Modeling the CREB Gene Cascade

Zachary Kilpatrick

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0) Introduction

So far, the topic at hand this summer have been the genetics of neuroscience, broadly. The biochemical processes that regulate cellular activity of the brain has been examined by many texts and papers over the past few decades. Although I had a strong background in computational, applied, and even pure mathematics, my background on the biosciences was weak. Through consultation of much background material, I gained enough knowledge of these processes to model them mathematically. The modeling was then based on the work of Dr. Paul D. Smolen, of the University of Houston, Texas Medical Center in the Department of Neuroscience. I was fortunate enough to work alongside and receive advice from Dr. Smolen.

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1) The Biochemistry behind Memory: Review of *Memory* by Squire and Kandel

For over a century, scientists have studied learning and memory as regulated by the human brain. Beginning with Herman Ebbinghaus (1850-1909), who pioneered laboratory study of the subject, functions of the mind were studied as a science of biological function as opposed to philosophical supposition.[1] Thus began the research of the processes within the human brain that regulate memory and learning. Pavlov is well known for his experiments in classical conditioning of dogs, and these were some of the first experiments on learning.

A critical experiment in human learning came with William Scoville and Brenda Milner's study of H.M. in 1957. H.M. had had part of his brain removed on account of epilepsy. H.M. could not convert short term memories to long term memories, as most people can. Yet, he could maintain practiced motor skills, such as tracing a star on a mirror, a facet of the mind known as nondeclarative memory. Nondeclarative memory allows one to never forget how to ride a bike or swing a tennis racket. Yet one could remember how to do these things in action, but not know how to explain them. The knowing how to explain is called declarative memory. H.M. lost his ability to make new declarative memories. As to how these are made, and how this ability can be lost, one can look to Ramón y Cajal's work at the beginning of the 20th century.

Cajal argued that neurons, the elementrary signaling mechansim of the brain, had the ability to modify their connections to their neighbors—a property named synaptic plasticity. Repetition allows for continued use of a synapse between two certain neurons, and eventually structural change in the connection. This he supposed through study of the neuronal networks of a human baby. In recent years, experiments have been done on *Drosophilia* (fruit fly), *Aplysia* (sea slug), and rat neural cells in order that a deeper understanding of this synaptic plasticity might be uncovered. Kandel and Kupferman's experiment on the sensitization of *Aplysia* under the siphon-gill withdrawal reflex was a major project in this area of neuroscience.

Touching an *Aplysia* upon its siphon causes it to withdraw its siphon-gill apparatus. Doing this repeatedly eventually habituates the sea slug to this stimulus such that it does not withdraw the apparatus as quickly eventually. Readings were made on synaptic potentials and there appeared a noted decrease as a result of this synaptic plasticity. The rememberance of habituation was soon built into the neural circuit. Bailey and Chen did similar experiments with similar results. Sensitization is on the flip side of synaptic plasticity, resulting from a noxious stimulus (NS), such as an electric shock. The *Aplysia* becomes more sensitive

to the touch when this NS accompanies the touch. In this case, the synapses change and are said to be "facilitated" by the sensitization. Modulatory neurons regulate transmitter release in this gill withdrawal circuit by tuning the strength of sensory neuron synapses. One of the most important of these modulatory neurons uses serotonin as its transmitter.

Other aspects of these biochemical neuronal systems include 2nd messengers that carry information about the exterior of the cell to the interior. Sutherland, Rall, and Greengard discovered these in 1959, including one 2nd messenger in particular, cyclic adenosine monophosphate (cAMP), that is synthesized from adenosine-tri-phosphate (ATP) by the enzyme adenylyl cyclase. cAMP activates protein kinase A (PKA). Brunelli, Castelucci, & Kandel did an experiment where they injected cAMP directly into sensory neurons and discovered this injection strengthened effectiveness of connection between sensory neurons and target cells. Kandel and Siegelbaum discovered that serotonin, cAMP, and PKA all act on a K^+ channel called the S channel that is open at rest and closed by cAMP. Increasing the concentration of either of these three produces a larger response on the K^+ channel, which is instrumental in information transmission.

Also during sensitization, a train of action potentials produced by the conditioned stimulus (touch) and unconditioned stimulus (shock) pairing depolarizes the motor neuron and thereby unplugs the N-methyl-D-aspartate (NMDA) receptor channel such that Ca^{2+} will flow in to activate the molecular steps necessary to send a signal back to the sensory neurons. This was investigated by Carew, Walters, Hawkins, and Kandel (1983). Benzer, Quinn, and Dudai in 1968 were experimenting on *Drosophilia* and discovered that cAMP is indeed instrumental in both short and long term learning.

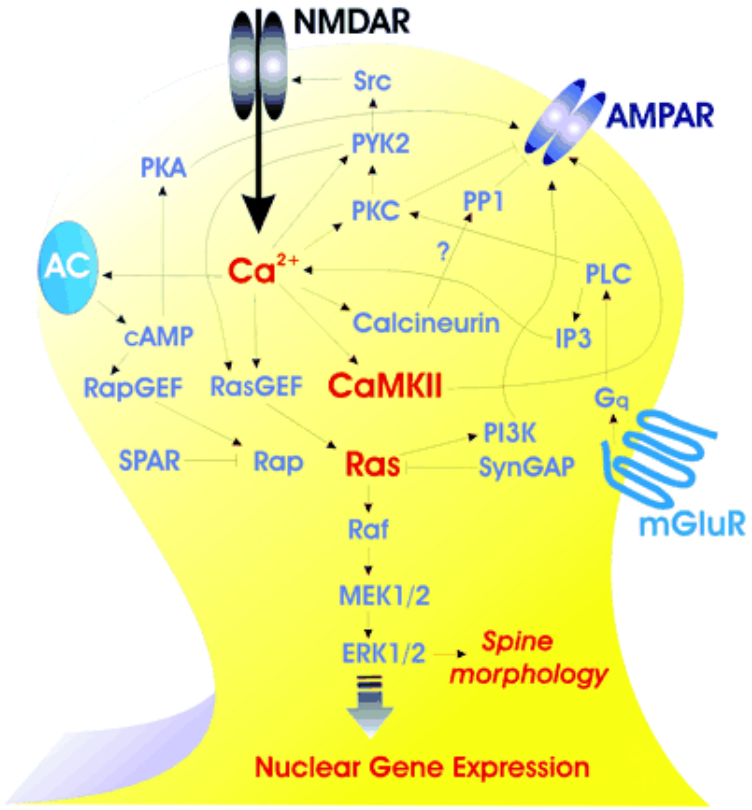

Figure 1: Postsynaptic glutamate receptor signaling pathways. The diagram focuses on the signaling pathways activated by calcium influx through NMDARs, and how these mechanisms interact with mGluRs, AMPARs, and adenylate cyclase (AC). Gq, G protein q. *Graphic taken from* ***Science*** [8]

On the subject of declarative memory, Fuster experimented with monkeys and short term working memory. He found that the portion of the brain concerned with knowledge is reactivated when declarative information is to be recalled. The tempral lobe houses recognition of shape, color, and vision. The medial temporal lobes are critical for the switch from short to long term memory. Alzheimer's disease affects the medial temporal lobe in humans. This initial insight into destruction of declarative memory came from the study of H.M. Amnesiac patients have damage done to the hippocampus, especially the CA1 region.

Declarative memory has been studied in monkeys by removing certain regions of the medial temporal lobe and testing the animals by Mishkin (1970). Monkeys performed a delayed nonmatching-to-sample task selecting between two colored objects, one they had seen before and one they had not. Monkey's that failed the tests then likely had portions of the brain missing required for declarative memory: the perirhinal cortex, entorhinal cortex, and parahippocampal cortex. It has also been shown that declarative memory has flexibility (Eichenbaum). McClelland, O'Reilly, and McNaughton investigated the fixation process of long term memory and found that connection increase stabilizes them. More repetition produces more synaptic connections between two neurons; memory is thus strengthened. The long term storage mechanism for declarative memory in mammals in found in the hippocampus as it has the binding function from connecting to so many cortical areas. Bliss and Lomo experimented in 1973 by stimulating the rat hippocampal pathway with a tetanus (a high frequency train of action potentials). If applied enough, this stimulus produced a long term increase in synaptic strength, a process known as long term potentiation (LTP).

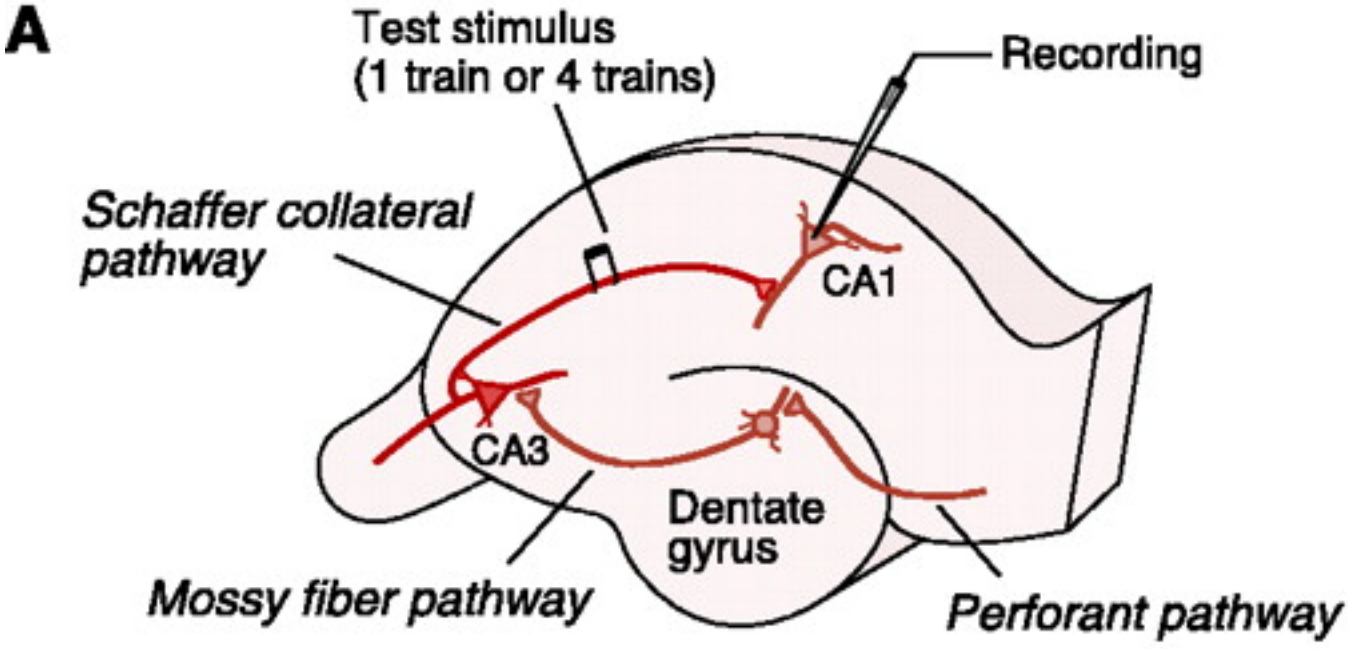

Figure 2: Long-term potentiation (LTP) in the hippocampus. (A) Three major pathways, each of which gives rise to LTP. The perforant pathway from the subiculum forms excitatory connections with the granule cells of the dentate gyrus. The mossy fiber pathway, formed by the axons of the granule cells of the dentate gyrus, connects the granule cells with the pyramidal cells in area CA3 of the hippocampus. The Schaffer collateral pathway connects the pyramidal cells of the CA3 region with the pyramidal cells in the CA1 region of the hippocampus. *Graphic taken from* ***Science***[11].

Long Term Potentiation occurs within 3 principal pathways through which information flows the hippocampus: (1)the perforant pathway; (2) the mossy fiber pathway; and (3) the Schaffer collateral pathway. LTP is induced rapidly— a single train of stimuli doubles synaptic strength. Once induced, the synapse is stable at the current strength for hours or even days, depending on the number of tetani. Methods for induction of LTP are of two types: associative and nonassociative. The dentate gyrus, a certain portion of the

brain connected to the hippocampus, receives information from the entorhinal cortex and conveys it to the hippocampus via granule cells. These granule cells send out axons by way of the mossy fiber pathway that terminate on pyramidial neurons of the CA3 region of the hippocampus. Mossy fibers then release glutamate as a transmitter. This is called nonassociative induction because it depends on brief, hi-frequency, neural activity in the presynaptic neurons and then an influx of Ca^{2+}. With the influx of Ca^{2+}, adenyl cyclase acts to increase the level of cAMP, which then activates PKA. Protein kinase A adds phosphate groups to proteins, thereby activating some proteins and inhibiting others. In *Aplysia*, this cascade is activated in the ganglion by the chemical messenger serotonin released by interneurons and leads to long term facilitation (LTF). When activated in rats and other mammals, mossy fiber LTP is not entirely influential in spatial and contextual memory. Yet, there is a correlation between LTP in the Schaffer collateral pathway and declarative memory. Pyramidial cells in the CA3 region send axons to cells in the CA1 region via the Schaffer collateral pathway and then glutamate acts as a transmitter. **Figure 3** shows a cartoon of the induction of LTP as it affects chemical messengers at the synapse.

Normally, channels are blocked with Mg^{2+}, but LTP creates hi-frequency synapse firing as the NMDA receptor channel is unblocked and Ca^{2+} can enter the cell much more freely. Richard Morris discovered that NMDA receptors are required for some forms of spatial learning and declarative memory. Synaptic efficiency also increases as glutamate transmitter levels also rise during LTP. Additionally, a higher level of intercellular Ca^{2+} provides for more active kinases to regulate more proteins. Among these kinases is CaM Kinase II, which inserts more AMPA receptors in the post synaptic membrane such that the principle ions (eg: Na^{+} and K^{+}), can be received at a higher rate across the synapse. Once a message is received by the postsynaptic neuron, a retrograde message is sent back to the presynaptic neuron. This retrograde message is usually in the form of substances such as nitrous oxide (NO). Yet, presynaptic activity is crucial for NO maintenance of LTP. Only those synapses where LTP is induced are then potentiated by this retrograde messenger. Thus is the surface bichemical regulation of LTP, but within the nucleus, there are gene regulatory networks that enhance LTP induction.

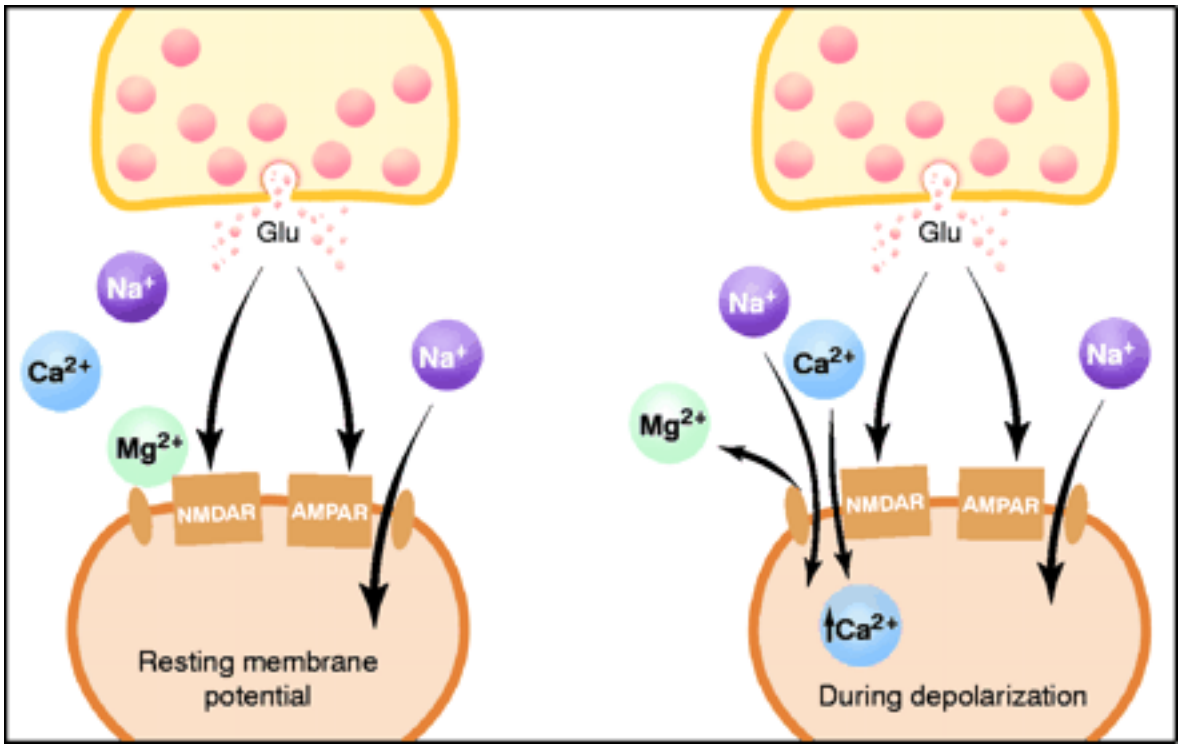

Figure 3: Model for the induction of LTP. During normal synaptic transmission, glutamate (Glu) is released from the presynaptic bouton and acts on both AMPA receptors (AMPARs) and NMDA receptors (NMDARs). However, Na^{+} flows only through

the AMPA receptor, but not the NMDA receptor, because Mg^{2+} blocks the channel of the NMDA receptor. Depolarization of the postsynaptic cell relieves the Mg^{2+} block of the NMDA receptor channel, allowing Na^{+} and Ca^{2+} to flow into the dendritic spine by means of the NMDA receptor. The resultant rise in Ca^{2+} within the dendritic spine is the critical trigger for LTP. *Graphic taken from* ***Science***[9]

Several methods have been developed to study the function of genes. One of these is gene knockout where a gene is removed completely; another is transgenic where a gene is injected into cells such that a gene's natural function is suppressed or enhanced. Gene knockout was used by Joe Tsien to remove a subunit of the NMDA receptor; he found this produced a deficit in LTP. He then used the transgenic method by injecting a CaM kinase promoter to overexpress a subunit of the NMDA receptor and discovered improvement in LTP. Findings have shown that NMDA receptors and LTP in the Schaffer collateral pathway are important for spatial memory.

Gene knockout has been restricted to certain regions in a process called the Cre/loxP system. Attatched to the gene to be knocked out are loxP "recognition sequences." In the case of Joe Tsien's experiment, the NMDAR1 gene in mice was to be knocked out. The Cre transgene, under the control of CaMK II, was then carried as a transgene in another set. These two sets were then mated and the offspring that ended up with both the Cre transgene and the NMDA R1 flanked by loxP have NMDA R1 snipped out in the CA1 region as Cre is only effective in the CA1 region of the hippocampus. The schematic for this method is given in **Figure 4**.

The brains of mice are also known to encode spatial information with place cells. The hippocampus may contain indeed a maplike representation of their environment not regulated by sensory input. Tsien found that disrupting the Schaffer collateral pathway and CaMKII overepression does not disrupt these place fields. Therefore, LTP must not be required for place field formation, but for fine tuning and functioning. Over time, these spatial maps become unstable. Indeed, overexpressing NMDA subunits improves the spatial memory. Mossy fiber and Schaffer collateral are used similarly in non-declarative memory though, both for short term storage and the conversion from short to long term memory.

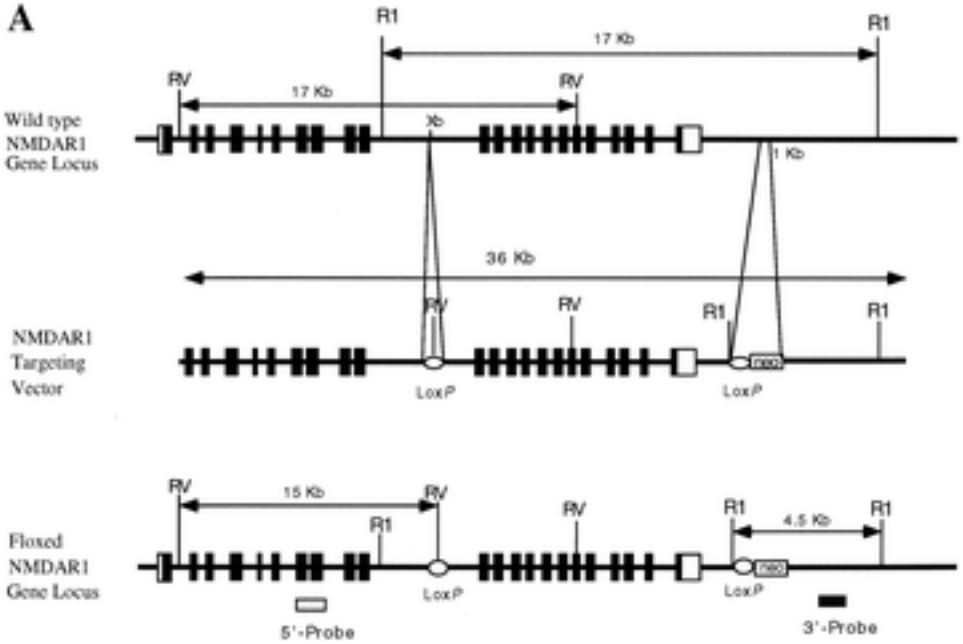

Figure 4: Targeted Disruption of the NMDAR1 Subunit Gene: (A) The NMDAR1 locus and targeting construct. (Top) The wild type genomic region that contains the NMDAR1 gene. (Middle) The NMDAR1 targeting vector that contains two loxP sequences. (Bottom) The floxed NMDAR1 gene. *Graphic taken from* ***Cell***[10]

The biological processes behind the conversion from short to long term memory is clearly a pressing question of neuroscience today. A concussion can disrupt this conversion as can weak formation of short term memories. Early scientists in the field, such as Ebbinghaus and James, uncovered that repetition is required for this conversion and that information is held ready in the short term while recall is required for long term. The three features of short term memory are: (1) it is transient; (2) it does not require anatomical change to retain; and (3) it does not require new protein synthesis. Long term memory requires new protein synthesis. A consolidation period, just after the short term memory is obtained, is required for stabilization of a long term memory. Retroactive interference after the short term memory is received, can disrupt the memory formation. C.P. Duncan experimented with seizure disruption on rats (1949). Sure enough, rats forgot their short term memories as the retrograde interference of the seizure had disrupted the consolidation period. Flexner, Agranoff, Barondes, and Squire inhibited protein synthesis in rats (1963). This also resulted in a lack of ability to convert short to long term memories. Schacher and Rayport went one step further to extract *Aplysia* nerve cells to examine this necessary protein synthesis. They constructed a more easily examinable circuit of this LTF. Similarly, Montarolo, Castelucci, Schacher, Goelet, and Kandel discovered in the tissue culture that more transmitter, specifically serotonin, applied to the circuit produced more synaptic facilitation. Certain genes needed to be switched on for this facilitation to take place.

Thus, serotonin acts to regulate gene expression in sensory neurons of the gill withdrawal circuit of *Aplysia* to establish new synaptic connections. An understanding of mechanisms that turn genes on and off is critical to exploring the switch from short to long term memory. The coding region of deoxyribonucleic acid (DNA), the building block of life, in which genes are imbedded, can be transcribed into messenger ribonucleic acid (mRNA), the delivery boy of genetics, and then translated into a protein. The control region comes before the coding region and is divided into the promoter and regulatory regions. The promoter region binds protein and determines the steady state level of transcription. The regulatory region is divided into response elements that bind to regulatory proteins that can be transcription regulators that either activate or repress. Second messengers signal transcription regulators to bind to response elements. PKA, as a kinase, is involved with genes and proteins for long term memory (LTM) by phosphorylating, as was described earlier, sites on transcription factors and genes themselves. There has been much experimentation in the last couple of decades on this specific area of genetics—memory genetics.

Tsien and Bacskai experimented with serotonin and *Aplysia*, and found that a single pulse of serotonin frees catalytic subunits of PKA for a few minutes and produces only a transient rise in cAMP. More pulses, though, led to longer freedom and more molecules sent to the nucleus. Genes critical for synaptic connections of long term memory are thus activated. What are called metabotropic receptors activate second messenger pathways and initiate modulatory action which modulates the strength of preexisting synapses. The architecture of the existing neuron is thus changed by sensitizing stimuli. Once PKA diffuses into the nucleus, it phosphorylates these transcription factors (eg: CREB-1, Squire & Kandel). Phosphorylated CREB-1 switches on genes for LTM and is in fact necessary and sufficient for LTM, as shown by the work of Bartsch, Casadio, and Kandel (1990). Squire and Kandel list the inhibitory regulator of LTM as CREB-2, which binds to CREB-1 and cAMP-response element (CRE), and is regulated by mitogen-activated-protein kinase (MAPK). Ghirardi, Bartsch, and Kandel found that blocking this repressor gives rise to long term facilitation for longer lasting synaptic connection growth. Yin and Tully found that overexpressing the activator CREB reduced the number of trials necessary to establish LTM.

One then wonders if the capability of controlling the expression of these genes could be beneficial in giving someone extreme memory skill. Those that have exceptional memory like the Talmund memorists need only see or here something once or twice to have it permanently engrained in their LTM. This seems like a skill many people would wish to have. The problem is that the hippocampus would be housing so much information that the faculties used to recall this information would be overloaded with information through which to sift. Therefore, there is no deep understanding for the memories contained, but only a myriad of stand alone facts and events. The process underlying flashbulb memory, where a traumatic or even just impressionable experience is deeply engrained in the mind, may involve a drastic and quick relief of CREB-2.

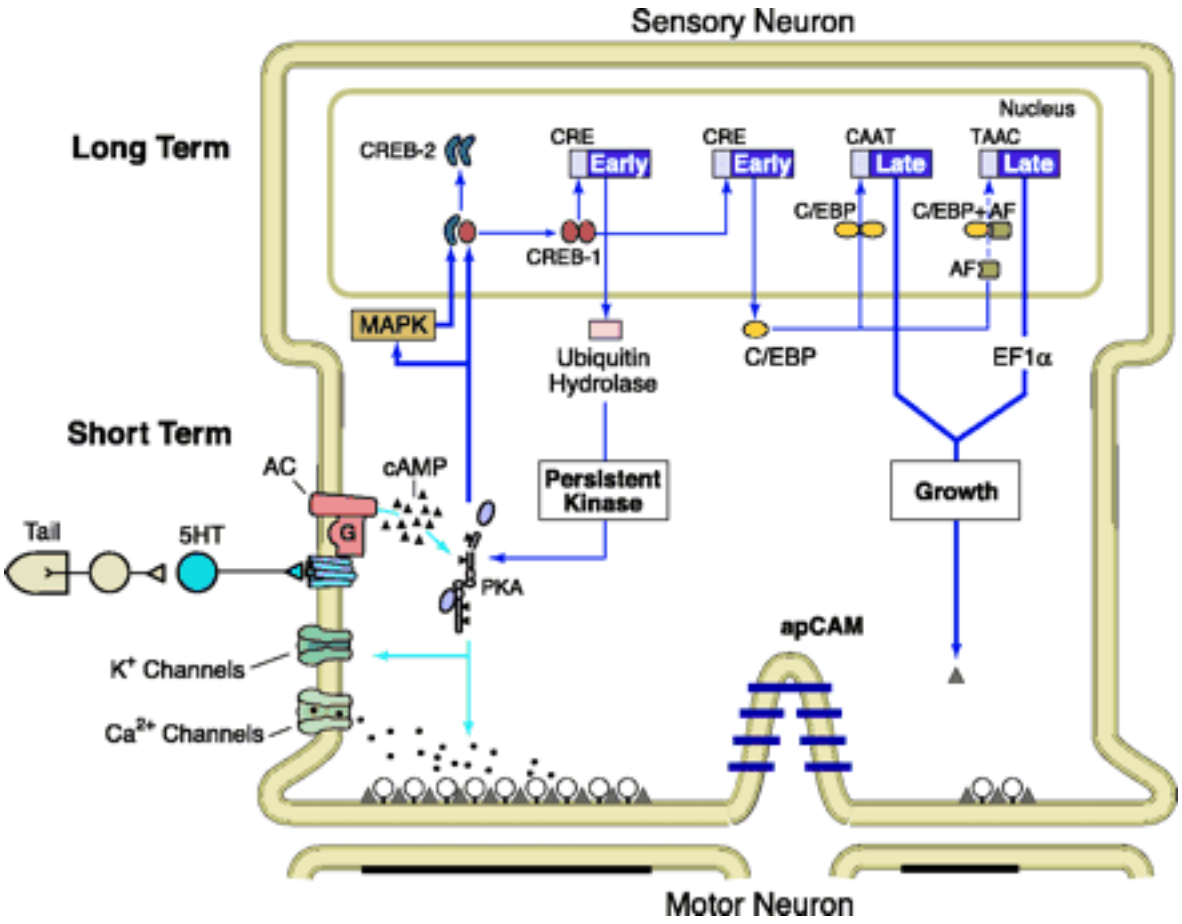

Figure 5: Effects of short- and long-term sensitization on the monosynaptic component of the gill-withdrawal reflex of Aplysia. In short-term sensitization (lasting minutes to hours) a single tail shock causes a transient release of serotonin that leads to covalent modification of preexisting proteins. The serotonin acts on a transmembrane serotonin receptor to activate the enzyme adenylyl cyclase (AC), which converts ATP to the second messenger cyclic AMP. In turn, cAMP recruits the cAMP-dependent protein kinase A (PKA) by binding to the regulatory subunits (spindles), causing them to dissociate from and free the catalytic subunits (ovals). These subunits can then phosphorylate substrates (channels and exocytosis machinery) in the presynaptic terminals, leading to enhanced transmitter availability and release. In long-term sensitization, repeated stimulation causes the level of cAMP to rise and persist for several minutes. The catalytic subunits can then translocate to the nucleus, and recruit the mitogen-activated protein kinase (MAPK). In the nucleus, PKA and MAPK phosphorylate and activate the cAMP response element-binding (CREB) protein and remove the repressive action of CREB-2, an inhibitor of CREB-1. CREB-1 in turn activates several immediate-response genes, including a ubiquitin hydrolase necessary for regulated proteolysis of the regulatory subunit of PKA. Cleavage of the (inhibitory) regulatory subunit results in persistent activity of PKA, leading to persistent phosphorylation of the substrate proteins of PKA. A second immediate-response gene activated by CREB-1 is C/EBP, which acts both as a homodimer and as a heterodimer with activating factor (AF) to activate downstream genes [including elongation factor 1 (EF1)] that lead to the growth of new synaptic connections. *Graphic taken from* ***Science***[11]

During the formation of long term memories, protein synthesis activated by CREB-1 is sensitive during the consolidation period. Immediate response genes are activated during this consolidation phase rapidly and transiently. For example, in *Aplysia* a ubiquitin hydrolase encoder and C/EBP are rapidly induced. Ubiquitin hydrolase destroys regulatory subunits of PKA that are a constraint on LTM. Once cAMP returns to its normal level, ubiquitin hydrolase overrides the phosphatase that is a limit upon PKA. In this way, ubiquitin hydrolase is a positive feedback loop. C/EBP works within the nucleus to activate genes that make

these new synaptic connections. LTM storage requires a structural change to pre- and post-synaptic cells as shown by Bailey and Chen. A single synapse can be altered for LTF while its neighbor is not.

There exists an early and late phase for LTP in the Schaffer collateral pathway of the mammalian hippocampus. Early phase is induced by tetanization and does not require protein synthesis. Late phase requires gene activation and, of course, protein synthesis. There is a high probability of transmitter release during late LTP and the post-synaptic spine grows to split the active zone in two. The level of cAMP rises and so does PKA and the activation of CREB-1. Transgenic mice with blocked PKA and CREB-1 have been shown to have defective late phase LTP. This places a high importance on the kinase, PKA, for short to long term memory conversion.

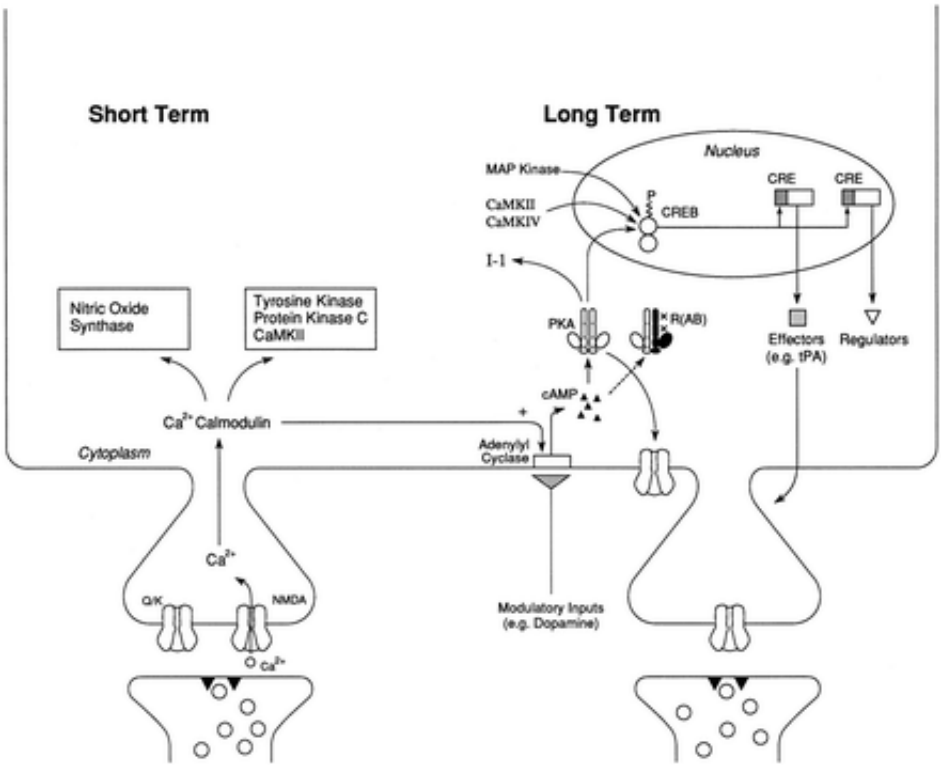

Figure 6: A Molecular Model for the Consolidation of the Late Phase of LTP and Hippocampus-Based Long-Term Memory: Calcium influx through NMDA receptors activates a variety of kinases, including CaMKII, and other enzymes such as nitric oxide synthase, to produce E-LTP (Short Term). L-LTP (Long Term) is produced when adenylyl cyclase is activated by Ca^{2+} increases or by neuromodulatory transmitters such as dopamine. The rise in cAMP activates PKA, which can then phosphorylate a variety of targets such as protein phosphatase inhibitor-1 (I-1), ion channels, and CREB. In the R(AB) transgenic mice, the expression of an inhibitory form of the regulatory subunit of PKA leads to reduced PKA levels and deficits in the L-LTP and in hippocampus-based long-term memory. *Graphic taken from* ***Cell*** [12]

Through these biochemical processes, information is stored long term in memory. In order that information might be fed into this sequence it must be learned. Learning takes place through repetition. The practice of priming shows that information previously identified by the brain is familiar enough to improve reaction time to that information. Squire and Raiche studied this and found that less activity was needed in the brain if information to be recalled had been previously seen. Perceptual learning is based on seeing a stimulus enough that it becomes familiar. Thus, prompted by this familiar stimulus (eg: a traffic light), the response is quicker. Emotional learning associates feeling with something caused by related experience.

This type of learning can be independent of conscious cognition. Conditioned fear, in mice, has been associated with the amygdala portion of the brain. Learned feelings exist independent of conscious declarative

memories. Yet from the amygdala comes hippocampal influence over declarative memory. An emotionally arousing event is remembered better than a neutral event because the amygdala is more active during a time of emotional arousal. Sander and Stern discovered that moments of meeting, times of implicit understanding and trust, unconsciously enhance and develop further a therapeutic relationship.

The motor cortex is responsible for learned skills. Specifically the caudate nucleus is influential in habit learning. Those with Huntington's or Parkinson's disease are unable to learn habits as their caudate nucleus is disrupted. Much intuition and categorization skill has been shown to be nondeclarative. Conditioning of category learning has been studied on the synaptic level.

The cerebellum is at the back of the brain (inside the human head), and is responsible for conditioned responses. For example, Richard Thompson found that the memory trace for eye-blink conditioning is formed and stored in the cerebellar cortex and interpositus nucleus of the cerebellum. In the cerebellum, climbing fibers synapse onto the Purkinje neurons. Mossy fibers synapse onto granule cells that send out parallel fibers that synapse onto Purkinje neurons. Purkinje neurons actually decrease firing as the skill is learned. This is called long term depression (LTD). Parallel fiber and climbing fiber inputs to the cerebellum are activated in close temporal proximity. This results is a decrease in the strength of parallel fiber synapses onto Purkinje neurons. This process has been shown in rabbits with a sound and then airpuff to the eyes. Eventually, rabbits would blink at the sound with less required activity in the Purkinje neurons. Evidently here is another facet of the complex mammalian system of memory and learning.

Since everyone grows up in a unique environment, each person's brain is modified uniquely. Most sensations come to "consciousness" in the cerebral cortex. As time goes by, all abilities of the brain start to fade. The elderly have trouble processing information from two sources; have impaired memory for temporal order; and forget daily tasks that need to be done. Time wears away at synaptic structure as well as pathway capacity for LTP. A similar but more drastic process occurs in Alzheimer's patients.

Alzheimer's disease targets the entorhinal cortex, the input region to the hippocampus, as well as the CA1 region of the hippocampus. Also, cell loss in the nucleus basalis, which contain cholinergic neurons impairs attention and high mental functions such as intellectuality. Age related memory loss does not produce dementia as Alzheimer's does. Rather, cell loss due to old age occurs in the dentate gyrus and subiculum. Alzheimer's disease has distinctive diagnostic features: (1) senile plaques in the brain, especially on the hippocampus and cerebral cortex; (2) neurofibrillary tangles; and (3) the loss of neurons. Senile plaques consist of amyloid protein extracellular deposits. They are surrounded by 3 cellular elements: (a) dendritic processes of neurons; (b) astrocytes— a support cell; and (c) inflammatory cells. Amyloid peptide is the principal component; it splits off from amyloid precursor protein. The gene identified for amyloid precursor protein is on the midportion of the long arm of human chromosome 21. A mutation is inherited to produce early onset. The cytoskeleton of cells is destroyed by neurofibrillary tangles. Other genes responsible for the disease include presenilin 1 on chromosome 14 and presenelin 2 on chromosome 1. These provide autosomal dominant conditions, meaning if one parent posesses the gene, half of the children will receive it. Down's Syndrome involves similar amyloid plaques as patients have an extra copy of chromosome 21.

Late onset Alzheimer's involves presence of specific alleles of a gene that encodes the glycoprotein ApoE on the proximal arm of chromosome 19. ApoE4 exists at four times the concentration of the normal population in Alzheimer's patients. The second risk factor for late phase is a variant of chromosome 12, which encodes α-2-macroglobulin (α2M). α2M acts as a part of a scavenger operation with ApoE2 and ApoE3 serving to flush various protein fragments out the synaptic region like toxic amyloid peptide. Mutations interfere with this flush, and excessive amyloid peptide results in cell death. There has been some success in drugs that boost cholingeric neuron transmission, which would limit synaptic failure. Some drugs have been found to delay the disease. Estrogen supplements help women. However the search still continues for an all out cure for this terminal illness.

Alzheimer's and other diseases of the memory are tragic results of failures in standard biological processes of the brain. Yet, the study of the genetics underlying these processes can aid in finding treatment for them. Much progress has been made in the past century in the way of research. There is still a long way to go though on studies in memory on a molecular level. Bridging the two sciences of molecular biology and

computational neuroscience has produced a plethora of valuable theory on the interation of the molecules of the mind.

2) A Closer Look at CREB Genetic engineering is useful in gaining a better understanding of the networks underlying many learning and memory processes. To this point, questions still remain as to treatment of terminal illness of memory and learning. Prediction of behavior within a human brain under the duress of certain stimuli to the long term memory is an ever broadening topic as well. The conversion from short to long term memory takes place, in humans, mostly within the hippocampus. One important gene network in particular involves the cAMP-responsive element binding protein.

This network consists of second messengers, kinase pathways, phosphorylation sites, genes, gene products and results in synaptic outgrowth. As has been discussed, second messengers act to carry information from the outside to the inside of the cell. Kinases are activated by these second messengers and work to carry phosphate groups to proteins and transcription factors and either phosphorylate or dephosphorylate them. Phosphorylation sites act as switches so that, when a site on a transcription factor increases in percentage phosphorylated, the rate of transcription increases. Thus, phosphorylation sites activate genes.

A certain second messenger—cyclic adenosine monophosphate (cAMP)—is activated when a stimulus is applied on the surface of the cell. Second messengers carry information from the exterior of a cell to the interior. In the case of this gene network, the stimulus may be the application of serotonin as in *Aplysia* or forskolin in rats. This directly activates cAMP signaling [1],[2]. Alternatively, phosphorylation of rat CREB sites (both Ser^{133} and P_{PKM}) can occur through electrical stimulus [3] or depolarization [4], by KCl for example. These are two particular protocols to activate the CREB gene cascade of particular interest.

When in fact these protocols are applied to a hippocampal neuron of a rat, the level of Ca^{2+} rises depending on the strength of the perturbation. The rise in the level of calcium then activates several kinases. Among them are a Ca^{2+}/calmodulin kinase referred to as CaM kinase IV (CAMKIV). CAMKIV is activated by both calcium and calmodulin. Another kinase pathway, known as Ras/mitogen-activated protein kinase pathway MEK^{pp} activates what is known as the extracellular signal-regulated protein kinase (ERK) mediated pathway. One of the kinases that also modulates this mini-cascade is known as protein kinase A. Once ERK is activated, it phosphorylates twice, its twice-phosphorylated form activates a kinase known as Rsk2. This pathway has been experimentally shown as critical to CREB phosphorylation [4]. Ca^{2+} works to activate protein kinase M ζ (PKM) [5]. Once the level of Ca^{2+} goes above a certain threshold, these kinase pathways are activated and so continues the CREB gene cascade.

Kinases can work to phosphorylate sites on transcription factors such that then genes will begin production of their respective product and act upon their function. These kinases in particular apply themselves to two target sites. The first is referred to as P_{CREB} or the $CREBSer^{133}$ site. This site's change in phosphorylation is proportional to the sum of the concentration of a ribosomal kinase (Rsk2), which is activated by ERK^{pp}, and that of CAMKIV. The P_{CREB} site is on the CREB gene itself [2]. A second phosphorylation site, P_{PKM}, is on transcription factor 2 (TF2). P_{PKM} is activated by PKM. As the level of phosphorylation of these sites rises, so too does the level of gene product, sigmoidally (ie: $P \longmapsto \frac{P^n}{(a+P^n)}$). With the rise of gene product (GeneProd1), comes synaptic outgrowth(w_{syn}). This synaptic outgrowth is the very reason for improved memory, better synaptic connections provide for a stronger hold of information in the hippocampus, all through this CREB gene cascade.

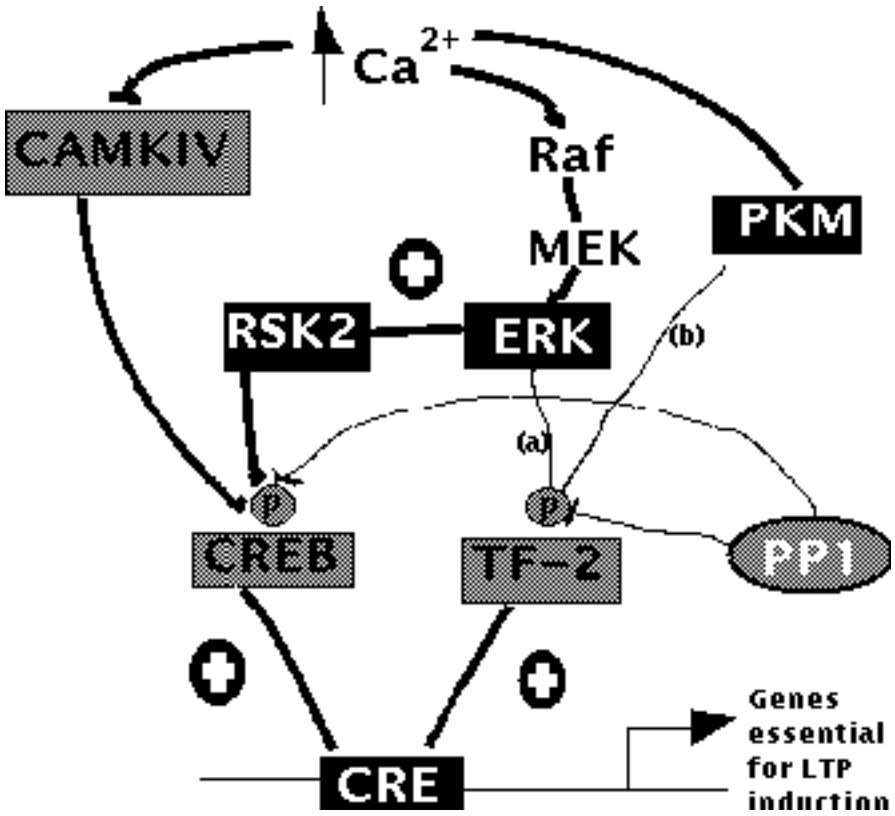

Figure 7: Schematic of the model for LTP induction. Ca^{2+} influx due to electrical stimulation activates Ca^{2+}/calmodulin-dependent kinases, represented by CAM kinase IV (CAMKIV) in the model. Ca^{2+} influx also activates MAP kinase kinase (MEK) which in turn activates ERK. Ca^{2+} influx also leads to the generation of a free, active catalytic subunit of the atypical protein kinase C isoform PKCζ. This active subunit is denoted PKM. ERK activates a CREB kinase, ribosomal S6 kinase - 2 (RSK-2). Both CAMKIV and RSK-2 can phosphorylate CREB, which is then able to activate transcription of immediate-early genes essential for LTP induction. In our model, these genes are represented by GeneProd1. Either a) ERK or b) PKM is assumed to catalyze a second phosphorylation event on a second transcription factor, here denoted TF-2. This second phosphorylation is also necessary for induction of GeneProd1 synthesis and LTP. Protein phosphatase 1, along with calcineurin, can dephosphorylate both CREB and TF-2. *Graphic caption by Smolen (2003)*

Clearly, some kinases and phosphorylation sites have been omitted so far in this discussion of the CREB gene cascade. Protein kinase C, which plays a role in the regulation of intermediate kinase pathways is important [5], but not in the work to be discussed. Also, a phosphorylation site P_{PKA} activated by PKA is involved in the increase of gene product that leads to synaptic growth (GeneProd1) [5], but it was omitted from the following model for simplicity's sake. As a matter of fact, long term potentiation can occur within a hippocampal neuron with only the phosphorylation of the two sites included [2].

Working with Dr. Smolen, a model of the gene network of kinases, phosphates, and gene products that represents the CREB gene cascade was perturbed by these protocols. Appropriate time courses were then fit according to experimental data in several papers. To follow is a short discussion of the derivation of the system of equations used within this model.

3) Modeling the CREB Gene Cascade The model begins with an equation for the change in the level of calcium with respect to time, as this chemical messenger activates all the kinase pathways within this gene cascade. When an electrical shock (tetanus) or depolarization with K^+ is applied to the system, then the level of Ca^{2+} rises within a matter of seconds to a plateau level. In the case of the two specific protocols studied (4 100Hz 1s tetani 5min apart & 90mM of extracellular K^+), the level of Ca^{2+} would plateau at around one μM based on experimental data [3]. Thus, for an ODE for Ca^{2+}, we have the standard form [6]:

$$\frac{d\mathrm{Ca}^{2+}_{\mathrm{free}}}{dt} = f\left(\lambda I_{\mathrm{Ca}} - k_{\mathrm{Ca}}\,\mathrm{Ca}^{2+}_{\mathrm{free}}\right) \tag{1}$$

Here f is the ratio of $\mathrm{Ca}_{\mathrm{free}}$ to $\mathrm{Ca}_{\mathrm{total}}$, experimentation shows $f \approx 0.001$ is appropriate [7]. The constant λ converts current to concentration units. I_{Ca} is current, which is a piecewise constant. Conversion from frequency or depolarizer concentration to current is necessary. k_{Ca} is also a constant. Hence, this ODE is easily solvable. Once solved, the intial condition of Ca^{2+} can be taken from experimental data standards [3] and thus, we get:

$$\mathrm{Ca}^{2+}_{\mathrm{free}}(t) = c\mathrm{e}^{-f k_{\mathrm{Ca}} t} + 50. \tag{2}$$

The model implements this function as decay after an instantaneous increase in calcium. General ODE's for concentration of a kinase or even a phosphorylation site have their first term as an activation term dependent on the activator messenger or kinase and sometimes itself. The second term is deactivation and is usually simply proportional to itself times a negative constant. Thus most kinase, phosphorylation site, and even gene product ODE's look something like:

$$\frac{d\mathrm{K}_1(t)}{dt} = k_{\mathrm{p}}\,\mathrm{K}_1(t)\,\mathrm{K}_2(t) - k_{\mathrm{d}}\,\mathrm{K}_1(t). \tag{3}$$

Ca^{2+} elevation recruits the calcium/calmodulin dependent kinase IV. The activation of this kinase can be modeled with an ODE which changes sigmoidally with Ca^{2+} and CaM (calmodulin) and decays quite slowly, as shown experimentally [2]:

$$\frac{d\mathrm{CAMKIV}_{\mathrm{act}}}{dt} = k_{\mathrm{act}}\left[\frac{[\mathrm{Ca}^{2+}]^4}{[\mathrm{Ca}^{2+}]^4 + {k_{\mathrm{Ca}}}^4}\right]\left[\frac{[\mathrm{CaM}]}{[\mathrm{CaM}] + \mathrm{K}_{\mathrm{CaM}}}\right] - \frac{[\mathrm{CAMKIV}_{\mathrm{act}}]}{\tau_{\mathrm{CAMK}}} \tag{4}$$

A second pathway is longer and more involved, the Ras/mitogen activated kinase pathway ($\mathrm{MEK}^{\mathrm{pp}}$) is represented as a mere piecewise function of the stimulus as the stimulus directly affects the level of Ca^{2+}, which directly affects this pathway [2]. T represents the time for which the stimulus is on, k_{MEK1} is the basal level of $\mathrm{MEK}^{\mathrm{pp}}$ before the stimulus, k_{MEK2} is its level during the stimulus, and after the stimulus, k_{MEKe} is the time constant for its decay:

$$\mathrm{MEK}^{\mathrm{pp}}(t) = \begin{cases} k_{\mathrm{MEK1}} & t \in [0, 100) \\ k_{\mathrm{MEK2}} & t \in [100, 100+T) \\ \mathrm{e}^{k_{\mathrm{MEKe}}(100+T-t)} & t > 100+T. \end{cases} \tag{5}$$

Extracellular-signal related kinases (ERK) have their pathways opened once the $\mathrm{MEK^{pp}}$ pathway is activated. They are phosphorylated twice by themselves. Then, the system of equations for ERK, phosphorylated ERK and twice phosphorylated ERK looks like:

$$\begin{aligned}
\frac{d\mathrm{ERK}}{dt} &= k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}}\,\mathrm{ERK} + k_{\mathrm{b,ERK}}\,\mathrm{ERK^{p}} && (6)\\
\mathrm{ERK^{p}} &= \mathrm{ERK_{tot}} - \mathrm{ERK} - \mathrm{ERK^{pp}} && (7)\\
\frac{d\mathrm{ERK^{pp}}}{dt} &= k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}}\,\mathrm{ERK^{p}} - k_{\mathrm{b,ERK}}\,\mathrm{ERK^{pp}}. && (8)
\end{aligned}$$

$\mathrm{ERK^{p}}$ is merely a balance term for the other two ODEs. Thus, this can be removed from the system as an equation by plugging it in where it appears in the other two ODEs, so that ERK is now a system of only two ODEs:

$$\begin{aligned}
\frac{d\mathrm{ERK}}{dt} &= -(k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}} + k_{\mathrm{b,ERK}})\,\mathrm{ERK} - k_{\mathrm{b,ERK}}\,\mathrm{ERK^{pp}} + k_{\mathrm{b,ERK}}\,\mathrm{ERK_{tot}} && (9)\\
\frac{d\mathrm{ERK^{pp}}}{dt} &= -k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}}\,\mathrm{ERK} - (k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}} + k_{\mathrm{b,ERK}})\,\mathrm{ERK^{pp}}\\
&\quad + k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}}\,\mathrm{ERK_{tot}}. && (10)
\end{aligned}$$

Rsk2, a ribsomal CREB kinase activated by $\mathrm{ERK^{pp}}$, is responsible for the phosphorylation of a site on CREB. We can represent the concentration of Rsk2 as follows:

$$\frac{d\mathrm{RSK2}}{dt} = k_{\mathrm{phos2}}\,\mathrm{ERK^{pp}}\,\mathrm{Rsk2} - k_{b}\,\mathrm{Rsk2}. \quad (11)$$

CAMKIV as well as Rsk2 alter the phosphorylated fraction of the CREB $\mathrm{Ser^{133}}$ site. The phosphorylation of the CREB $\mathrm{Ser^{133}}$ site is represented as $\mathrm{P_{CREB}}$ and represents the part of this site that is saturated with activation phosphate groups at that point in time. PP1 represents the level of protein phosphotase that cleaves phosphate off of the site and is assumed constant for simplification purposes:

$$\frac{d\mathrm{P_{CREB}}}{dt} = k_{\mathrm{phos1}}\,(\mathrm{CAMKIV_{act}} + \mathrm{Rsk2})(1 - \mathrm{P_{CREB}}) - k_{\mathrm{dephos1}}(\mathrm{PP1} + \mathrm{PP_{basal}})\mathrm{P_{CREB}}. \quad (12)$$

Concentration of $\mathrm{PKM_{act}}$ (an active catalytic subunit of the protein kinase C) is represented simply as a first order decay equation with a stimulus-dependent rate constant $k_{\mathrm{f,PKM}}(t)$. This constant is almost identical to $\mathrm{MEK^{pp}}$ except that its internal constants will have different values. Thus $\mathrm{PKM_{act}}$ is represented as such:

$$\frac{d\mathrm{PKM_{act}}}{dt} = k_{\mathrm{f,PKM}}(t)\mathrm{PKM_{act}} - k_{\mathrm{b,PKM}}\mathrm{PKM_{act}} \quad (13)$$

$$k_{\mathrm{f,PKM}}(t) = \begin{cases} k_{\mathrm{f,PKM1}} & t \in [0, 100) \\ k_{\mathrm{f,PKM2}} & t \in [100, 100+T) \\ \mathrm{e}^{k_{\mathrm{PKMe}}(100+T-t)} & t > 100+T \end{cases} \quad (14)$$

Increasing $\mathrm{PKM_{act}}$ leads to the phosphorylation of sites on CREB. The site specific to $\mathrm{PKM_{act}}$ will be referred to as $\mathrm{P_{PKM}}$ and the change in the fraction phosphorylated is a function of $\mathrm{PKM_{act}}$ and those sites remaining to be phosphorylated, similar to the previous site ODE:

$$\frac{dP_{\mathrm{PKM}}}{dt} = k_{\mathrm{phos2}}\,\mathrm{PKM_{act}}(1 - P_{\mathrm{PKM}}) - k_{\mathrm{dephos2}}(\mathrm{PP_1} + PP_{\mathrm{basal}})P_{\mathrm{CREB}}. \quad (15)$$

Thus, all of these components affecting gene production must be implement in an ODE for Gene Product that affects LTP. All of the phosphorylation sites are thought of as sigmoidally affecting gene product in this case. Then dephosphoylation occurs on the level of first-order decay. This produces the following for $\mathrm{GeneProd_1}$:

$$\frac{d\mathrm{GeneProd_1}}{dt} = k_{\mathrm{trans}}\Big[\frac{{P_{\mathrm{CREB}}}^2}{{P_{CREB}}^2 + {k_{\mathrm{CREB}}}^2}\Big]\Big[\frac{{P_{\mathrm{PKM}}}^2}{{P_{\mathrm{PKM}}}^2 + {k_{\mathrm{PKM}}}^2}\Big] - \frac{[\mathrm{GeneProd_1}]}{\tau_{\mathrm{GP_1}}}. \quad (16)$$

It can be of interest to examine the behavior resultant of this gene cascade. Thus, the synaptic weight variable takes into account the level of $\mathrm{GeneProd_1}$ and deteriorates in a time-delayed first-order decay fashion:

$$\frac{dw_{\mathrm{syn}}}{dt} = k_{\mathrm{LTF}}\,\mathrm{GeneProd_1} - \frac{w_{\mathrm{syn}} - w_{\mathrm{bas}}}{\tau_{\mathrm{syn}}}. \quad (17)$$

And thus the model is constructed. Using the system of ODEs (and a function for $\mathrm{Ca^{2+}}$), the program solves these equations for concentrations of the kinases, phosphorylation sites, and products. MATLAB, through use of the *forward Euler method*:

$$f(n+1) = f(n) + \mathrm{h}\,f'(n)$$

solved this system:

$$\mathrm{Ca^{2+}_{free}}(t) = c\mathrm{e}^{-fk_{\mathrm{Ca}}t} + c\frac{\lambda I_{\mathrm{Ca}}}{k_{\mathrm{Ca}}}$$

$$\frac{d\mathrm{CAMKIV_{act}}}{dt} = k_{\mathrm{act}}\left[\frac{[\mathrm{Ca^{2+}}]^4}{[\mathrm{Ca^{2+}}]^4 + {\mathrm{k_{Ca}}}^4}\right]\left[\frac{[\mathrm{CaM}]}{[\mathrm{CaM}] + \mathrm{K_{CaM}}}\right] - \frac{[\mathrm{CAMKIV_{act}}]}{\tau_{\mathrm{CAMK}}}$$

$$\mathrm{MEK^{pp}(t)} = \begin{cases} k_{\mathrm{MEK1}} & t \in [0, 100) \\ k_{\mathrm{MEK2}} & t \in [100, 100+T) \\ \mathrm{e}^{k_{\mathrm{MEKe}}(100+T-t)} & t > 100+T \end{cases}$$

$$
\begin{aligned}
\frac{d\mathrm{ERK}}{dt} &= -(k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}} + k_{\mathrm{b,ERK}})\,\mathrm{ERK} - k_{\mathrm{b,ERK}}\,\mathrm{ERK^{pp}} + k\mathrm{b},\mathrm{ERK}\,\mathrm{ERK_{tot}} \\
\frac{d\mathrm{ERK^{pp}}}{dt} &= -k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}}\,\mathrm{ERK} - (k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}} + k_{\mathrm{b,ERK}})\,\mathrm{ERK^{pp}} \\
&\quad + k_{\mathrm{f,ERK}}\,\mathrm{MEK^{pp}}\,\mathrm{ERK_{tot}} \\
\frac{d\mathrm{RSK2}}{dt} &= k_{\mathrm{phos2}}\,\mathrm{ERK^{pp}}\,\mathrm{Rsk2} - k_b\,\mathrm{Rsk2} \\
\frac{d\mathrm{P_{CREB}}}{dt} &= k_{\mathrm{phos1}}\,(\mathrm{CAMKIV_{act}} + \mathrm{Rsk2})(1 - \mathrm{P_{CREB}}) - k_{\mathrm{dephos1}}(\mathrm{PP1} + \mathrm{PP_{basal}})\mathrm{P_{CREB}} \\
\frac{d\mathrm{PKM_{act}}}{dt} &= k_{\mathrm{f,PKM}}(t)(\mathrm{PKM_{tot}} - \mathrm{PKM_{act}}) - k_{\mathrm{b,PKM}}\mathrm{PKM_{act}} \\
k_{\mathrm{f,PKM}}(t) &= \begin{cases} k_{\mathrm{PKM1}} & t \in [0, 100) \\ k_{\mathrm{PKM2}} & t \in [100, 100 + T) \\ \mathrm{e}^{k_{\mathrm{PKMe}}(100+T-t)} & t > 100 + T \end{cases} \\
\frac{d\mathrm{P_{PKM}}}{dt} &= k_{\mathrm{phos2}}\,\mathrm{PKM_{act}}(1 - \mathrm{P_{PKM}}) - k_{\mathrm{dephos2}}(\mathrm{PP_1} + PP_{\mathrm{basal}})P_{\mathrm{CREB}} \\
\frac{d\mathrm{GeneProd_1}}{dt} &= k_{\mathrm{trans}}\Big[\frac{{P_{\mathrm{CREB}}}^2}{{P_{CREB}}^2 + {k_{\mathrm{CREB}}}^2}\Big]\Big[\frac{{P_{\mathrm{PKM}}}^2}{{P_{\mathrm{PKM}}}^2 + {k_{\mathrm{PKM}}}^2}\Big] - \frac{[\mathrm{GeneProd_1}]}{\tau_{\mathrm{GP_1}}} \\
\frac{dw_{\mathrm{syn}}}{dt} &= k_{\mathrm{LTF}}\,\mathrm{GeneProd_1} - \frac{w_{\mathrm{syn}} - w_{\mathrm{bas}}}{\tau_{\mathrm{syn}}}
\end{aligned}
$$

Plots of all of these concentrations can be given. Total time to run the simulation is also passed as are the step size (h) for forward Euler and the plot increment (iterations between point plots). Whether or not a small enough step size has been chosen can be tested by resetting h to $\frac{h}{2}$ and seeing if the plot changes. The model is malleable as there are many constants in the system of differential equations. Source material is then examined for experimental data regarding these kinase, site, and gene concentrations. [2], [3], [4], [5], and [7] were all instrumental in attaining appropriate timecourse fits.

4) Time Course Fits to Data As Ca^{2+} is the first concentration timecourse in the cascade, it was the first plot to be fit to experimental data. Bito [3] had several plots of Ca^{2+} concentration time-courses as they responded to tetani. This fit was made by giving Ca^{2+} the near instantaneous rise (around a couple of seconds) as it appeared in [3] and from there modeling the quick decay to give spike trains similar to potentials of neurons. The first spike in the following image from [3] is comparable to the shape that the tentani train would induce on the model and thus it was modeled so with a peak at 1 μM instead:

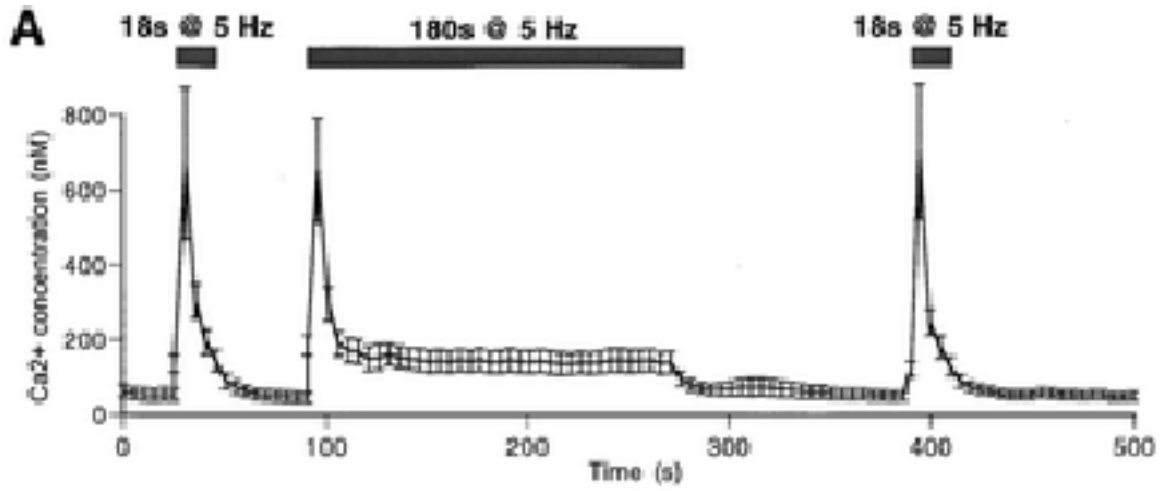

Figure 8: Evidence for Involvement of Synaptic Activity? Induced Oxidative Activity in Regulation of pCREB Dephosphorylation: (A) Changes in bulk [Ca^{2+}] during short and prolonged stimulation at 5 Hz. Mean ± SEM, $n = 13$. *Graphic taken from* ***Cell***[3]

This behavior was reflected in the model by beginning the level of Ca^{2+} at 50nM and then, during a tetanus, have the concentration rise and fall to drive the kinase pathway activations. Thus, under the stimulus of 4 100Hz 1s tetani 5min apart, the activity of Ca^{2+} within the model of a rat hippocampal neuron appears like:

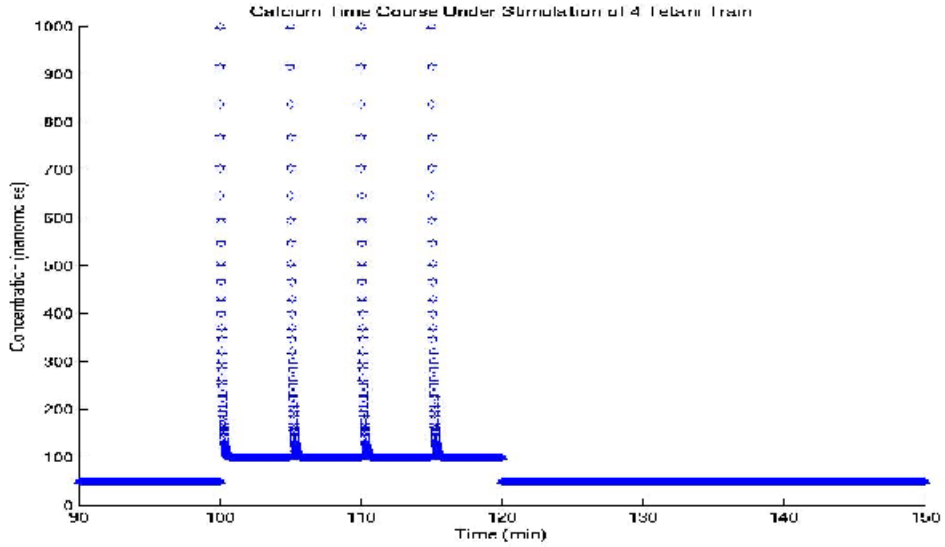

Figure 9: Calcium Time-Course in a Model of a Rat Hippocampal Neuron Under Stimulation by a 4 Tetani Train: This shows what our model uses as its Ca^{2+} time-course during the 4 100Hz 1s tetani 5min apart. The clear spiking reflects the behavior of the data in [3]. The specific constants used were $f = 1, k_{\text{ca}} = 1000, \lambda = 1000 and I_{\text{ca}} = 50$ during the stimulus.

After the wellspring of this model (Ca^{2+}), the next timecourses to be fit are those of the kinase pathways. CAMKIV, under depolarization by K^+, experiences rapid concentration increase at first and then attenuation and slow decay. This attenuation does not happen during tetanization as levels of messengers, especially Ca^{2+}, and kinases fall quite quickly. Data in [4] gives time data for the fold increase of CAMKIV during depolarization by 60mM of KCl.

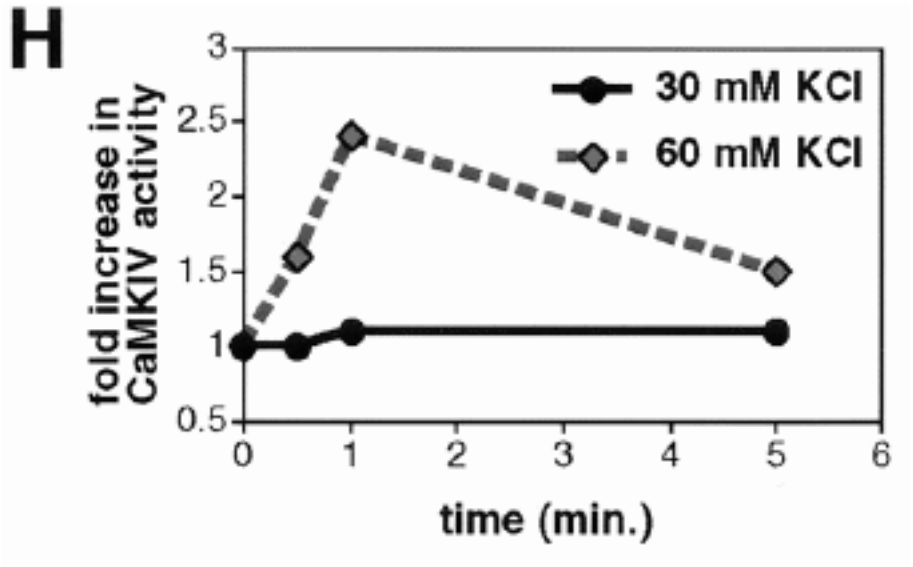

Figure 10: CaMKIV Activity Is Dispensable for Ca^{2+}-Stimulated CREB-Dependent Gene Expression: (H) Hippocampal neurons were treated with 30 mM or 60 mM, and CaMKIV was assayed for kinase activity. Experiments are averages of duplicate determinations. *Graphic taken from* ***Neuron*** [4]

A fit to that data was run in MATLAB through a trial and error method of determining the appropriate constants to fit the shape presented by [4] for activation of CAMKIV.

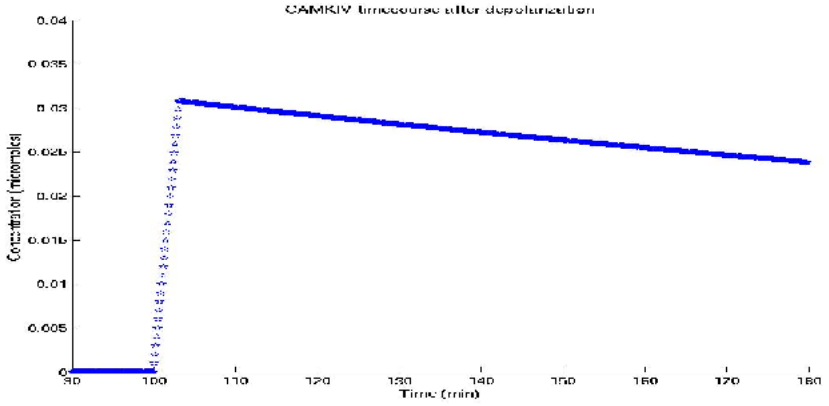

Figure 11: CAMKIV Time-Course fit in an ODE system model for CREB. This was fit using the data of the experiments presented in [4]. The constants were set at the following values: $k_{\text{act}} = 0.01, k_{\text{ca}} = 1000, \text{CaM} = 1, k_{\text{cam}} = 1,$ and $\tau_{\text{CAMK}} = 300$.

The third in a chain of kinases that form a pathway to a phosphorylation site, ERK, is a subsystem of this CREB system. In fact the kinases must be phosphorylated twice to affect the later kinase, Rsk-2. In practice, experimentally, it is enough to give one measurement for both ERK^{p} and ERK^{pp} to describe the state of this subsystem. Here, more detail would be ideal for model fitting, but [4] gives only a time course for what the author referrs to as pERK.

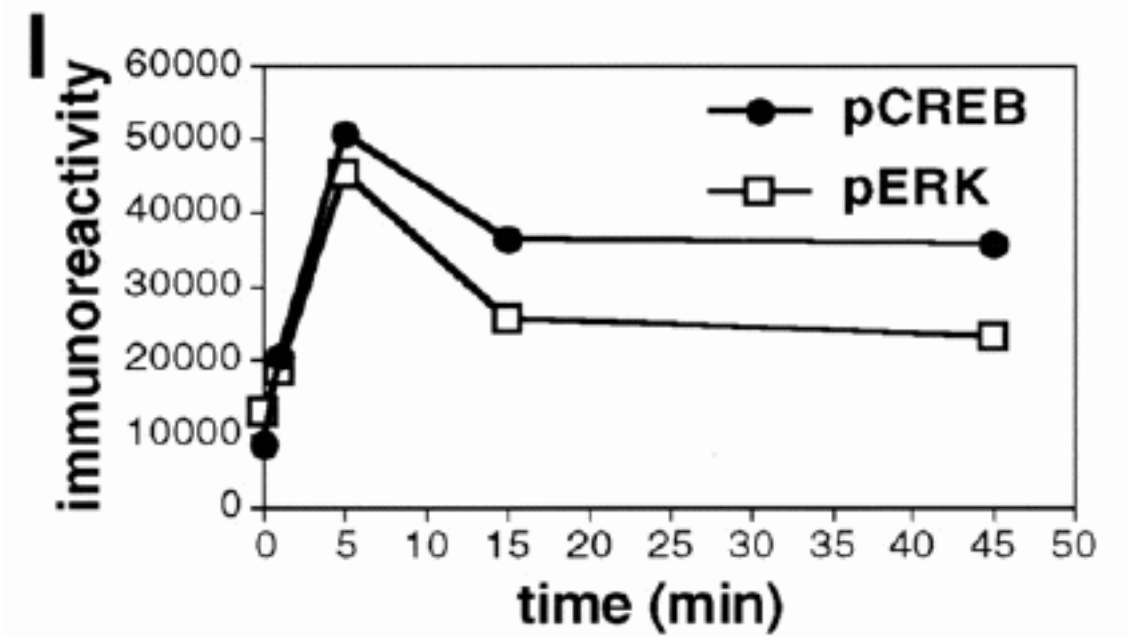

Figure 12: CREB Phosphorylation at Ser-133 Depends on ERK/MAP Kinase Signaling: (I) Transverse slices of hippocampal area CA1 were treated as in (G). The slices were fixed and processed immunohistochemically for phospho-CREB and phospho-ERK. The graph depicts the averaged immunoreactivity in area CA1 for phospho-CREB and phospho-ERK (n = 5,6). *Graphic taken from* ***Neuron*** [4]

This is interpreted to be a fold time-course for the concentration of both $\mathrm{ERK^p}$ and $\mathrm{ERK^{pp}}$. The fit for $\mathrm{ERK^{pp}}$ is the most important of these two as it directly affects the concentration of Rsk-2. Again, this is under depolarization as opposed to a tetanus.

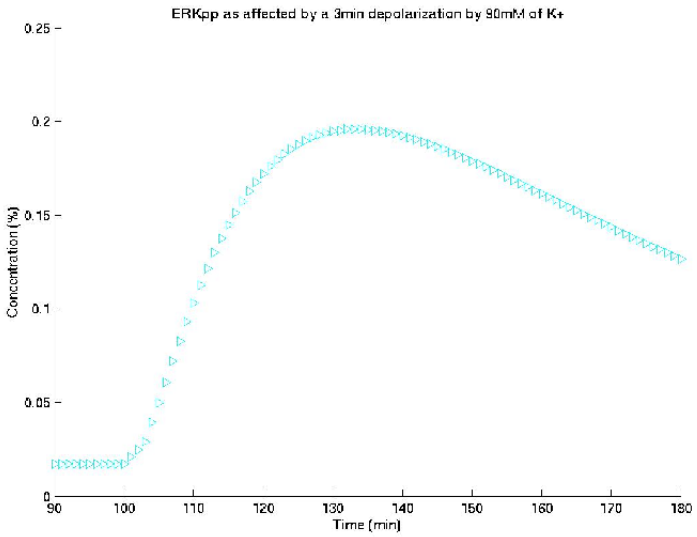

Figure 13: $\mathrm{ERK^{pp}}$ Concentration Before, During, and After a 3min depolarization by $\mathrm{K^+}$. Time Constants: $k_{\mathrm{f,ERK}} = 0.03, k_{\mathrm{b,ERK}} = 0.02, \mathrm{ERK_{tot}} = 1, k_{\mathrm{MEK1}} = 0.1, k_{\mathrm{MEK2}} = 1,$ and $k_{\mathrm{MEKe}} = 0.06$.

A couple of difficulties that have arisen have been that the system is underdetermined—there are more

constants to determine than there are data points. Therefore, even if a solution of suitable constants are found to fit data, it is almost certain that another solution exists that works too. Though some of these other solutions can be removed as extraneous—that they do not make biological sense—the problem still remains. Also, data that is given is usually not simply concentration of, say, a kinase plotted against time. It is usually given as immunoreactivity or fold increase is also popular. A simple point to point correlation has not been done, "rules" of the time course have been kept in mind. Thus far, this system has been fit mostly using one of the most inefficient tools in the world for solving large mathematical systems, the human brain. However, a least squares solve for parameters has been recently developing. If the program succeeds, it will be able to take in all sorts of data simulateously and solve for appropriate parameters. With enough data points or fold increase information, the fits will be successful throughout the system.

5) Future Directions

The model contains many issues still to be resolved as to fitting, accurately, all timecourses of all components of the ODE system. Further data is necessary in order that the parameters found as the constants of the system will then be unique. With a limited data set, it is difficult to uncover the single solution to the extensive least squares problem that is appropriate considering all natural conditions of the system. The process of finding these would be expedited by a more efficient least squares solver.

Dr. Steve Cox of Rice University, Department of Compuational and Applied Mathematics, is developing a least squares solver requiring only 2 function evaluations per iteration, where as usually $n+1$ (n=number of parameters) function evalutions are required. Function evaluations involve solving the whole ODE system with MATLAB's Runge-Kutta solver, which adds up to a lengthy run of the full program if it iterates upwards of 10 times.

Another way in which to specify further this model, is to predetermine the time constants τ for each point in time for each decay. By examining experimental time-course data, one can fit more than simply the shape of the graph. The time constant τ is determined by the curvature of rise or decay. For example, once ERK^{pp} is at a low steady state, Rsk-2 depends only on itself. It would appear essentially as an exponential decay function ($e^{\tau t}$). Integrating this idea into the model makes it less likely that least squares will terminate on what is only a local minimum. There will be fewer local minima as the model parameters are being more specifically determined. Achieving accurate timecourses and unique constants will provide for a sound model which would then be of use for progress in clinical neurology [13].

Integrating several of these models of genes into one massive model of an area of the brain or simply the hippocampus would be invaluable in determining the method of progression for neurological disorder. Polygenetic disorders of the brain include Alzheimer's, autism, schizophrenia, reading disability, and attention-deficit/hyperactivity disorder. Some of the genes for Alzheimer's have been identified already [1]. Schizophrenia's contributing genes have also been uncovered in recent years [14]. In order that therapy and treatment for these disorders might be supposed and tested on a molecular level, the first patient would ideally be a mathematical system of equations as opposed to the brain of a live human.

In order to predict the effects of different dosages of multiple drugs used to treat mental disorders, models for the multiple gene networks are necessary. Thus far, mathematical biologists have used these models already to determine the effect of Alzheimer treatment on the Aβ burden in the human brain [15]. The door is open wide for exploration in the field of mathematical models of gene regulatory networks of the memory.

Bibliography

[1] Squire, L.R., and Kandel, E.R. *Memory: From Mind to Molecules.* Scientific American Library, New York, 2000.

[2] Wu, G., Deisseroth, K., and Tsien, R. *Activity-dependent CREB phosphorylation: Convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway.* PNAS 98, 2808-2813 (2001).

[3] Bito, H., Deisseroth, K., and Tein, R.W. *CREB Phosphorylation and Dephosphorylation: A Ca^{2+} - and Stimulus Duration - Dependent Switch for Hippocampal Gene Expression.* Cell 87, 1203-1214 (1996).

[4] Impey, S., Obrietan, K., Wong, S., Poser, S., Yano, S., Wayman, G., Deloulme, J.C., Chan, G., and Storm, D. *Cross Talk between ERK and PKA Is Required for Ca^{2+} Stimulation of CREB-Dependent Transcription and ERK Nuclear Translocation* Neuron 21, 869-883 (1998).

[5] Ling, D., Bernardo, L., Serrano, P., Blace, N., Kelly, M., Crary, J., and Sacktor, T. *Protein Kinase M ζ is Necessary and Sufficient for LTP Maintenance.* Nature Neuroscience 5, 295-296 (2002).

[6] DeSchutter, E., and Smolen, P. "Calcium Dynamics in Large Neuronal Models." In: *Methods in Neuronal Modeling: from Synapses to Networks* 2nd ed. Eds., Koch, C., and Segev, I., MIT Press, Cambridge (1998).

[7] Lee, S.H., Rosenmund,C., Scwaller, B., and Neher, E. *Differences in* Ca^{2+} *buffering properties between excitatory and inhibitory hippocammpal neurons from the rat.* J. Physiology 525.2, 405-418 (2000).

[8] Sheng, M., and Kim, M. *Postsynaptic Signaling and Plasticity Mechanisms.* Science 298, 776-780 (2002).

[9] Malenka, R., and Nicoll, R. *Long-Term Potentiation–A Decade of Progress?* Science 285, 1870-1874 (1999).

[10] Tsien, J., Huerta, P., and Tonegawa, S. *The Essential Role of Hippocampal CA1 NMDA Receptor? Dependent Synaptic Plasticity in Spatial Memory.* Cell 87, 1327-1338 (1996).

[11] Kandel, E.R. *The Molecular Biology of Memory Storage: A Dialogue Between Genes and Synapses.* Science 294, 1030-1038 (2001).

[12] Abel, T., Nguyen, P., Barad, M., Deuel, T., Kandel, E., and Bourtchouladze, R. *Genetic Demonstration of a Role for PKA in the Late Phase of LTP and in Hippocampus-Based Long-Term Memory.* Cell 88, 615-626 (1997).

[13] Smolen, P., and Byrne, J. *Support of Progress in Clinical Neurology by Models of Genetic Regulation.* Arch. Neurology 60 (in press, 2003).

[14] Cloninger, C.R. *The discovery of susceptibility genes for mental disorders.* PNAS 99, 13365-13367 (2002).

[15] Craft, D., Wein, L., Selkoe, D. *A mathematical model of the impact of novel treatments on the $A\beta$ burden in the Alzheimer's brain, CSG and plasma.* Bull. Math. Biol. 64, 1011-1031 (2002).