment does not reveal any new significant information, then we can assume my proposal within the discussion was correct and we can further specify NMDAR's role in protection via NE by blocking the source of depotentiation: protein phosphatase 1 (PP1) [Figure 16].

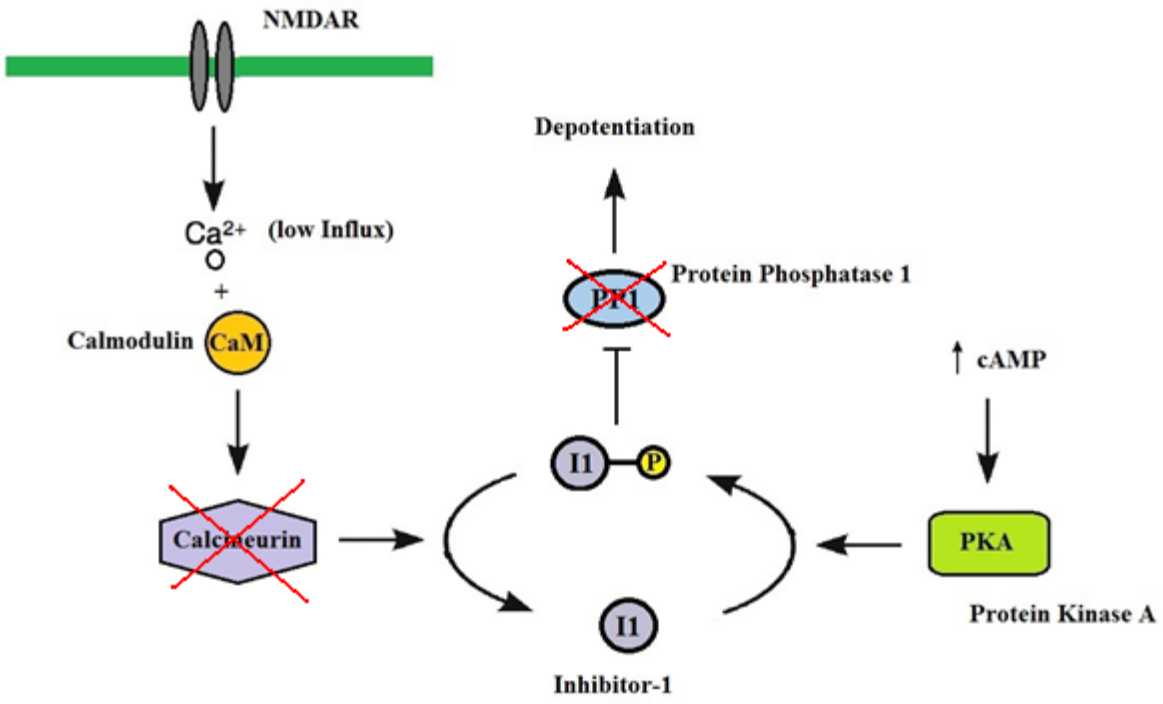

Figure 16. Future work. Potential targets to examine the role of NMDAR in depotentiation after NE application.

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