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A New Interpretation of the Drug Levodopa in the Treatment of Parkinson's Disease

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A NEW INTERPRETATION OF THE DRUG LEVODOPA IN THE TREATMENT OF PARKINSON'S DISEASE

A Thesis Presented

by

Joanne T. Vannah

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ABSTRACT

A NEW INTERPRETATION OF THE DRUG LEVODOPA IN THE TREATMENT OF PARKINSON'S DISEASE

June 2005

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An argument for levodopa induced dyskinesias (LID) as an expression of long term potentiation (LTP) in the striatum is presented. Normally, with an intact nigrostriatal dopamine (DA) input, long term depression (LTD) is expressed in the striatum, however, striatal LTD is lost following DA denervation and chronic levodopa treatment. It is possible that these changes in synaptic efficacy are due to two other chemical modulators, achetylcholine (ACh) and glutamate, which in turn leads to the induction of striatal LTP. ACh and glutamate may produce these changes in motor behavior by affecting the striosomal pathway in addition to their effect on the direct (striatonigral) pathway and the indirect (striatopallidal) pathway of the basal ganglia. This thesis postulates that by blocking LTP expression in the DA depleted striatum, that the occurrence of LID will be reduced. Heterosynaptic plasticity is a mechanism in which long-term facilitation proceeds in the state of LTD or LTP depending on the levels of intracellular calcium

present. Artificial neural networks (ANN) may be used to speculate and predict the most accurate and precise combinations of levodopa, ACh antagonist and glutamate antagonists that will achieve the goal of preventing LTP in DA denervated and chronic levodopa treated biological systems to reduce/eliminate LID.

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A special note of appreciation to my parents, Joanne M. and Vincent J. Vannah, they have always extended support and encouragement. A deep and warm thanks to my nephew, Gus Blake, who inspires me tremendously and in his presence all goals achieved are elevated to a new level.

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CHAPTER 1

INTRODUCTION

Parkinson's disease (PD) is a movement disorder that affects more than half a million people in the United States (Langer A and Lozano R, 1998). Individuals with PD experience motor abnormalities as a result of this neurodegenerative disease (Langer A and Lozano R, 1998). Early motor disturbances are presented as tremors usually in one of the extremities. As the disease progresses over time, additional motor complications appear. These motor complications include patients becoming stiff and their actions become delayed. Some PD patients begin to have trouble initiating movements, losing coordination or complete lack of mobility. Non-motor symptoms associated with PD such as mood, sleep and cognitive disturbances are also prevalent but will not be discussed in this paper (Langer A and Lozano R, 1998). The typical onset of PD occurs in patients between the ages of 50-60 (Jellinger KA, 2001). Because some of the initial motor disturbances are subtle, the diagnosis may not be immediately recognized. As the neurodegenerative progression builds steadily, PD symptoms become obvious and very confining for patients who have lived with the disease for an average of ten years (Klawans HL, 1996).

The primary brain regions affected in PD are the basal ganglia (Pollack AE, 2001). A substantial decrease in the neurotransmitter, dopamine (DA), as is the case in PD has dramatic effects on the basal ganglia. As a result, the symptoms of PD are treatable by pharmaceutical agents (Langer A and Lozano R, 1998). The most commonly used drug treatment for PD is DA replacement therapy with the most effective drug being

levodopa, the chemical precursor to DA (Calon F. et al., 2000). Levodopa is absorbed in the gastrointestinal tract in the small bowel and is rapidly distributed to other tissues with a half-life of 5-10 minutes (Koller WC, 2000). Levodopa readily crosses the blood brain barrier whereas exogenously administered DA itself is unable to do so. Although initially effective, chronic treatment with levodopa results in debilitating side effects such as the uncontrollable, involuntary movements known as dyskinesias (Calon F. et al., 2000; Hirsch E., 2001).

The purpose of this thesis is to present a new hypothesis for the mechanism underlying levodopa induced dykinesias (LID), which posits that these abnormal, involuntary movements are the result of disturbances of a memory mechanism in the basal ganglia that promotes long term changes ultimately leading to LID. Because the neurotransmitters in the basal ganglia involved in LID and their interactions with one another are not clearly defined, an artificial neural network (ANN) model will be presented as an appropriate method for assessing these neurotransmitter relationships. Once developed, the ANN can be trained to prevent these long-term memory changes from occurring within the model system, thereby potentially uncovering therapeutic regiments to control LID. In order to understand this hypothesis, an overview of the anatomy and physiology of the basal ganglia, the brain regions involved in PD, is presented. This anatomical insight provides a foundation for understanding the cellular mechanisms in the basal ganglia that are activated during LID.

Basal Ganglia Anatomy

The basal ganglia consist of brain regions that serve as a key processing center in the control of movement as well as the processing of a certain types of motor memory associated with conditioned responses (Graybiel AM et al., 2000). The basal ganglia structures are compromised of five extensively interconnected subcortical nuclei in the forebrain that wrap around the thalamus beneath the anterior portion of the lateral ventricles (Graybiel AM, 1990). The individual structures are specifically termed: caudate nucleus, putamen, globus pallidus, subthalamic nucleus and substantia nigra (Figure 1) (Graybiel AM, 1990). The basal ganglia structures form an intricate network of parallel loops that integrate and redistribute information from the cortex, and in turn, project this information to the thalamus that affects the motor cortex in the frontal lobe (Smith D. et al., 1997).

Information coming into the basal ganglia originates from a variety of locations in the cerebral cortex, thalamus and the limbic system (Parent A. et al., 2001). This input enters the basal ganglia in the striatum (Parent A. et al., 2001), which is a collective term for the caudate and putamen (Parent A. et al., 2001). Inputs to the striatum from the thalamus and cortex are excitatory and use the neurotransmitter glutamate (Pollack AE, 2001; Smith A. et al., 1998). Other inputs include serotonin from the Raphe nucleus, achetylcholine (ACh) from striatal interneurons and DA from the substantia nigra pars compacta (SNc) as described below (Pollack AE, 2001).

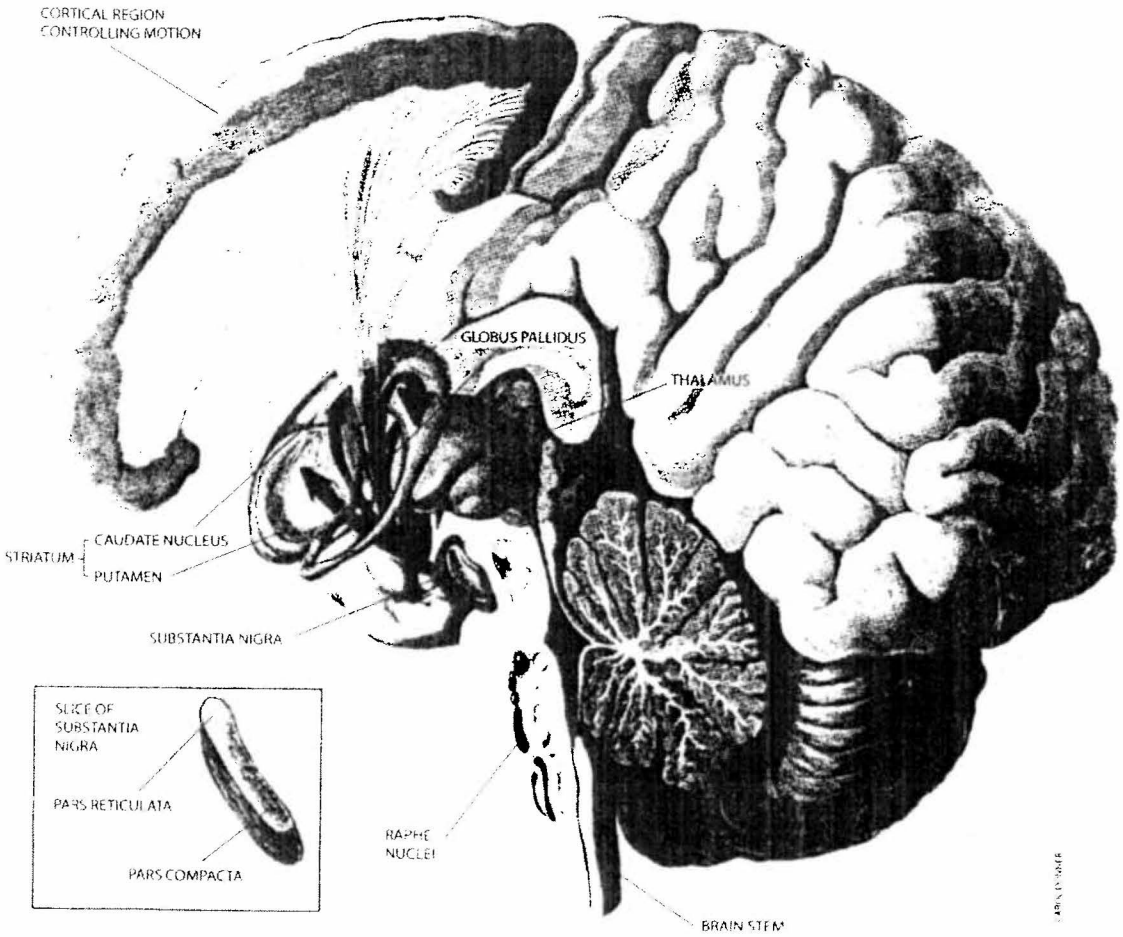

Figure 1: (modified from MoussaBH and Riederer Youdim and Peter, 1997) Basal Ganglia Structures: caudate nucleus, putamen, globus pallidus, subthalamic nucleus and substantia nigra. The pars compacta of the substantia nigra loses dopamine neurons which disturbs motor control in PD. The substantia nigra DA neurons form the nigrostriatal pathway as they project to the striatum. The raphe nuclei which supplies serotonin to the basal ganglia loses neurons that produces nonmotor disturbances (e.g. depression) in PD.

Once information has reached the striatum it is processed through the basal ganglia circuitry before exiting the thalamus and brainstem via the basal ganglia output structures: the globus pallidus (GPi) and the substantia nigra reticulata (SNr) (Starr MS, 1995). The GP is divided into internal (GPi) and external (GPe) segments (Albin RL et al., 1998). Similarly, the substantia nigra, which is located in the midbrain, has several components, the SNr and the SNc (Albin RL et al., 1998). The SNc contains the DA neurons that project to the striatum and form the nigrostriatal pathway (Smith A. et al., 1997). It is the DA neurons of the SNc that degenerate in PD.

Basal Ganglia Circuitry

Between the basal ganglia inputs and outputs are two major pathways of projection neurons whose cell bodies are located in the striatum (Smith A. et al., 1997). These are referred to as the "direct" and "indirect" pathways. In the current model of basal ganglia function, all information conveyed through the basal ganglia input structures is processed within the basal ganglia and flows out through one of these pathways to the basal ganglia output structures (Figure 2) (Smith A. et al., 1997). The concerted and contrasting effects of the "direct" and "indirect" pathways function to control motor behavior in the basal ganglia by supplying both a break and an accelerator on the thalamus.

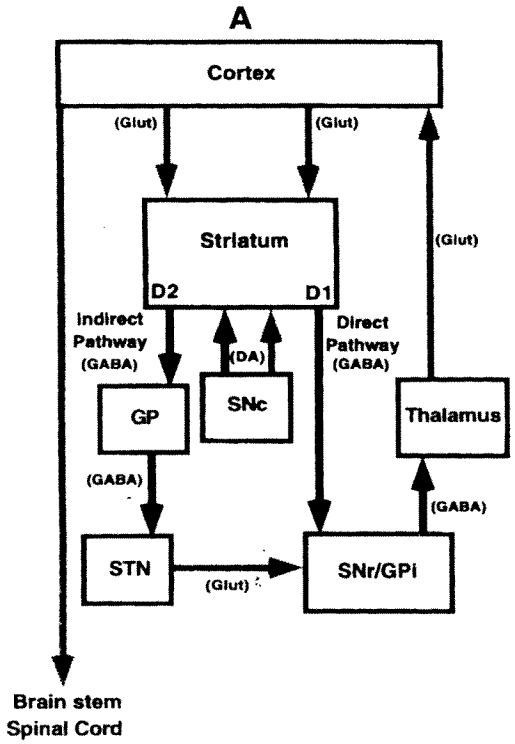

Figure 2: (modified from Pollack AE, 2001) Basal Ganglia Circuitry: Connections and Neurochemical Modulators: Glutamate (Glut:excitatory) connections lead from the cortex to the striatum, from the STN to the SNr/GPi and from the thalamus to the cortex. All other connections are inhibitory and utilize gamma-aminobutyric acid (GABA; inhibitory).

All the connections within the basal ganglia are inhibitory with the exception of the projection from STN to SNr/GPi which is excitatory (Figure 2) (Gerfen CR et al., 1990). The direct pathway consists of a direct inhibitory projection to the SNr/GPi which allows the inhibition directed to the thalamus from the SNr/GPi to be reduced (Smith A. et al., 1997). The indirect pathway has an excitatory connection to the GPi/SNr, the STN, thereby causing greater inhibition to the thalamus (Smith A. et al., 1997). As information passes through the basal ganglia and becomes modified, this information projects to three thalamic nuclei: the ventral lateral, the ventral anterior and the mediodorsal (Smith A. et al., 1997). These thalamic nuclei then projects to the prefrontal cortex, the premotor

cortex (PMC), the supplementary motor area (SMA) and the primary motor cortex (Smith A. et al., 1997). The SMA is a major input to the basal ganglia and therefore creates a loop between the SMA and the basal ganglia (Smith A. et al., 1997). The SMA is involved with planning sequences of movement (Smith A. et al., 1997). Without the thalamus receiving information from the basal ganglia and conveying this information back to the cortex, the cortex would not be able to properly direct voluntary motor control (Gerfen CR, 1990). This Cortico-Basal Ganglia-Loop is maintained by two neurotransmitters: glutamate (excitatory) and gamma-amino butyric acid (GABA) (inhibitory) (Figure 3) (Rascol O. et al., 2001).

The DA that is released by the neurons in the SNc has excitatory effects on the direct pathway acting through D1 receptors and inhibitory effects on the indirect pathway acting through D2 receptors (Rascol O. et al., 2001). These striatal projection neurons in the direct and indirect pathways comprise 90-95% of the neuronal populations of the striatum and are medium spiny neurons that contain the inhibitory neurotransmitter GABA. The one excitatory connection in the basal ganglia, from the STN to the SNr/GPi, uses glutamate as its excitatory neurotransmitter (Gerfen CR, 1990). Input from the cortex to the striatum is also excitatory and uses glutamate as its neurotransmitter (Gerfen CR, 1990).

Five different types of DA receptors have been cloned (Parent A. et al., 2001). D1 like receptors (D1 and D5) stimulate intracellular adenosine 3'5'-cyclic phosphate (cAMP) production, whereas D2 like receptors (D2-D4) inhibit cAMP production (Gerfen et al., 1990). Primarily D1 and D2 receptors are located on the shafts of dendritic spines of GABA containing striatal projection neurons (Pollack AE, 2001).

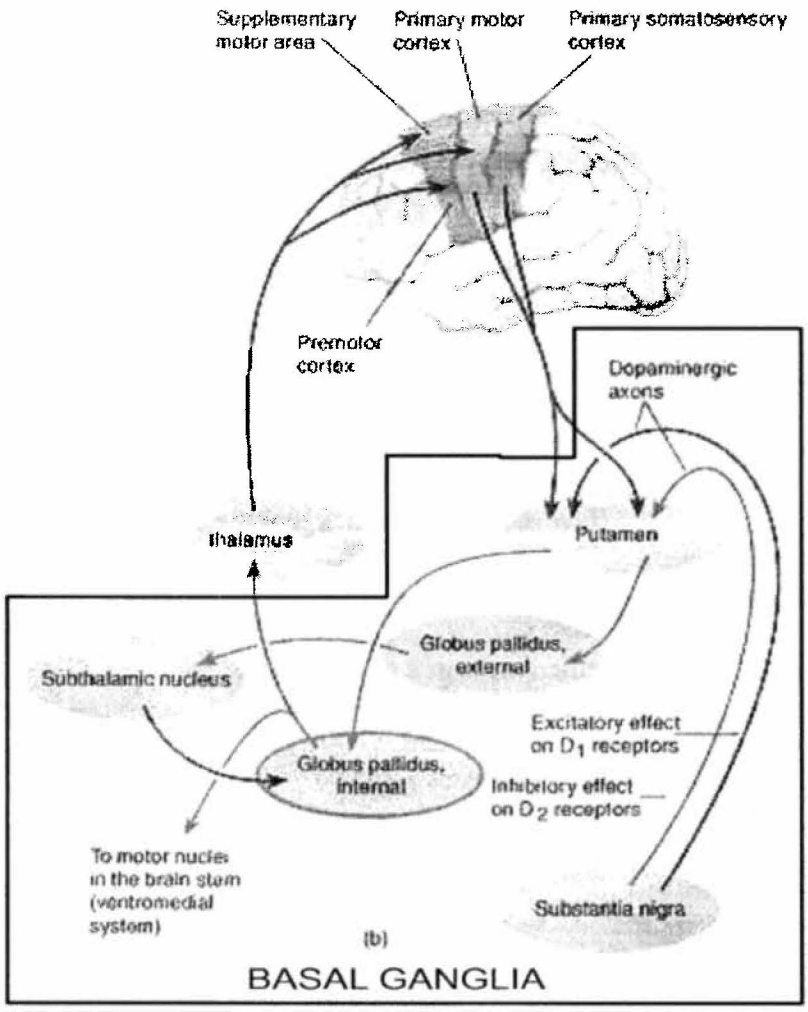

Figure 3: (modified from Carlson NR, 2001) The Cortico-Basal Ganglia Loop. The connections of the excitatory D1 receptor activity and inhibitory D2 receptor activity are shown. The cortico-basal ganglia motor loop, maintained by information about planned movements executed by the primary motor cortex (PMC) and the primary somatosensory cortex (PSC). Inputs from the PMC and PSC project to the putamen. The signal then proceeds to the caudate and globus pallidus and returns back to the motor cortex via the ventrolateral thalamus. The loop is completed when the information from the PMC and PSC are sent to the putamen.

Excitatory glutamatergic inputs synapse on the heads of these same dendritic spines (Pollack AE, 2001). It has been proposed that due to their close proximity and anatomical arrangement, that DA synaptic activity may modulate glutamatergic input on these medium spiny neurons (Pollack AE, 2001).

Approximately 1-2% of the neuronal population in the striatum are the large , aspiny interneurons with extensive collaterals that use ACh as a neurotransmitter (Starr MS, 1995). Three different types of ACh muscarinic receptors are found in the striatum: M1, M2 aand M4 (Blanchet F. et al., 1997; Pollack AE, 2001). M1 and M4 muscarinic receptors are located on both cholinergic interneurons as well as on medium spiny neurons (Pollack AE, 2001). M2 muscarinic receptors are located on cholinergic interneurons as are D2 & D5 receptors and to a much smaller degree D1 receptors (Blanchet F. et al., 1997; Pollack AE, 2001). ACh synapses have been found on the dendritic spines and cell bodies of medium spiny neurons (Pollack AE, 2001). These ACh interneurons, as well as medium spiny neurons, also express ionotropic glutamate receptors, N-methyl-D-aspartate (NMDA) receptors and Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors.

Recent evidence suggests that muscarinic receptor activation has a role in stabilizing the physiological states of striatal neurons (Suzuki T. et al., 1998). Calabresi P. et al. (1999) described striatal neurons a having two physiological states denoted as "down" and "up." A "down" state describes a neuron that is hyperpolarized and an "up" state describes a depolarized striatal neuron (Calabresi P. et al., 1999). In this thesis, the

physiological states of striatal neurons will be referred to as hyperpolarized and depolarized. Normally, the striatal neurons show very little excitability due to the large number of potassium channels that oppose the influx of calcium (Nisenbaum ES et al., 1996). Therefore, the typical state for a striatal neuron is hyperpolarized (Calabresi P. et al., 1999). Following DA denervation, there is a rise in the excitatory neurotransmitter glutamate in the striatum and striatal neurons spend less time in their hyperpolarized state and enter an oscillating state where the neurons frequently shift from a hyperpolarized state to a depolarized state (Calabresi P. et al., 1997). Since these oscillatory striatal states are unable to be reproduced *in vitro* this suggests that an intact corticostriatal system is necessary to induce this oscillatory behavior (Calabresi P. et al., 1990). NMDA and AMPA glutamate receptors located on medium spiny neurons are also differentially activated in the striatum depending upon which physiological state the striatal neurons are in (Calabresi P. et al, 1997). The striatal depolarized state is correlated with NMDA receptor activation, whereas the striatal hyperpolarized state is correlated with AMPA receptor activation (Calabresi P. et al., 1997). ACh has also been found to enhance the NMDA mediated responses recorded from medium spiny cells in the striatum (Guebellini et al., 1998), suggesting a possible role of ACh in inducing a long lasting elevation of corticostriatal glutamatergic transmission (Guebellini P. et al., 1998). The increase of ACh in the striatum, elevates NMDA receptor activation on striatal medium spiny neurons, in turn, enlargens the influx of calcium in the striatal medium spiny neurons. Thus, ACh in the striatum has a role in controlling intracellular levels of calcium in medium spiny neurons. Therefore, ACh in striatal interneurons serves a significant role in modulating chemical activity in this brain region.

DA has a differential effect on the activity of ACh interneurons depending upon which DA receptors are activated (Blanchet F. et al., 1997). For example D1 receptors facilitate the release of ACh whereas D2 receptors inhibit ACh release (Blanchet F. et al., 1997). Differential effects on behavior in DA receptor deficient mice have also been noted, with D2 receptor deficient mice expressing behaviors that resemble Parkinsonian symptoms, whereas D1 receptor deficient mice do not (Baik JH et al., 1995; Drago J. et al., 1994). Since D2 receptors inhibit ACh release, the question arises in the D2 receptor deficient mice if it is the absence of D2 receptors that affects motor behavior directly or whether it is through an indirect effect of D2 receptors on cholinergic interneurons. It is important to understand if ACh or DA has a more dominant role in affecting motor behavior because such insight may provide a better therapeutic regiment for PD.

Taken together, ACh has two significant roles in the striatum. ACh interneurons appear to have a modulatory role in stabilizing the physiological states of striatal neurons and they also have a role in regulating NMDA responses on medium spiny neurons (Guebellini P. et al., 1998). The role of ACh in the striatum is one that is central to the physiology of the basal ganglia and therefore may be intimately involved in the onset of LID.

Striatum: Matrix and Striosomes

The striatum, based upon its neurochemical design, has been separated into two fundamental compartments: the matrix and the striosomes (Graybiel AM et al., 2000). Most of past and current PD research has only considered the role of the matrix

compartments, where the cell bodies from the direct and indirect pathways are located, for exploring LID (Graybiel AM et al., 2001). The striosomes are surrounded by the larger matrix and its cells project to the dopamine containing neurons of the SNc (Figure 4). Interestingly, neurons in the striosomes compartment are naturally activated by pulsatile stimulation of DA receptors in response to new stimuli in the environment (Graybiel AM et al., 2000). But, exploration of how striosomes affect the cells of the matrix has been only marginally investigated (Graybiel AM et al., 2000). Therefore, investigating the influence of the striosome compartment on the matrix neurons could lead to a new understanding of the underlying mechanisms in LID.

The matrix and striosome compartments have distinct afferent and efferent connections (Dure LS IV et al., 1992). The striosomes receive inputs from the prefrontal cortex, the insular cortex and the amygdala and the matrix receives its major input from the association and sensorimotor cortex (Dure LS IV et al., 1992). Striatal circuitry differs for efferent connections from each compartment as well; striosomal neurons project primarily to the SNc whereas the matrix neurons project to the GPe, GPi and SNr. Ligand binding for the D1 receptor subtype is more pronounced in the striosomes as is acetylcholinesterase histochemistry. Conversely, D2 receptor binding and immunostaining for tyrosine hydroxlase are more concentrated in the matrix (Graybiel AM and Langer LF, 1989). Therefore, D1 receptors and ACh are more prominent in the striosomes compared to D2 receptors which are contained in the matrix.

The potential influence of the striosomal pathway on the basal ganglia has largely been overlooked, but it may hold significance since this pathway is in a position to regulate input to the striatum. This is a key point because the activity of the striosomal

pathway could have a direct effect on motor behavior because the projections from the striosomal pathway reach the SNc where they could influence the DA projection back to the striatum (Graybiel AM et al., 2000). To date, major theories of PD and LID take into account only the direct and indirect pathways that primarily comprise the matrix compartment of the striatum without considering the possible effects of the striosomal pathway on LID.

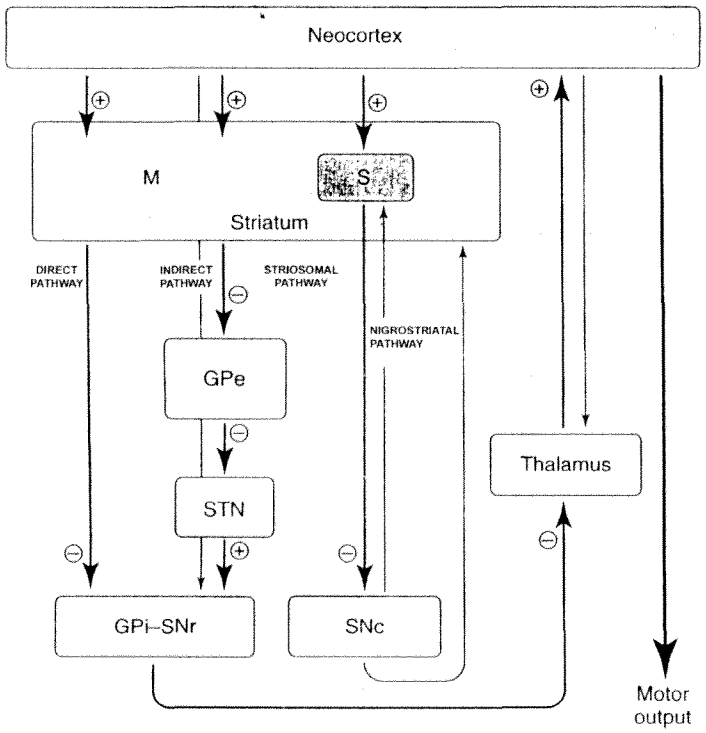

Figure 4: (modified from Graybiel AM et al., 2000) Three Pathway Model in the Basal Ganglia: The striosomal pathway: through the striosomal compartment of the striatum to the SNc. The direct pathway: through the matrix compartment of the striatum to the GPi & SNr and then to the thalamus and neocortex. The indirect pathway: through the matrix compartment of the striatum to the GPe, then to the STN, and to the GPi & SNr and then to the thalamus and neocortex. The nigrostriatal pathway that leads from the SNc to the striatum which degenerates in PD is also depicted.

However, because the DA pathway of the SNc is destroyed in PD, there must be another means for the striosomes to interact with the matrix after the onset of PD. One candidate serving as a link between the striosome and matrix compartments is the ACh interneuron. Even though cell bodies of the ACh interneurons are primarily located in the matrix compartment, their extensive neurites innervate both the matrix and the striosomes of the striatum (Calabresi P. et al., 1998). In addition, the density of ACh interneurons is about twice a high in regions where the matrix *borders* the striosomes (Blanchet F. et al., 1997). This anatomical arrangement suggests that ACh interneurons are in the position to serve a role in transferring information from one striatal compartment to another. Therefore, it is plausible that ACh interneurons may serve as a communicator between the striosomal pathway and medium spiny neurons that comprise the matrix (Figure 5).

In the intact basal ganglia, another way for the matrix and striosomes to interact is through the SNc. The striosomal pathway extends from the striosomes in the striatum to the SNc and is activated with pulsatile DA treatments. The neurons from the SNc then project to the matrix in the striatum through the nigrostriatal pathway, thus the activation of the striosomal pathway could enhance the activation of the nigrostriatal pathway and influence the matrix of the striatum. However, in DA denervated SNc that characterizes PD, this arrangement seems less likely.

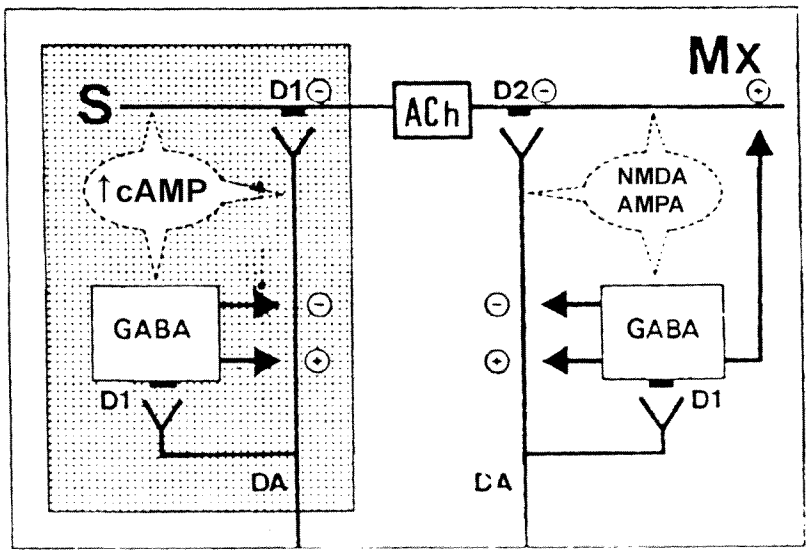

Figure 5: (modified from Blanchet F. et al., 1997)ACh borders the Matrix and Striosomes in the Striatum. Striosomes (S) and matrix (Mx) compartments are separated by ACh interneurons which may serve as a communicator between compartments. Both compartments have GABA projection neurons. The striosomes are comprised of mainly D1 DA receptors that facilitate cAMP activation and the matrix has both D1 DA (direct pathway) and D2 DA (indirect pathway) receptors. The striosomes could influence the matrix following levodopa treatment by acting via the ACh interneurons and affecting the medium spiny neurons (that contain NMDA and AMPA receptors) of the matrix.

CHAPTER 2

BASAL GANGLIA RESEARCH: MEMORY AND MOTOR STUDIES HAVE BEEN KEPT SEPARATE

ACh has been recognized as an important molecule in learning and memory (Calabresi P. et al., 1998). Since the striatum has the highest concentration of ACh found in the brain, it seems likely that the basal ganglia are an area where ACh may be positioned to secure new memories (Calabresi P. et al., 1998). In the past, basal ganglia research has traditionally maintained two separate tracks of scientific investigation (Graybiel AM et al., 1998). One track has been focused on the role of basal ganglia in learning and memory, and the other track pertains to the role of the basal ganglia in the control of movement (Graybiel AM, 1998). However, minimal research has focused on the potential connection between these two tracks. The current explanations of LID as a purely motor abnormality are evident in this separation of research tracks because there is minimal investigation into the possibility that alterations in long-term memory patterns in the basal ganglia underlie the origin of LID. The next section will discuss each of the two research tracks, the roles of the basal ganglia in learning and memory and the control of movement, first separately and then in the context of an argument for the relationship between the two. It may be that the relationship between learning and memory and motor behavior could convey new insights into PD treatments and LID.

Basal Ganglia and Motor Control: The Current Model of PD and LID

The role of the basal ganglia in motor control has largely been viewed from a clinical standpoint. Much research has been focused on how to understand and therefore treat motor disturbances of the basal ganglia. PD is the most common motor disorder that affects these brain structures (Jellinger KA, 2001). The motor dysfunction observed in PD is the result of a decrease in DA in the SNc. These DA neurons normally project from the SNc to the striatum forming the nigrostriatal pathway (Pollack AE, 2001). Although a small percentage of DA neurons naturally die off as part of the aging process, the DA levels in PD are reduced to 30% of their normal concentration (5-10nM) in the striatum (Calon F. et al., 2000; Yahr MD, 1993). The reduction in DA in the striatum, in turn, decreases the activity in the direct pathway and increases the activity in the indirect pathway (Graybiel AM et al., 2001). Since the direct pathway has an inhibitory effect on the basal ganglia output, SNr/GPi, therefore more inhibition directed to the thalamus (Figure 2). In contrast the indirect pathway has an excitatory connection to the GPi/SNr via the STN (Gerfen CR et al., 1990). The loss of DA in the indirect pathway results in less inhibition on the STN which leads to more excitation to the GPi/SNr output structures and greater inhibition on the thalamus (Gerfen CR et al., 1990). Since the basal ganglia output, GPi/SNr, has an inhibitory connection to the thalamus, changes in the activity of both direct and indirect pathways as a result of DA depletion, produces further

inhibition of the thalamus which, in turn, decreases thalamic excitation of the motor areas of the cortex (Gerfen CR et al., 1990). This results in a decrease in motor activity.

The most commonly used symptomatic medical treatment for PD is levodopa, the chemical precursor to DA, which after years of treatment often results in the disabling side effects of uncontrollable, involuntary movements known as LID (Calon F. et al., 2000). Existing theories on the manifestation of LID have been based upon clinical observations of PD patients as well as on animal models of PD. One theory of LID is based upon experiments that used an animal model of PD in which monkeys were treated with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) and then given chronic levodopa treatment. The results indicated that the abnormal movements observed after chronic treatment with levodopa were a result of the reduced activity in the GPi/SNr (Obeso JA and Okanow CW, 2000). Levodopa treatment was thought to cause too much inhibition of the projections from the striatum to the GPe neurons, which result in excessive inhibition of the STN, which, in turn, reduces the excitatory input to the GPi/SNr (Obeso JA and Okanow CW, 2000). The reduced activity of the GABA neurons in the GPi disinhibits the thalamus resulting in an increase in the activity of the thalamocortical inputs (Obeso JA and Okanow CW, 2000). This increase in thalamocortical inputs could result in LID because amplified inputs promoting movement would be sent from the thalamus to the cortex (Obeso JA and Okanow CW, 2000). However, this theory is flawed because when the GPi is lesioned it improves LID and PD motor functions suggesting that a decrease in GPi activity does not account for LID (Calabresi P. et al., 2000).

A second theory of LID suggests that it is due to the overactivity of the STN and that treating with glutamatergic antagonists will relieve the LID because of the potential of these antagonists to inhibit the excitatory effects of the STN on the output structures, GPi/SNr (Wichmann T. et al., 1994). However, because the effect of systemically administered glutamate antagonists is not localized to one specific region of the basal ganglia, these glutamate antagonists could be working to inhibit glutamate receptors involved in memory formation and not on the glutamatergic pathway from the STN to the GPi/SNr. In fact, motor fluctuations have been found to be dramatically reduced in 6-OHDA lesioned , levodopa treated rats when injected intrastriatally with the NMDA antagonist, MK801 (Calabresi P. et al., 2000). This suggests the glutamate activity responsible for motor fluctuations is localized in the striatum.

A third hypothesis for a mechanism underlying LID, the subject of this thesis posits that the pulsatile administration of levodopa in the DA depleted striatum interacts with ACh and glutamate and produces a change in the amount of calcium influx into striatal medium spiny neurons, which in turn, intensifies the strengthening of synapses on medium spiny neurons. This synaptic change may activate pathways and cellular mechanisms similar to those involved in forming long term memories in the basal ganglia. This thesis examines the possibility that LID are a manifestation of a long term memory pattern via synaptic mechanisms that occur during learning and memory in the basal ganglia. Such mechanisms are hypothesized to be induced by DA denervation and subsequent chronic levodopa treatment that, in turn, leads to the abnormal motor behavior that characterizes LID.

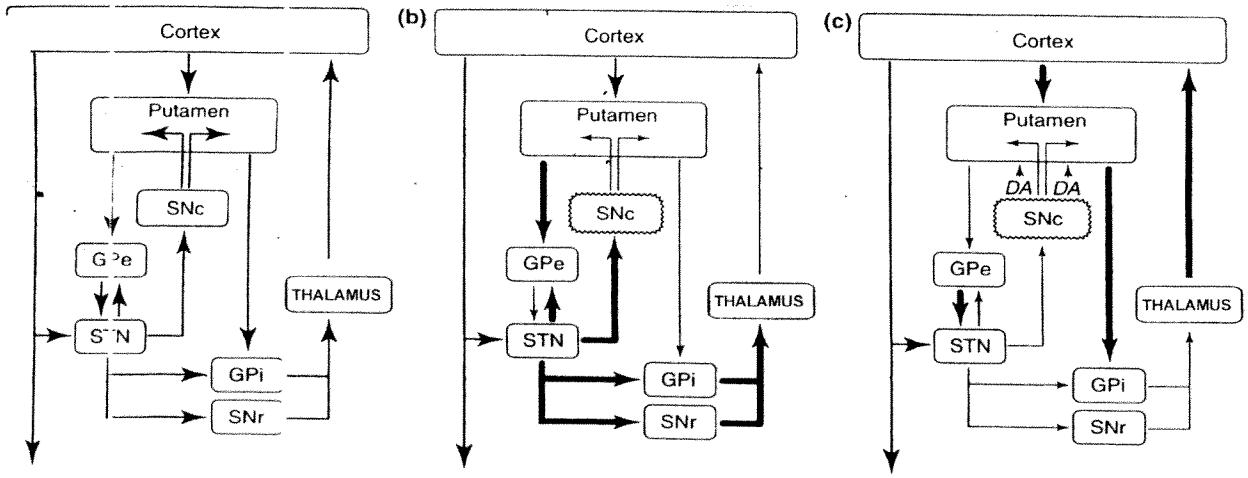

Figure 6: (modified from Obeso JA and Olanow CW, 2000) Connection Differences in Normal, PD and LID Basal Ganglia. A) normal, B) PD and C) LID basal ganglia can be seen in the diagram. Size of lines and arrow heads indicate amounts of activity. Bold lines represent an increase in activity, thinner and smaller arrow heads indicate a reduction in activity.

What has been clearly realized is that the development of LID depends on two main factors: pulsatile administration of DA replacement therapy and the degree of DA denervation (Hirsch E, 2001). Studies comparing intermittent levodopa administration to continuous DA administration showed LID does not arise with continuous levodopa administration (Calabresi P. et al., 2000). Continuous pharmacological stimulation of DA receptors results in desensitization of motor behavior whereas intermittent stimulation of these receptors produces increased sensitization of motor behavior (Calabresi P. et al., 2000). When rats are administered the DA agonist, apomorphine, their locomotive responses differ depending upon *how* the apomorphine is administered (Castro R. et al., 1985). Castro R. et al. (1985) compared DA denervated rats receiving continuous

administration of apomorphine and noted the locomotive responses increases after intermittent injections as opposed to continuous administration. In contrast, the locomotive effect of apomorphine declines when the following injection is given before the preceding dose is completely metabolized. In addition, the greater the DA depletion, the larger the motor response to DA pulsatile administration (Castro R. et al., 1985). Speculations on why this may be are presented below.

Early studies of neuronal activity in the SNc noted a slow and steady firing rate of DA neurons which serve to produce stable movements (De Long R. et al., 1983). This observation suggests that the SNc exerts a tonic (i.e. continuous) influence on basal ganglia circuits (Obeso JA and Olanow CW, 2000). Under normal circumstances in the intact striatum, DA receptors on striatal neurons are at a basal level of continuous activation (Calabresi P. et al., 2000). In the DA depleted system of PD, the synaptic terminals that store and regulate the release of DA grow dependent upon the peripheral supply of exogenously administered levodopa (Calon F. et al., 2000). With increasing loss of DA in advancing PD, there is a dramatic loss of DA terminals as well as greater difficulty in buffering the fluctuations in plasma levels of levodopa (Obeso JA and Olanow CW, 2000). In such instances, fluctuations in the plasma concentrations of levodopa may expose DA receptors to alternating high and low concentrations of DA, therefore disrupting the continuous activation of DA receptors observed in a normal system (Obeso JA and Olanow CW, 2000). However, because levodopa has a short half life in circulation it must be administered in a chronic intermittent fashion (Figure 7) (Graybiel et al., 2000).

Pulsatile stimulation of DA receptors in the striatum has clearly been implicated in the onset of LID, however, the exact mechanism underlying LID remains unclear (Obeso JA and Olanow CW, 2000). What is known is that DA concentration in the intact nigrostriatal pathway, is at a constant level and is maintained with little variation (Calon F. et al., 2000).

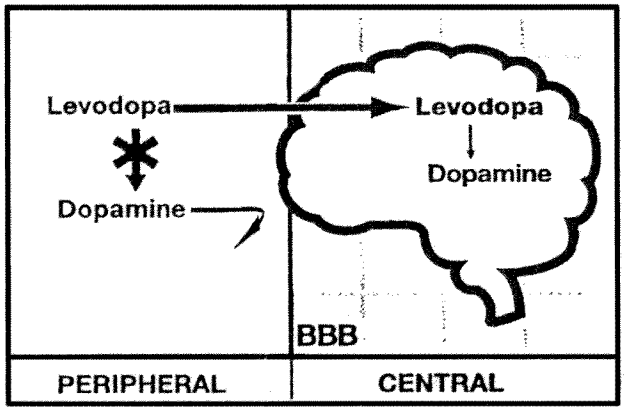

Figure 7: (modified from Koller WC, 2000) Levodopa and absorption in the PNS and CNS. Central nervous system relies on the periphery for DA supply.The diagram depicts levodopa being administered in the periphery and transformed into DA once it crosses the blood brain barrier (BBB) and enters the central nervous system.

Therefore, pulsatile administration of levodopa contrasts dramatically with this continuous flow of DA in the intact striatum. However, recent studies have suggested a link between the onset of LID and activation of the striosomes in the striatum (Graybiel AM et al., 2001). Past research has shown that the striosomes are involved in the processing of long-term memory of reinforcement/stimulus response and reward learning (Graybiel AM, 2000). In fact, pulsatile release of DA and subsequent DA receptor stimulation in the striosomes occurs naturally during theses types of learning in the basal ganglia (Graybiel AM, 2000). Therefore, the pulsatile stimulation induced by levodopa

treatment for PD may activate the same region in the basal ganglia involved in reinforcement and stimulus/response learning, the striosomes, and subsequently affect the activity of the medium spiny neurons of the matrix. A closer look into how the reinforcement and stimulus/response mechanisms function in the basal ganglia may shed some insight into what may be occurring during repeated levodopa treatments and subsequent LID in PD.

Basal Ganglia in Learning and Memory

In recent decades investigation of the basal ganglia has become an active area of research in the study of learning and memory (Compton, 2001). For example, studies have substantial evidence that supports the claim that the basal ganglia plays a significant role in the formation of habits and in stimulus/response learning (Compton DM et al., 2001). Researchers have found that the basal ganglia provide a pathway, the striosomal pathway, that allows the coupling of sensory information/stimuli with a conditioned motor behavior (Calabresi P. et al., 1997). Inputs to the striosomes are primarily routed from the prefrontal cortex, insular cortex and the amygdala (Dure LS IV et al., 1992). These same regions are important in processing memory (Dure LS IV et al., 1992). An example of stimulus/response and habitual learning was provided by Pavlov's classical conditioning experiments which was the first model of learning studied in psychology (Mackintosh, 2003). Pavlov trained dogs to salivate at the sound of a bell by forming a reflexive behavior to a newly learned association. During the training period a bell became the "conditioned" stimulus for the dogs following repetitive trials of food

rewards after the bell was sounded (Mackintosh NS, 2003). A newly learned association was expressed by behavior. Similarly, the reinforced behaviors on a cellular level appear to be processed in the striosomes (Graybiel AM et al., 2000). After Pavlov, some of the earliest work in stimulus/response learning was done by Divac I. (1967, 1968) who showed that lesions in various parts of the caudate nucleus in rats, monkeys and cats impaired memory retention. In attempts to train rats to respond by lever presses by using light as the stimulus and food as a reward for pressing, rats with caudoputamen lesions were unable to retain this information (Divac I., 1967). Such findings are consistent with memory consolidation for stimulus/response learning occurring in the striatum which supports the idea that learned stimulus/response information is held in a labile state and may be influenced for a brief period following its initial acquisition (McGaugh JL and Herz MJ, 1972). These observations led to the belief that some functions of the caudate (striatum) cannot be explained by mere sensory and motor systems alone but rather that memory was involved for learned motor behavior (Packard, MG. et al., 1989). White NM et al. (1996) presented evidence that the dopaminergic nigrostriatal pathway was involved in the consolidation of memories. In addition, Divac I. (1968) showed that posttraining injections of DA agonists into selective regions of the caudoputamen increased retention of memory tasks. Such work is suggestive that DA in the caudoputamen is necessary in forming conditioned responses through reinforcement. In fact, distinct parts of the caudoputamen may differ in their memory-related functions (Graybiel AM, 1995). The medial part of the caudoputamen has been associated with conditioned reward responses and the lateral part is correlated with stimulus/response learning (Graybiel AM, 1995). Thus, distinct regions of the striatum are associated with different learning processes.

Similar to stimulus/response learning is the concept of conditioned reward responses in the basal ganglia. Anticipation of conditioned rewards as a form of memory was studied by Schultz W. et al. (1997) in the DA neurons of the nigrostriatal pathway. The reward pathway is activated by DA pulsatile stimulation. Once this DA pulsatile activation has begun, the brain is prepared to receive novel stimuli. The DA neurons were activated by unanticipated reward stimuli compared to conditioned stimuli where rewards were expected. In the cases where rewards were expected the DA neurons were not activated. Assessment of reward stimuli is proposed to work by the activation of phasic, that is, pulsatile, not continuous, DA activity (Schultz W., 1997). Therefore, the initiation of this phasic DA activity would indicate novel reward stimuli and errors with respect to the normal/conditioned reward responses. These prediction errors would be translated as new situations leading to rewards that would require the learning of a novel stimulus (Schultz W., 1997). DA would be released in response to this new stimulus and serve to reinforce and consolidate it (Figure 8) (Schultz W., 1997). Such activity is similar to what occurs in LID which is also a manifestation of pulsatile DA activation in the basal ganglia. Recent research presents evidence that the striatum is differentially activated dependent upon the learning of new or familiar behavioral routines (Graybiel AM, 1995). Studies that support the differential activation of the striatum found that novel behavior/ new learning takes place in the anterior striatum (i.e. caudate nucleus), whereas pre-learned or repetitive tasks activate the posterior striatal regions (i.e. putamen). Based on these data, it is clear that the basal ganglia seem to have a strong role in forming types of memory involved in conditioned responses. In order to understand how these memories.

could be formed on a cellular level, it is helpful to review some current theories on the molecular basis of forms of memory mechanisms

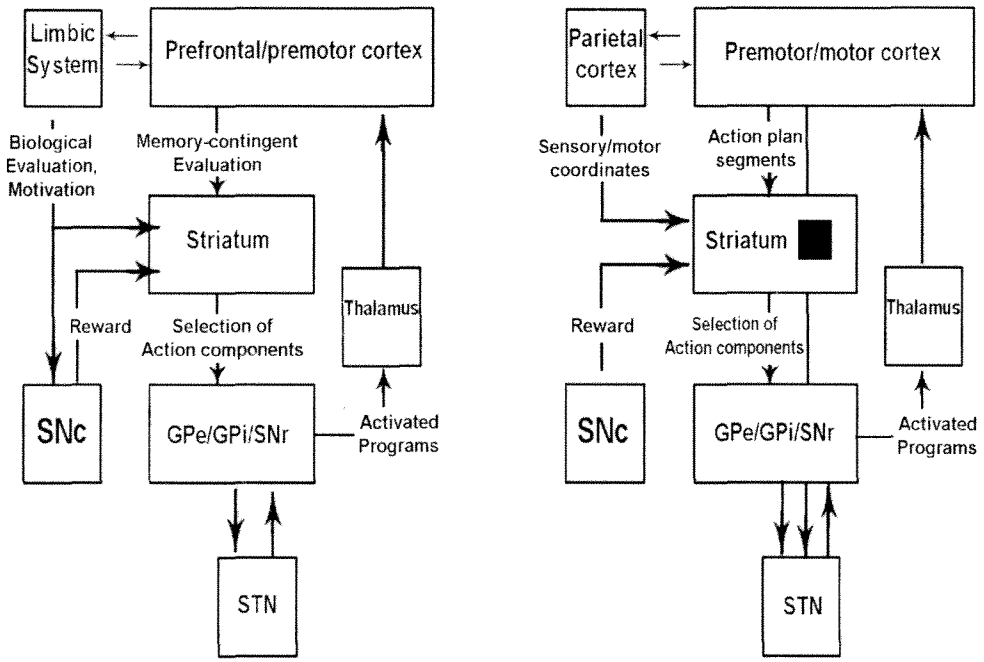

Figure 8: (modified from Graybiel AM, 1995). Learning and Expression of Behavioral Routines Flow Chart. A) Information inputs from the prefrontal cortex, limbic system and SNc are processed through the striatum. Processing through pallidonigral-subthalamic circuitry differentially activates programs that are sent to the cerebral cortex and to pattern generators in the brain stem/spinal cord. As time progresses these programs build behavioral routines. Pale squares in the striatum indicate striosomes, which are more prominent in the caudate nucleus.

B) Once behavioral programs are learned, their behavioral sequences are ready to be expressed. Information about action plans are sent from the premotor/motor cortex and other inputs are sent from the parietal cortex which supplies sensorimotor/spatial information which is then processed in the striatum (putamen and parts of the caudate nucleus). This activates selected programs through the pallidonigral-subthalamic circuitry which are sent to the cerebral cortex and to the brain stem/spinal cord. This system interacts with the learning system and is modifiable dependent upon evaluation signals that may be received by the SNc. The dark square in the striatum represents the striosomal organization of the putamen and caudate nucleus.

CHAPTER 3

HETEROSYNAPTIC PLASTICITY IN THE STRIATUM

Synaptic plasticity is a long lasting change in the functional efficacy of synaptic transmission that is prompted by certain patterns of brain stimulation (Calabresi P.et al., 2000). Long term memory has been hypothesized to be a function of this synaptic plasticity (Silva MA et al., 1997). Therefore, synaptic plasticity occurring in the striatum may be modulated by the basal ganglia (Calabresi P. et al., 1996). In the most general terms, synaptic plasticity can be broken down into two basic components, homosynaptic plasticity and heterosynapic plasticity (Blais BS et al., 1999). Homosynaptic plasticity involves a single synapse, which with repeated activation either becomes stronger or weaker (Blais BS et al., 1999). In heterosynaptic plasticity, activity in one neuron modulates activity in another by either increasing or decreasing its presynaptic strength (Blais BS et al., 1999). The presynaptic strength would be increased if action potentials are positively correlated with the postsynaptic cell activity and decreased if the action potentials are not correlated with the postsynaptic cell activity. The basic idea in heterosynaptic plasticity is that the synaptic strength of one neuron is modified by the activity in another neuron (Houk JC, 1995).

In the basal ganglia, heterosynaptic plasticity could be demonstrated by the striosomal projection neurons possibly affecting the activity of the matrix neurons at several potential locations. The most probable of these sites is through the ACh interneurons that line the border between the matrix and striosomes.

As stated earlier, the striatal output neurons comprise two distinct compartments, the striosomes and matrix, with ACh interneurons located at the striosome/matrix border (Graybiel AM et al., 2000).This anatomical arrangement may allow the striosomes to influence, via ACh interneurons, the projection neurons in the matrix that comprise the direct and indirect pathways. This synaptic organization illustrates on a cellular level how reinforcement by heteroynaptic plasticity might work (Houk JC, 1995).

While DA has been associated with positive reinforcement, such memory mechanisms contributing to LID have been marginally investigated. Positive reinforcement could activate a heterosynaptic plasticity mechanism in the basal ganglia. In principle heterosynaptic plasticity combines three factors: presynaptic activity, postsynaptic activity and a neurochemical reinforcement (Houk JC, 1995). A neurochemical reinforcement refers to the release of a chemical agent during the reinforcing activity. Such neurochemical reinforcement has been attributed to monoamines, such as DA (Calabresi P. et al., 2000). Studies have demonstrated that the release of monoamine neurotransmitters from one group of neurons can influence long term changes in transmission efficiency in another group of neurons, even if the neurotransmitter used in the both pathways are not the same (Houk JC, 1995). For instance DA in one pathway could influence glutamate cell activity in another pathway. In general, heterosynaptic plasticity is facilitated by a monamine mediated increase of the intracellular concentrations of cAMP (Houk JC, 1995). For example, Frey U. et al. (1993) found that a brief application of a cAMP analog induces a long lasting potentiation in the neurons of the striatum. Activation of D1 receptors which promotes an increase in cAMP may be responsible for heterosynaptic long term changes in synaptic efficacy of medium

spiny neurons in the striatum (Houk JC, 1995). Bouyer JJ et al. (1984) found that striatal afferents from the cerebral cortex and DA afferents from the SNc often terminate in close proximity to one another, their synapses are often on the same dendrites and/or on the same dendritic spines. Tyrosine hydroxylase (TH) staining showed that these DA synapses found on dendrites and spines were located on the striatal medium spiny neurons (Freund TF et al., 1984). Thus, the convergence of dopaminergic, glutamatergic and cholinergic synapses on medium spiny neurons includes the criterion for heterosynaptic modification. Following DA denervation, levodopa administered in a pulsatile manner could possibly activate the striosomes, thereby disrupting the normal synaptic mechanism in the striatum (Graybiel AM, 2000). In turn, the striosomal compartment could influence the medium spiny neurons of the matrix by acting through the ACh interneurons. The potential influence of the striosomes on the matrix via the ACh interneurons would incorporate factors necessary to form heterosynaptic plasticity resulting in the production of long term memory.

LTP and LTD in the Striatum

The heterosynaptic arrangement of striosomes, ACh and the matrix in the striatum allows for a bimodal (i.e., weak or strong) stimulation mechanism to change synaptic efficacy. The two most fundamental molecular theories of long term memory formation are long term potentiation (LTP) and long term depression (LTD). LTP is an increase in synaptic strength whereas LTD is a reduction in synaptic strength (Baudry M. et al., 1993). Research suggests there are no synapses exclusively expressing LTP or LTD,

instead, plasticity in synapses is evoked by either strong stimulation in the case of LTP or weak stimulation in the case of LTD (Calabresi P. et al., 1996). Such stimulation would proceed upwards (LTP) or downwards (LTD) in an extended continuum (Calabresi P. et al., 1996; Linden DJ, 1998; Zucker RS et al., 1989). On a molecular level, stimulation intensity determines the amount of calcium influx, which in turn, determines whether LTP or LTD is triggered (Figure 9) (Zucker RS et al., 2001). Therefore, the amount of intracellular calcium determines the bimodal state of heterosynaptic plasticity: a lesser amount of calcium influx triggers LTD whereas a more substantial amount of calcium influx triggers LTP (Bailey CH et al., 2000). This mechanism may allow for memory storage to be organized in an array of synaptic weights of different strengths. Synaptic connections may be driven to their minimal (LTD) and maximum (LTP) strengths within the neural array and may function to contribute to the regulation of synaptic plasticity by determining the synaptic connections to present the state of LTP or LTD based upon the amount of calcium influx (Berke JD and Hyman SE, 2000). Both LTD and LTP mechanisms have the potential to be expressed in the striatum because of the low excitability of striatal neurons. However, the normal synaptic mechanism in the striatum favors LTD, with LTP rarely expressed (Calabresi P. et al., 1997; Hirsch E, 2001). LTD and LTP both requires activation of striatal glutamate and DA receptors (Calabresi P. et al., 1994). LTD is triggered in striatal neurons when DA receptors are activated in addition to AMPA receptor activation, while LTP is triggered when DA agonist administration is accompanied by NMDA glutamate receptor activation. This may be due to NMDA receptor's influence on calcium influx.

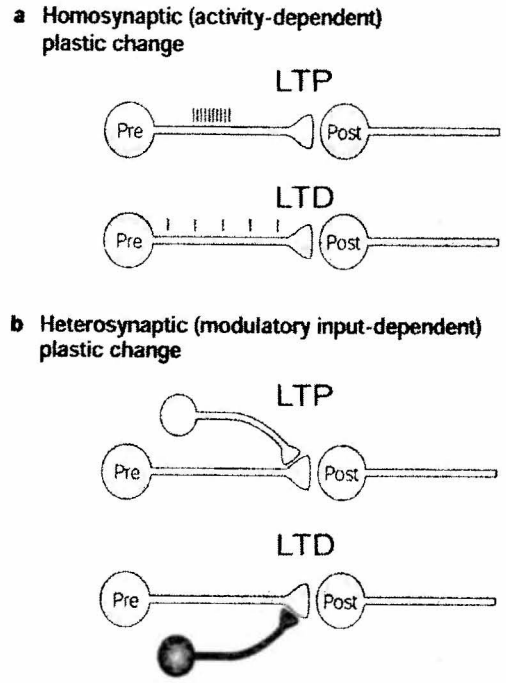

Figure 9: (modified from Bailey CH et al., 2000) Homosynaptic and Heterosynaptic Mechanisms. A) homosynaptic: synaptic strengthening occur at the same synapse as it is being strengthened. B) heterosynaptic: synaptic strengthening between a presynaptic and postsynaptic cell results as a third neuron, a modulatory interneuron, regulates the strength of the synaptic strength

The state of synaptic plasticity, LTD or LTP, depends upon the amount of intracellular calcium in the striatum (Calabresi P. et al., 1994). AMPA and NMDA glutamate receptors appear linked to LTD and LTP, respectively. Therefore, it may be useful to analyze these glutamate receptors and assess what specifically in their individual structure determines these distinct functions to promote LTD or LTP. Castellani GC et al. (2001) addressed these questions by the examination of subunit characteristics of AMPA and NMDA glutamate receptors. Previous studies had provided evidence supporting the idea that calcium concentration determines the state of synaptic

plasticity, LTD or LTP (Calabresi P. et al., 1994; Carrol FI et al., 1999). Studies have confirmed that high frequency stimulation (HFS) of presynaptic afferents leads to LTP whereas low frequency stimulation (LFS) of presynaptic afferents leads to LTD (Castellani GC et al., 2001). It has been suggested that subunits of AMPA receptors directly influence the amount of calcium influx and therefore serve as a regulating component of thee bimodality of synaptic plasticity, LTD or LTP (Carrol FI et al., 1999). AMPA receptors are composed of subunits termed GluR1-GluR4 (Castellani GC et al., 2001). Properties of AMPA receptors are dependent upon the composition of individual GluR1-GluR4 subunits and the phosphorylation/dephosphorylation state of the subunit proteins (Castellani GC et al., 2001). Specific sites on these AMPA subunit proteins have been linked to synaptic plasticity, e.g. Serine 831 (S-831) and Serine 845 (S-845) on the GluR1 subunit which can be phosphorylated by CaMKII/protein kinase C and protein kinase A, respectively (Carrol FI et al., 1999). Expression of LTP correlates with a two-fold increase in phosphorylation of S-831 and the expression of LTD is correlated with a decrease in the phosphorylation of S-845 (Carrol FI et al., 1999). Therefore, knowing the phosphorylation states the effect they have on LTD/LTP expression may help predict the state of synaptic plasticity. For example, if LTP is correlated with an increase in phophorylation of S-831, then removing the phosphate at this particular site may block LTP expression.

NMDA receptor antagonists inhibit the induction of both LTP and LTD that suggests that NMDA receptors may be an entry point for calcium in the postsynaptic neuron. This in turn would affect the state of synaptic plasticity (Castellani GC et al., 2001). NMDA receptors are composed of the subunits: NR1 and NR2. NR2 subunits

have four distinct subtypes, 2A-2D, which have specific functions for mediating ionic currents in the NMDA receptor (Castellani GC et al., 2001). NR1 and NR2B subunits mediate currents at ~ 250 milliseconds whereas NR2A subunit mediates faster currents at ~ 50 milliseconds (Castellani GC et al., 2001). Castellani GC et al. (2001) presented a model which combines the changes of AMPA receptors with the changes of NMDA receptors in order to predict the directionality of synaptic plasticity, LTD or LTP, based upon the amount of intracellular calcium present within the system being measured. The combination of particular subunits on glutamate receptors caused modality changes in the synaptic plasticity. A similar case may be true in the striatum because neurotransmitters that seem to have a role in synaptic plasticity in the striatum are DA, glutamate and ACh. These are the neurotransmitters found in the pathway leading from the striosomes > ACh interneurons > matrix. However, the interaction of these neurotransmitters and their affect on calcium levels in the medium spiny neurons in the striatum has not been investigated. If their interaction can be shown to affect calcium levels in the striatum, then it is likely that LTP expression in the striatum could be regulated and as a consequence LID suppressed.

The imbalance that erupts in the basal ganglia between the DA and glutamate following DA denervation may contribute to the change in synaptic efficacy in the striatum (Calabresi P. et al., 2000). Following DA denervation, the striatum experiences an elevation in both glutamate and ACh (Starr MS, 1995). Both of these neurotransmitters are modulated by DA (Starr MS, 1995). Thus as DA levels decrease in the striatum, so does DA's ability to modulate glutamate and ACh. An increase in glutamate has been proposed to compensate for the depletion of DA in the striatum (Starr

MS, 1995). The increase in glutamate causes excitation in the striatum whereby the striatal neurons enter the depolarized state and NMDA receptors are activated. The NMDA receptor activation allows more calcium to enter the cells for LTP to be expressed. This disruption of striatal neurotransmitters leads to a difference in synaptic efficacy in medium spiny neurons that is modulated by an increase in intracellular calcium levels that results in LTP expression.

Following DA denervation, LTD in the striatum is no longer expressed and chronic pulsatile administration of levodopa treatment produces LTP in the striatum (Calabresi P.et al., 2000). Calabresi P. et al. (2000) hypothesizes that after DA denervation the LTP that is induced by pulsatile levodopa involves a change in gene expression that leads to altered metabolic functions of postsynaptic striatal neurons (Figure 10). These altered metabolic functions would ultimately result in apoptosis (Calabresi P. et al., 2000). Additional alterations noted in Calabresi's (2000) investigations were changes in NMDA subunits that alter phosphorylation processes and correlate with LID. Activation of these altered subunits would cause an extensive elevation in intracellular calcium in striatal spiny neurons, leading to an alteration of the metabolic state of the cell. These alterations have been thought to promote depolarization and further NMDA receptor activity in a cyclical manner that is correlated with apoptosis (Calabresi P. et al., 2000).

Since D2 receptors have a regulating function over glutamate and DA release, the role of the D2 receptors in regulating synaptic plasticity has been examined. Maura G. et al. (1988) showed that D2 receptors and possibly D4 (D2 like) receptors are located on corticostriatal terminals and these receptors inhibit the release of glutamate. Calabresi P.

et al. (1997) compared D2 receptor knock out mice to wild type mice to further investigate the role of DA receptors in synaptic plasticity. Since HFS has been previously shown to induce synaptic plasticity in the cerebral cortex, Calabresi P. et al. (1997) decided to use HFS to investigate LTD and LTP in striatal brain slices. *In vitro* HFS of brain slices from wild type mice produced LTD, whereas this same stimulation in slices from D2 receptor deficient mice produced LTP. This seems to suggest that in D2 receptor deficient mice, either there is a change in synaptic efficacy following repetitive activation by HFS of corticostriatal projections or that D2 receptors are required for LTD. Freedman JE and Weight FF (1988) found postsynaptic D2 receptors are capable of blocking cAMP activation, activating potassium channels and preventing calcium currents from entering isolated striatal neurons. This suggests that D2 receptors may be associated with LTD and in the absence of D2 receptors LTP is expressed in the striatum. Therefore, D2 receptors could serve to suppress calcium influx in the striatum so that in their absence, calcium influx occurs and subsequent LTP is produced. Drago J. et al. (1994) reached similar conclusions during experiments that explored the effects of D1 and D2 receptor deficient mice on motor behavior. While D1 receptor deficient mice have normal coordination and mild hyperactivity (Drago J. et al., 1994), D2 receptor deficient mice present with motor symptoms of PD (Baik JH et al., 1995). These data indicates that D2 receptors play an instrumental role in long term changes in synaptic efficacy in the striatum and may be necessary for LTD expression in the striatum.

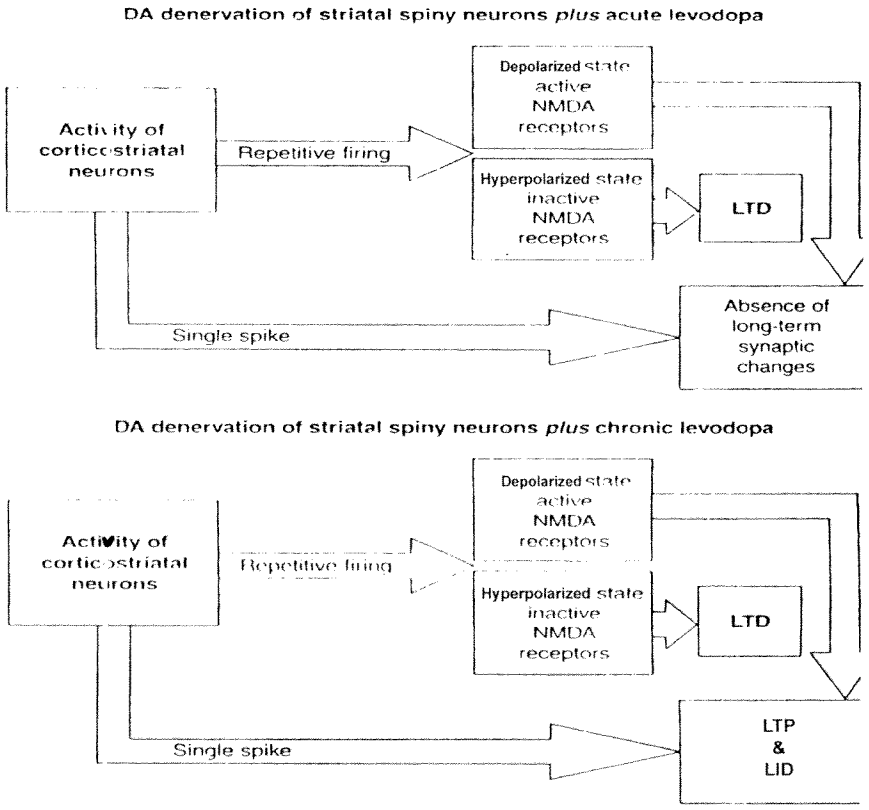

Figure 10: (modified from Calabresi P. et al., 2000) Long Term Mechanisms Following Acute and Chronic Treatment with Levodopa. The top half of the diagram depicts DA denervation plus acute levodopa treatment, LTD is produced when striatal neurons are hyperpolarized (refererred to as "down" state in diagram) and NMDA receptors are inactive. No LTP is produced during acute levodopa treatment. The bottom half of the diagram illustrates chronic levodopa treatment post DA denervation. NMDA receptor activation plus striatal neurons in the depolarized state produce LTP and LID. NMDA inactivation and striatal neurons in the hyperpolarized state produces LTD and no LID.

CHAPTER 4

CORRELATIONS BETWEEN MEMORY AND MOTOR PATTERNS IN THE BASAL GANGLIA AND THE ONSET OF LID

Until very recently, most basal ganglia research has not investigated in depth the possibility that LID, which greatly compromises the effectiveness of PD treatments, could be the result of a memory pattern generated in the basal ganglia. When DA denervation occurs in PD, the normal continuous flow of DA to the striatum is interrupted. At the same time levels of glutamate and ACh increase within the striatum. DA has a modulatory role over both of these neurotransmitters and it is also hypothesized that the increase in glutamate serves a compensatory role in the striatum when DA levels decline (Starr MS, 1995). This change in the neurochemistry of the basal ganglia following DA depletion causes the striatal neurons to transition from a hyperpolarized state to a depolarized state. As a result of this transition, NMDA receptors on medium spiny neurons are activated and this fosters an elevation in calcium influx in the medium spiny neurons of the striatum leading to either LTP or LTD. What is not known is to what degree the interactions among ACh, DA and glutamate regulates intracellular calcium influx in the medium spiny neurons. In addition, D1 receptor activation in the neurons of the striosomes and the matrix produces an increase in cAMP production in the medium spiny neurons of the striatum. The result of ACh interneurons mediated calcium influx may activate PKC and PKA kinases associated with cAMP responsive-element binding protein (CREB) transcription changes (Silva et al., 1997). Transcription factors are nuclear proteins that bind to specific base sequences of DNA in the regulatory regions of

particular genes so as to regulate their expression. Both the production of cAMP by D1 receptor activation and the increase in calcium can activate CREB transcription factors (Silva MA et al., 1998). Memory mechanism, in particular, LTP, have been linked to the activation of CREB transcription factors (Silva MA et al., 1997). Such protein synthesis and gene expression changes are necessary elements in long term synaptic changes that are the necessary for long term memory (Silva MA et al., 1997). The molecular changes that produce long term synaptic changes within the striatum are essentially unknown (Calon F. et al., 2000). Molecular changes have been observed in the hippocampus during LTP but there is no conclusive evidence that specific transcription factors produce a form of long term memory that is manifested as LID in the striatum (Figure 11).

The underlying mechanism for the interaction between glutamate and DA in the striatum is not well understood, but evidence suggests their involvement in cAMP activation and the identity of sets of genes encoding transcription factors such as CREB which affect the expression of members of the Fos-Jun family of transcription factors (Canales JJ et al., 2002). Expression patterns of immediate early genes (IEG) coding for the Fos-Jun family of transcription factors are activated during DA-glutamate interaction in the striatum (Canales JJ et al., 2002). Following DA denervation, the expression of IEGs changes throughout the course of chronic levodopa treatment and IEG expression shifts from the matrix region of the striatum to the striosomes (Figure 12).

The shift in IEG expression in the striatum also correlates with the occurrence of LTD and LTP in the striatum. The cortical inputs to the matrix are innervated by the association cortex in the medial parts of the striatum and by the sensorimotor cortex in

the lateral parts of the striatum (Graybiel AM et al., 2000). Cortical inputs to the striosomes are innervated by the limbic system (Graybiel AM et al., 2000)

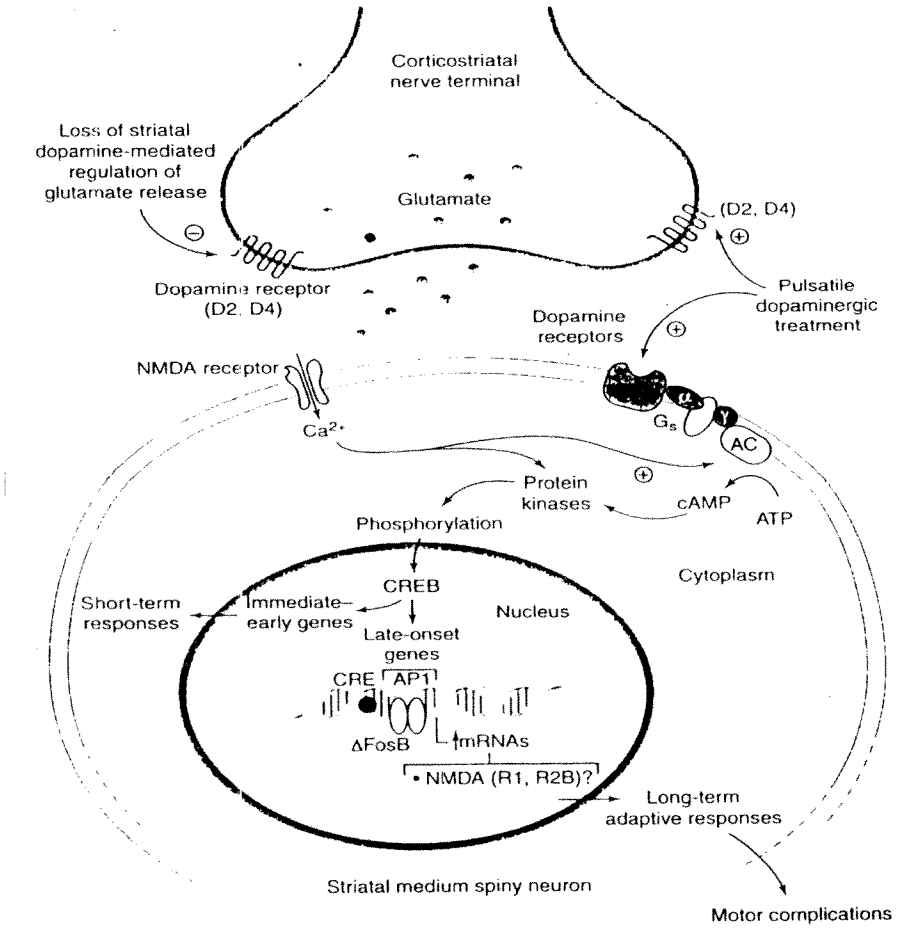

Figure 11: (modified from Calon F. et al., 2000) Flow of Molecular Events presenting LID as Abnormal Learning. DA and glutamate interaction produce molecular events. LID are depicted as a result of abnormal learning brought about by DA denervation and pulsatile L-Dopa treatment which elicits changes in the basal ganglia that result in poorly regulated responses to DA agonists

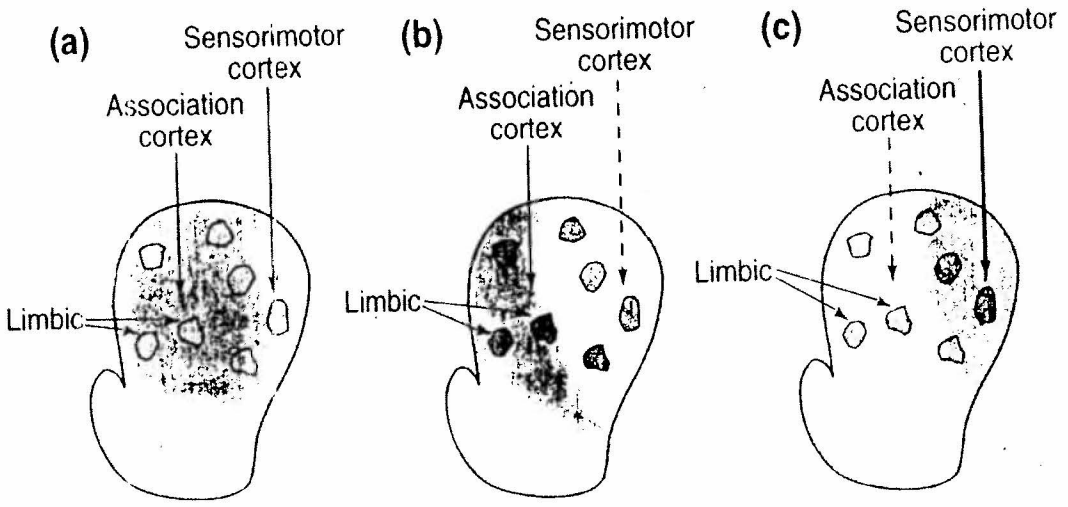

Figure 12: (modified from Graybiel AM et al., 2000). Shift of IEG Expression from Matrix to Striosomes in Chronic Levodopa Treatment. Shaded areas are activated IEGs (Fos family) in response to levodopa treatment over time. A) activation of IEG expression is widespread in the striatum during acute levodopa treatment. B) activation of IEG expression is predominant in the striosome, as levodopa treatment becomes chronic and intermittently administered striatal DA is reduced substantially, but some DA still intact in the striatum. C) activation of IEG expression is found in the striosomes and in the medial striatum as levodopa treatment becomes chronic and intermittently administered and the striatal DA has become completely depleted. LID are correlated with medial striatum/striosome activation (Graybiel et al., 2000). The dashed lines indicate areas where LTD is expressed and the bold lines indicate where LTP is expressed.

Acute levodopa treatment correlates with widespread IEG activation in the striatum (Graybiel AM et al., 2000). As chronic levodopa treatment proceeds over time a striosome predominant pattern of IEG activation emerges in the anterior and lateral striatum and LTP is expressed (Graybiel AM et al., 2000). Interestingly, NMDA glutamate antagonists have been shown to improve LID (Calabresi P.et al., 2000); their

effectiveness could be a consequence of blocking the LTP mechanism that may be a direct result of limiting the calcium influx via NMDA receptors. Blocking the LTP mechanism in the striatum could then prevent the formation of the motor memories that constitute LID. Since all the neurotransmitter interactions needed to express striatal LTP are found in ACh interneurons, it would be interesting to observe if cholinergic antagonists also have the potential to block striatal LTP. Indeed, cholinergic antagonists were the first effective therapeutic agents used to treat PD (Yahr MD, 1993). However, these anticholinergic treatments were eventually discontinued due to memory impairments that PD patients encountered. As noted, DA differentially modulates ACh release through D1 and D2 receptor stimulation (Calabresi P. et al., 1998). Following the loss of DA in PD, ACh modulation is altered resulting in a substantial increase in the levels of ACh in the striatum. In fact, studies have suggested the oscillatory phase that striatal neurons present after DA depletion is the result of the increase in ACh which enables the depolarized state of the striatal neuron and produces LTP in the striatum (Burgard EC and Sarvey JM, 1990). Thus, by blocking ACh receptors, this may prevent LTP in the striatum from being formed and, in turn, LID that accompanies chronic levodopa treatment may potentially be controlled.

Although monoamine mediated heterosynaptic plasticity has been studied in a number of neural systems, its potential to produce motor memories resulting in LID in the striatum has not been thoroughly explored. In other neural systems outside the striatum, the release of monoamine neurotransmitters from one set of synapses can elicit long term changes in efficacy of transmission in another set of synapses that use a different neurotransmitter (Schwartz and Greenberg, 1987). In many instances this

monoamine mediated heterosynaptic plasticity is brought about by the increase in the intracellular concentration of cAMP (Greenberg SM and Schwartz SH, 1987). In a wide range of species, these actions of monoamines underlie the storage of memory in the form of long lasting changes in synaptic efficacy (Greenberg SM and Schwartz SH, 1987). Monoamines mediating the changes in synaptic efficacy in the striatum may be responsible for the striosomes producing heterosynaptic plasticity in the matrix through ACh interneurons. The striosomes have a large number of D1 receptors that are known to increase cAMP responses. Therefore, levodopa acting in the striosomes synapses on the ACh interneurons, that in turn, synapse on neurons in the matrix. Since activation of D1 receptors increases cAMP, the pulsatile administration of levodopa acting through D1 receptors in the striosomes may increase cAMP in the cells of the striosomes that, in turn, activates protein kinases leading to phosphorylation of IEG that activate Fos. Fos/Jun AP1 complexes induce changes in NMDA receptor expression in the medium spiny neurons facilitated through ACh interneurons. These NMDA receptor changes would result in an increase in NMDA receptor activation that would subsequently trigger LTP in the medium spiny neurons. This example of heterosynaptic plasticity would yield a change from LTD to LTP in the medium spiny neurons.

In the striatum, there are several possible sites with an anatomical basis for heterosynaptic plasticity. The most probable place this heterosynaptic plasticity is taking place is on medium spiny neurons. Levodopa treatment activates the striosomes that, in turn, increases cAMP through D1 receptor activation in the striosomes. The increase in cAMP activates IEG expression and when coupled with AP1 complexes lead to NMDA receptor changes in the medium spiny neurons. Indeed medium spiny neurons express

DA, glutamate and mucarinic receptors and therefore a likely region for heterosynaptic plasticity to be expressed.

DA denervation results in an increase in glutamate (Starr MS, 1995). Some investigators have examined the possibility that chronic levodopa treatment further changes NMDA and AMPA glutamate receptor populations in the striatum, but evidence has been inconclusive (Starr MS, 1995). Following DA denervation and chronic levodopa treatment, an increase in tyrosine residue phosphorylation on NMDA subunits which indicates an increase in NMDA receptor activation and possibly NMDA receptor concentration has been reported from animal experiments (Menegoz M. et al., 1995). Similar phosphorylation changes on NMDA subunits have been found during LTP (Rosemblum K. et al., 1996). It has been noted in postmortem analyses of PD patients that there is an increase in ligand binding to NMDA receptors suggesting an increase in glutamate function in the basal ganglia which is correlated with calcium influx and second messenger cascades (Ulas J. et al., 1994). However, it is not clear whether this increase in binding of NMDA receptors is due to the DA depletion or the chronic levodopa treatment, but pharmacological evidence implies that an abnormal glutaminergic excitation in the striatum may contribute to the pathophysiology of LID (Papa SM et al., 1995). When an NMDA receptor antagonist, such as MK 801, is administered with levodopa to unilaterally 6-hydroxydopaminee lesioned rats, there is a substantial decrease in motor fluctuations (Papa SM et al., 1995); This effect is maximized when MK 801 is injected intrastriatally as opposed to systemically or injected into other regions of the basal ganglia (Marin C. et al., 1996). This suggests that glutamate acting in the striatum has a major role in inducing LID. Thus, if NMDA

antagonists such as MK 801 were administered with levodopa, it might switch the striatal neurons to a hyperpolarized state and by doing so, induce LTD which is the normal long term memory mechanism in the striatum (Calabresi P. et al., 2000). Therefore, by switching the heterosynaptic plasticity to favor LTD over LTP, the objective would be to minimize LID.

Because of the difficulty assessing new treatments for human diseases, noninvasive methods such as computer modeling have been employed in recent years in medical research. By designing an artificial neural network (ANN) to promote a bias in the modality of heterosynaptic plasticity in the striatum to favor LTD it may be possible to limit striatal LTP and thereby control LID which occurs during levodopa treatment in PD. Because the location of the proposed heterosynaptic mechanism is not certain, an ANN should assess the neurotransmitters to evaluate the importance of ACh, glutamate and DA in producing striatal LTP. The effect each of these neurotransmitters has on LTP expression may provide insight into where the heterosynaptic plasticity is taking place.

CHAPTER 5

ARTIFICIAL NEURAL NETWORKS AS A RESEARCH TOOL IN PARKINSON'S DISEASE

ANN are sets of algorithms that are modeled after the human brain (Hinton GE, 1992). Generally speaking, they contain mathematical equations that model biological processes such as learning and memory. Such models representing brain functions provide an important tool in neuroscience research because they offer a noninvasive exploration into measuring neuronal function in human pathologies. Neural networks differ from other types of programming techniques in that they have the unique ability to learn mathematical relationships between a series of inputs and their corresponding outputs. For a better understanding of neural network models, Tu JV(1996) provides an adequate summary. A comparison between ANN and standard statistics is outlined below in Figure 13. One significant difference between statistics and ANN is the level of expertise needed in building models.

In a standard statistical format there is a linear relationship between the independent and dependent variables. The ANN is designed to take complicated nonlinear relationships and find patterns between the input and output data. These differences can be depicted graphically by a diagram of a logistic regression model (Figure 14) and a basic ANN model (Figure 15). In the past ten years computational models of PD have begun to be used to complement other investigative methods. Current researchers have begun to see the importance of neural network modeling as a research tool in neuropharmacological investigations such as PD and therapeutic dosing

Neural networks	Statistics
Input	Independent (predictor) variable
Output	Dependent (outcome) variable, predicted value
Connection weights	Regression coefficients
Bias weight	Intercept parameter
Error	Residuals
Learning, training	Parameter estimation
Training case, pattern	Observation
Cross-entropy	Maximum likelihood estimation

Figure 13: (modified from Tu JV 1996) Comparative Terms Between ANN and Standard Statistics

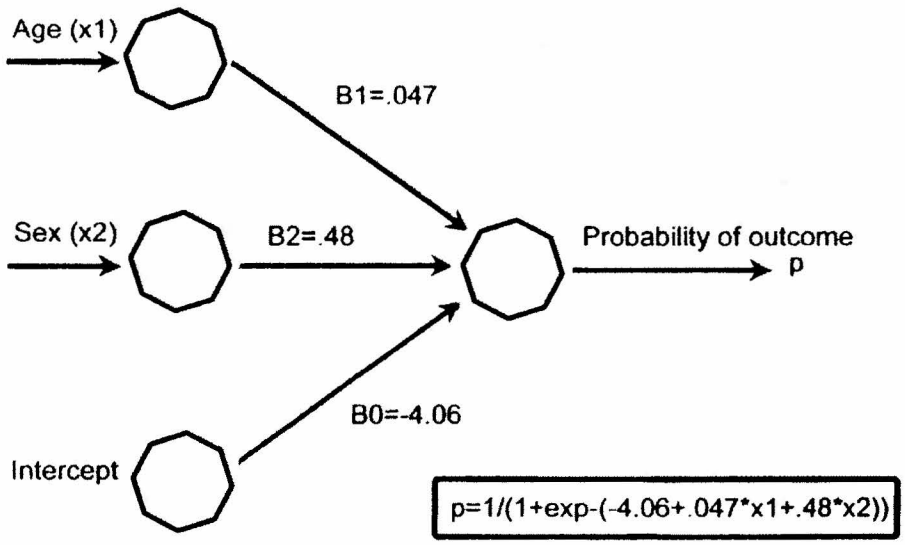

Figure 14 (modified from Tu JV, 1996) A Basic Logistic Regression Model. Logistic regression is a commonly used in statistical modeling. The probability of an outcome relates to a series of possible predictor variables. The usual assumption is that these predictor variables are related in a linear manner. This particular diagram is designed to predict the probability (p) of a patient dying from a hypothetical disease on the basis of the age (x1) and sex (x2) of the patient. The values of the beta coefficients are shown.

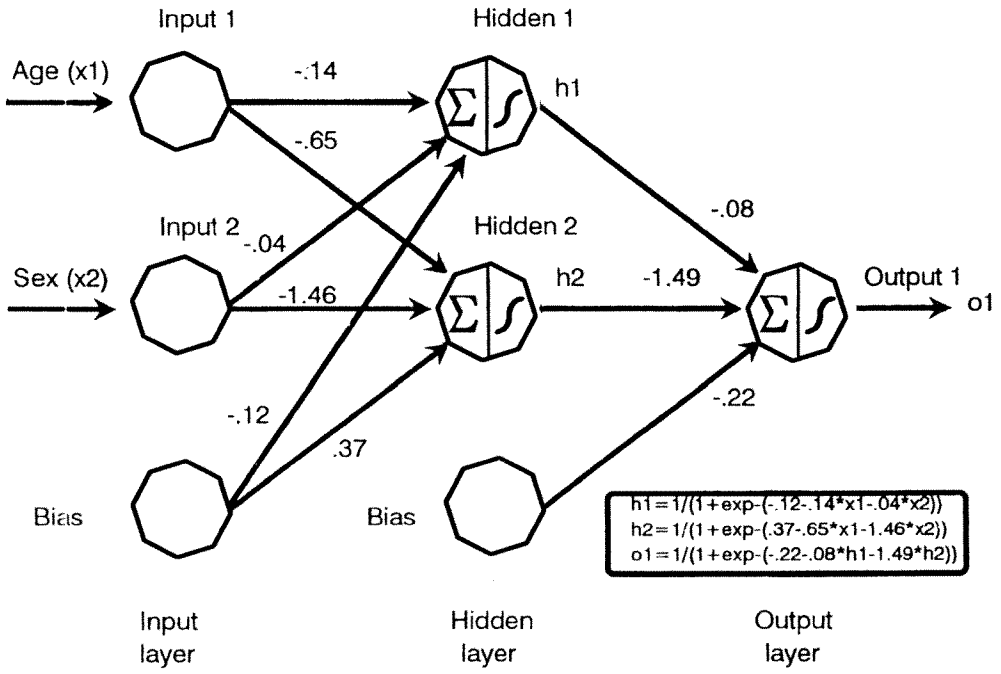

Figure 15: (modified from Tu JV 1996) A Basic ANN Model. Diagram of an ANN trained to predict the probability of a patient dying from a hypothetical disease on the basis of the age (x1) and sex (x2) of the patient. Each circle represents a node, each line represents a connection weight. The nodes of the network are in three layers (input, hidden, output). A logistic activation function is used in both the hidden (h1, h2) and output (01). H1 and h2 are the activation of hidden nodes 1 and 2 and the predicted output node 01. At each hidden and output node, a weighted linear combination of the inputs is summed and then a logistic transformation is applied.

Modeling the neural mechanisms that may be responsible for LID in PD may help to understand how the basal ganglia interact in healthy versus diseased subjects and may lead to experimental designs to verify theoretical methods. A variety of plausible mechanisms of drug action can be examined by designing an ANN system and varying constraints between neural units. Because ANN are trained rather than programmed, this leads to further refinement of the model being tested (Tu JV, 1996). Fahn S (1982)

purported that pathophyiological complications of PD should be divided into pharmacokinetic and pharmacodynamic mechanisms.

Pharmacokinetic complications relate to problems with absorption and the metabolism of levodopa (Spencer SE and Wooten GF, 1984). For instance the use of continuous DA treatment by controlled release capsules helps reduce pharmacokinetic problems to advanced PD (Chase TN. et al., 1989). However, the pharmacokinetic aspects alone fail to explain the changes in effectiveness associated with drug therapies for PD (Fabbrini G. et al., 1987).

Pharmacodynamic complications relate to the variables conditioning the response of DA receptors that mediate motor behaviors (Spencer SE and Wooten GF, 1984). An ANN model of PD was developed by Jamieson PW et al.(1991) that assessed the effects of levodopa pharmacodynamics in PD. Jamieson PW et al. (1991) investigated the actions of DA to determine: 1) if DA alone was responsible for the LID produced during chronic treatment of levodopa 2) how the degree of DA depletion affects the reactivity in the striatum 3) if two pharmacological mechanisms exist to explain the dyskinetic effects of levodopa, and 4) if both D1 and D2 receptor types are necessary to produce these effects. They developed a number of related models to test the system using input units based on the two necessary components of LID: degree of DA depletion and pulsatile administration of levodopa (Jamieson PW et al., 1991). Motor behavior was the output (Jamieson PW et al., 1991). The models were tested using data from studies of response fluctuations with levodopa treatment (Mouradian MM, 1988). The results from Jamieson PW et al. (1991) indicate that the effects of DA depletion and levodopa dose acting on DA receptors could not solely be responsible for the dyskinetic effects. The most

consistent data of the Jamieson study was the correlation of increased DA receptor stimulation to levodopa treatment with increased DA depletion (Jamieson PW et al., 1991). These results are supported in many experiments that show a greater degree of DA denervation correlates with a greater expression of LID (Bertorelli R and Consolo S 1990). The results of the Jamieson study also implied that distinct mechanisms produce the antiparkinsonian effect of levodopa and LID (Jamieson PW et al., 1991).

A more recent study by Wickens J and Kotter R (1998) used a computer model to explore the striatal mechanisms in PD. This study aimed to clarify some issues that were not addressed with earlier PD models (Wickens J and Kotter R, 1998). One such issue is the changes in patterns of neural firing in the basal ganglia that are likely to underlie the symptoms of PD (Wickens J and Kotter R, 1998). Much of their focus was on dendritic spines of striatal neurons, regarding them as isolated biological compartments (Wickens J and Kotter R, 1998). The use of dendritic spines in this manner allowed the researchers to study the postsynaptic interactions between glutamatergic and dopaminergic afferents in the striatum (Wickens J and Kotter R, 1998). They noted that following DA depletion, the morphology of striatal neurons is altered leading to a reduction in the length of individual spines and a decrease in the overall number of spines (Ingham CA et al., 1993). They hypothesize the change in firing patterns in the striatum is one way to compensate for the morphological and physiological changes in the basal ganglia that occur after DA denervation (Wickens J and Kotter R, 1998). Their computer simulations predict that there is a limit to such compensatory mechanisms in the basal ganglia and indicate that the effect of levodopa may be to prolong existing bursts in firing patterns in striatal neurons but not to generate new burst (Wickens J and Kotter R, 1998). Therefore,

they propose the compensatory mechanisms following DA denervation fail to be effective when corticostriatal excitation drops below a critical level needed to increase firing rates in striatal neurons (Wickens J and Kotter R, 1998). This suggests that changes in firing patterns of striatal neurons induce changes in synaptic plasticity that may alter the expression in the striatum from LTD to LTP. This has been the case in previously studied animal models of continuous versus pulsatile levodopa administration where firing patterns determine whether or not LID developed. These changes in firing patterns could also correlate with LTD (no LID) and LTP (LID).

A similar study was done by Terman D. et al. (2002), using a computational network model of activity patterns in the subthalamopallidal circuit of the basal ganglia. They were particularly interested in assessing the dynamic interaction between STN and GPe that lead to oscillatory activity patterns within this subthalamopallidal circuit (Terman D. et al., 2002). They predict that the STN and GPe neurons have traits similar to those of a central pattern generator and therefore may be able to self-sustain oscillatory activity. Terman D. et al (2002) followed STN and GPe activity over time in contrast to most basal ganglia models that are static. Simulations were run to investigate the design correlating the network with the associated synaptic conductances that may be modulating the activity of these networks over time (Terman D. et al., 2002). Terman's models found that this subthalamopallidal circuit is capable of producing both correlated rhythmic activity and irregular autonomous patterns of activity (Terman D. et al., 2002). Because neurons in both of these areas have similar membrane properties it was expected that they would be predisposed to rhythmic firing (Terman D. et al., 2002). Rhythmic firing refers to correlated patterns between STN and GPe occurring through an elevation

of striatal input coupled with a decrease of intrapallidal inhibition (Terman D. et al., 2002). Understanding what causes the change in the circuitry following changes in striatal input could shed insight into what causes the striatal circuitry change following DA denervation. An increase in striatal input and weakened intrapallidal inhibition within the indirect pathway can change the pattern of the circuit from irregular to rhythmic (Terman D. et al., 2002). The investigators speculate this may be why oscillatory activity in the subthalamopallidal circuit arises after DA denervation in PD (Terman D. et al., 2002). This is similar to the matrix circuitry involved in LID which creates an oscillation of physiological states of striatal neurons following levodopa treatment. This is another example of a subtle change in basal ganglia circuitry, in this case changes in firing patterns between GPe and STN, that has a strong influence on the activity of striatal neurons.

It seem reasonable to suspect the changes in firing patterns that occur after DA denervation in the striatal neurons is due to the interaction of glutamate and ACh coupled with levodopa treatment. Pulsatile administration of levodopa activates the striosomal pathway and therefore produces new signaling patterns in the basal ganglia circuitry (Graybiel AM et al., 2000). Because the striatum is capable of producing LTD and LTP, an appropriate neural network model would need to be bimodal in order to account for the expression of these two synaptic plasticity mechanisms.

A similar model was described in the hippocampus by Huerta and Lisman (1995) which described a bimodal heterosynaptic plasticity induced during cholinergic oscillation *in vitro*. The frequency of synaptic stimulation appears to determine whether CA1 synapses express LTD or LTP (Huerta PT and Lisman JE, 1995). The investigators

found that cholinergic oscillatory bursts were responsible for inducing LTP or LTD in the CA1 area of the hippocampus (Huerta PT and Lisman JE, 1995). The cholinergic system originating in the medial septum innervates the whole hippocampus and is known to enhance synaptic plasticity (Huerta PT and Lisman JE, 1995). Studies have shown that low doses of cholinergic agonists produce a slight enhancement of LTP in the hippocampus whereas high doses of cholinergic agonists produce a network oscillation (Burgard EC and Sarvey JM, 1990). Following DA denervation, there is an elevation of ACh in the striatum that may be responsible for LTP expression in the striatum in a manner similar to that observed in Huerta and Lisman's study. Huerta PT and Lisman JE(1995) looked specifically at the type of synaptic plasticity produced during very brief bursts of stimulation in the hippocampus (4 shocks at 100 Hz) *in vitro*. Previous studies had suggested the frequency of synaptic stimulation determines whether LTD or LTP is produced (Artola A and Singer W, 1993; Teyler TJ et al., 1994). The results of Huerta PT and Lisman JE (1995) showed that during the cholinergic oscillations the timing of the stimulation relative to the oscillation phase predicted the type of synaptic plasticity, which determined whether LTD or LTP was expressed (Huerta PT and Lisman JE, 1995). For instance when excitatory postsynaptic potentials (EPSP) spikes were plotted over time they found LTP was expressed when these cholinergic oscillations were at the peak and LTD when they were at their trough (Huerta PT and Lisman JE, 1995). This suggests a correlation between excitatory stimulation and LTP and this excitation may be directly proportional to calcium influx. During the network oscillation, CA1 neurons of the hippocampus display subthreshold and suprathreshold oscillations, with amplitudes of 5-25 mV (Leung LS and Yim CY, 1986; MacVicar BA and Tse FW, 1989). Such

postsynaptic voltage fluctuations could influence plasticity by increasing stimulation intensity. The oscillating behavior itself is induced by cholinergic action and in its absence synaptic plasticity is greatly reduced (Huerta PT and Lisman JE, 1995). This suggests ACh plays a strong role in synaptic plasticity. In addition, cholinergic innervation is able to depolarize pyramidal neurons by blocking potassium channels that reduces calcium influx inhibition (Huerta PT and Lisman JE, 1995). Therefore, the study by Huerta PT and Lisman JE (1995) supports the idea that ACh interneurons have a strong role in heterosynaptic plasticity in the hippocampus. It is reasonable to suggest that a similar mechanism may be taking place in the striatum since the anatomical arrangement that allows heterosynaptic plasticity in the striatum has the potential for bimodal synaptic modification: LTD and LTP. By designing a network that represents the neurotransmitters involved in striatal heterosynaptic plasticity that would produce LTD and inhibit LTP, a network model for controlling LID may lay the foundation for future experimental analysis.

A bidirectional neural network was modeled by Wakuya H and Zurada JM (2001) in tracking sun spot data. The beauty of this model is that it has the ability to portray two subnetworks performing two types of signal transformations bidirectionally. In this model it takes past information and compares it to new input information. For systems that rely on past information to predict future outcomes, this model is particularly useful (Figure 16). In addition, the subnetworks receive complementary signals through mutual connections (Wakuya H and Zurada JM, 2001).

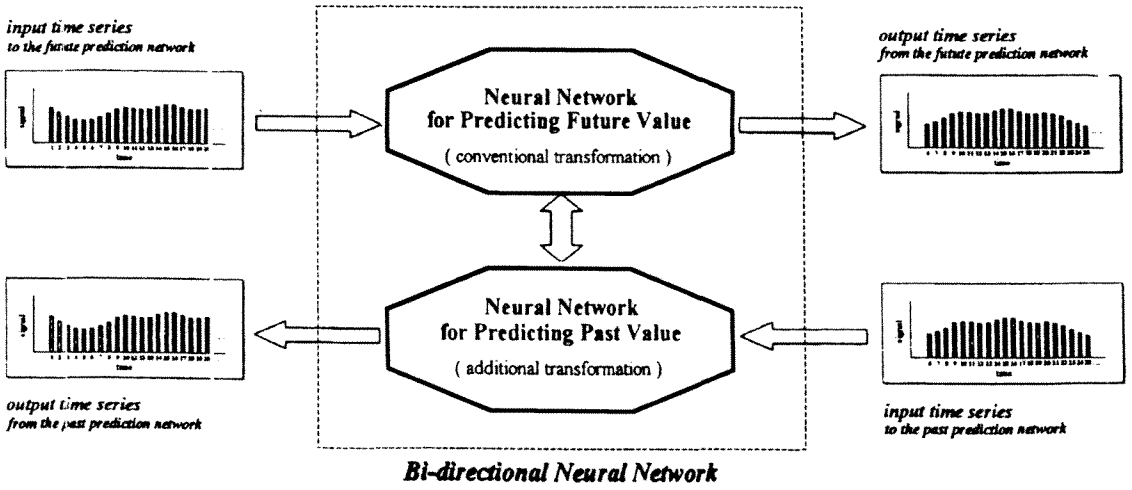

Figure 16 (modified from Wakuya H and Zurada JM, 2001). Bidirectional ANN Model Based Upon Past and Present Values to Predict Future Values. Outline of the signal flow within the bidirectional computing structure. In this figure, the upper half deals with the future prediction task, a transformation from the present signal to the future signal. The lower half deals with the past prediction task, a transformation from the desired future signals to the present signal. These two transformations cooperate with each other through their mutual connections.

Such a model seems appropriate for illustrating the heterosynaptic bimodal synaptic plasticity in the striatum. The bimodality illustrates the ability of the striatal medium spiny neurons to oscillate towards a depolarized or hyperpolarized state and depicts the synaptic plasticity correlated with these states. The depolarized state has been associated with LTP, a mechanism induced by DA administration following DA denervation (Calabresi P. et al., 1999). The optimal neural network design would then seem to be one that used DA agonist, glutamate antagonists and ACh antagonist as input components and trained the network by varying their concentrations and effects on synaptic plasticity based on one particular neurotransmitter (e.g. ACh, DA or glutamate), while assessing their collaborative effects. The goal would be to determine which

combinations produce the output (LTD) and which produce the output (LTP). An illustration of such an ANN of bimodal heterosynaptic plasticity in the striatum similar to the bidirectional model used by Wakuya H. and Zurada JM is depicted in Figure 17.

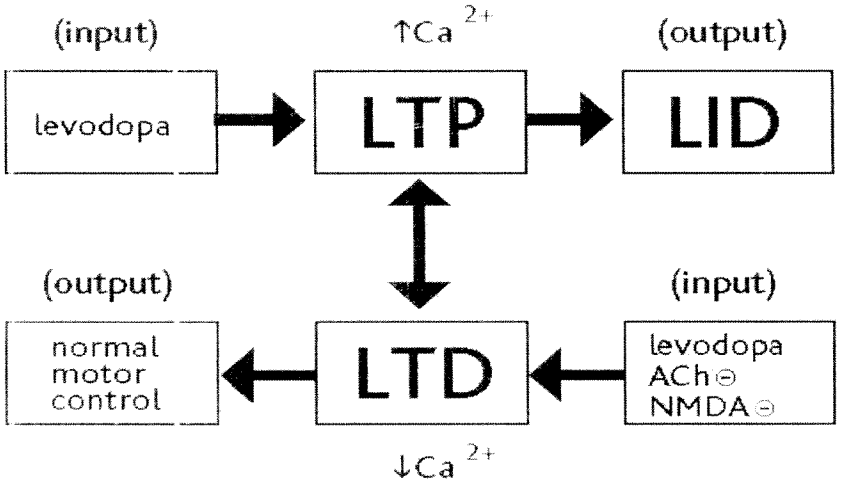

Figure 17: ANN Model of Bimodal Heterosynaptic Mechanism in the Striatum. The upper half of the diagram shows chronic levodopa that leads to LTP and LID, the bottom half illustrates the hypothesis that ACh and/or glutamate antagonists may inhibit LTP/LID and produce LTD. The middle components, LTD and LTP, represent the fact these two states of synaptic plasticity are flexible and exist in an extended continuum based upon the amount of calcium in the system.

If the model predicts that activation of certain combinations of neurotransmitters produces LTD along with the simultaneous inhibition of LTP, it may be advantageous to further investigate the results by a laboratory experiment to verify the neural network model. Confirmation of this model may lay the groundwork for improved pharmacological intervention in the treatment of PD. It has been observed that following DA denervation, changes in striatal levels of ACh and glutamate are observed. Both glutamate and ACh interact and are modulated by DA. Both LTD and LTP depend upon

DA and glutamate in the striatum. However, the role of ACh in producing either of these two long term mechanisms, LTP and LTD, has not been thoroughly addressed. These three neurotransmitters: DA, glutamate and ACh need to be assessed individually first, followed by the effects of their interaction to see how they might direct the heterosynaptic plasticity towards LTD or LTP.

A similar design was modeled by Castellani GC et al. (2001). Researchers looked at bidirectional synaptic plasticity in the mammalian cortex and its dependence on AMPA and NMDA receptors. Their model denoted the particular states of LTD and LTP as a function of calcium concentration (Castellani GC et al., 2001). Their model claims that by knowing the local postsynaptic concentration of intracellular calcium one may deduce the status of NMDA receptor subunit phosphorylation and thus synaptic efficacy. They conclude that changes in the number and/or subunit composition of NMDA glutamate receptors in the postsynaptic membrane determine the calcium concentration and therefore LTD or LTP (Castellani GC et al., 2001). Phosphorylation sites on NMDA and AMPA receptors can therefore serve as a predictor of a change in the direction of synaptic strengthening or weakening (Figure 18) (Castellani GC et al., 2001).

Clearly ANN have a useful place in scientific research. As mentioned ANN can be designed with less formal training than in standard statistical modeling and offer superior assessment of nonlinear relationships. ANN are limited in their oversimplification of biological processes but remain a good starting point for assessing variables and laying the foundation for laboratory experimentation. A few negative facts to note about ANN are their limitations in validating causal relationships (Tu JV, 1996).

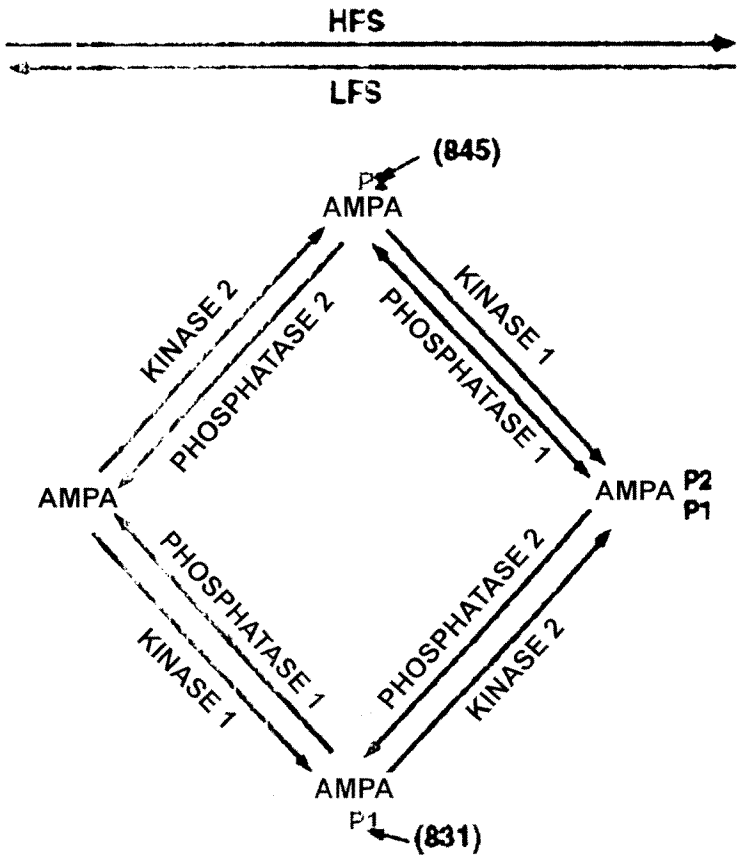

Figure 18: (modified from Castellani GC et al., 2001) Direction of Plasticity: LTD or LTP Depends Upon Phosphorylation Sites. Phosphorylation sites influence the amount of calcium present at the AMPA receptors (from Castellani et al.,2001). A model of Glu R1 phosphorylation/dephosphorylation at two sites. The model assumes two specific kinases (kinase1, kinase2) and two opposing specific phosphatases (phosphatase1 ,phosphatase2). Presumably, high frequency stimulation preferentially stimulates the activity of protein kinases resulting in GluR1 phosphorylation. Low frequency stimulation preferentially stimulates the activity of protein phosphatases resulting in GluR1 dephosphorylation

In other words, it is often difficult to be certain of the deeper relationships that may be hidden in the data or conversely, relationships may appear to exist on the surface but may just be false positives. In this respect, logistical models from standard statistics are far better at and often focus on inferring causal relationships (Tu JV, 1996). In logistic

regression, variables may be determined which are the strongest predictors of outcomes based on odds ratio and beta-coefficients (Tu JV, 1996). In addition, in a stepwise manner, independent variables may be eliminated if they are not correlated to a particular outcome (Tu JV, 1996). In an ANN, the training data are fed into the system and the network trains itself by finding the input variables that produces a particular outcome (Tu JV, 1996). In this arrangement, insignificant predictor variables may not be recognized since no reliable criteria exist for interpreting weights in a connection weight matrix (Tu JV, 1996). The intent of the ANN for the LID model may be at a significant disadvantage because relationships between neurotransmitters and their respective agonists and antagonists may show a correlation with LTP without being mechanistically linked. However, these relationships could be verified through laboratory experimentation.

CHAPTER 6

FACTORS IN ASSESSING STRIATAL HETEROSYNAPTIC LTP PLASTICITY

DA and ACh

Although many studies have been conducted that examines the relationship between DA and glutamate and other studies have probed the relationship between DA and ACh, little research exists that has combined the interaction of all three of these neurochemicals and their effects on LID. In recent years, researchers have verified the significance of both pulsatile administration of DA in the DA denervated system and an increase in glutamate in producing striatal LTP over LTD (Calabresi P. et al., 1997). However, the role of ACh in striatal LTD and LTP have not been clearly defined. It is thought that DA, glutamate and ACh interactions may produce LID as a possible result of inducing LTP in the striatum. By designing a neural network that will combine aspects of research data including: levodopa levels, ACh antagonist levels, glutamate antagonist levels, and their effects on inhibiting LTP, possible insight into controlling striatal LID may result. Such correlations would allow laboratory experimentations to further investigate this hypothesis. In designing this model, it may be advantageous to review what is known about each independent variable first, then decide on the best design to assess the variable DA-ACh-glutamate interactions.

Initial observations of the interactions between DA and ACh in the extrapyramidal system were based upon clinical investigations of PD patients in the early 1960s (Lehman J and Langer SZ, 1983). Muscarinic receptor antagonists that decrease

cholinergic function, relieve PD symptoms in a manner similar to DA agonists (Lehman J and Langer SZ, 1983). Therefore it was proposed by researchers that the endogenous neurotransmitters, DA and ACh, had opposite functions in the extrapyramidal system (Lehman J and Langer SZ, 1983). It was soon postulated that under normal conditions, ACh and DA are in a state of balance (Barbeau A, 1962).

The DA and ACh interaction is also observed in biochemical studies of neurotransmitter turnover. Administering DA receptor agonists produces a decrease in ACh turnover in the striatum (Lehman J and Langer SZ, 1983). In response to DA receptor agonist, apomorphine, there is a decrease in ACh release and turnover in the intact system. The first model to explain the DA and ACh interactions emerged in the 1970s which claimed DA terminals in the striatum form inhibitory connections on the dendrites of the striatal cholinergic interneurons. This allowed DA receptor stimulation to inhibit the activity of cholinergic interneurons by acting as a link between DA terminals and other interneurons or to efferent neurons in the striatum (Lehman J and Langer SZ, 1983). This would allow ACh interneurons to act as a trans-synaptic "second-messenger" of DA receptor stimulation. However, anatomical and pharmacological data conflicted with the idea that ACh dendrites were the exclusive or even most abundant postsynaptic target of DA terminals (Lehman J and Langer SZ, 1983). Thus, although a relationship between DA and ACh exists, the details of this interaction appear to be more elusive.

Huerta PT and Lisman JE (1993) found cholinergic agonists depolarized striatal neurons by blocking potassium channels. The depolarization of striatal neurons is also a factor in eliciting striatal LTP. As noted earlier, striatal neurons are usually at a low level of excitation and express LTD due to the numerous open potassium channels that

counteract the influx of calcium. Therefore blocking ACh receptors may be one factor affecting striatal LTP formation following DA denervation and levodopa treatment systems. Other evidence suggests muscarinic receptor activation has a role in stabilizing striatal transition states (Suzuki T. et al., 1998). While the majority of cholinergic interneurons are located in the matrix of the striatum, their neurites extend into the striosomes and therefore could serve as a link between these two striatal compartments (Blanchet F. et al., 1997). The density of cholinergic interneurons is about two fold higher along the matrix/striosome border compared to regions outside of this border (Blanchet F. et al., 1997). Thus, ACh seems to be ideally positioned to serve in the heterosynaptic plasticity between the striosomes and the matrix.

DA and Glutamate

Studies have shown both DA and glutamate are necessary elements for LTD and LTP expression (Calabresi P. et al., 1997). DA denervation is accompanied by an increase in glutamate that affects the striatal physiological states of neurons. Following chronic levodopa treatment LTP is expressed over LTD. NMDA and AMPA receptors are located on ACh interneurons and medium spiny neurons in the matrix of the striatum (Calabresi P. et al., 1999). Scientists have found a relationship between NMDA receptor activation on medium spiny neurons and LTP expression in these same neurons in DA denervated systems treated with levodopa (Calabresi P. et al., 1999). Following DA denervation, there is an increase in striatal levels of ACh and glutamate (Starr MS, 1995). Such excitatory chemical input could alter physiological striatal states. Interestingly,

Gubellini P. et al. (1997) found that endogenously produced ACh enhanced NMDA responses, which increased protein kinase C (PKC) activation in medium spiny neurons. This suggests a possible role of ACh inducing a long lasting elevation of corticostriatal glutamatergic transmission (Gubellini P. et al., 1997). Studies have shown that the increase in ACh may allow for more binding of glutamate at NMDA receptors (Gubellini P. et al., 1997). They also documented that glutamate binding to NMDA receptors stimulates the release of ACh in striatal compartments indicating that there seems to be some role of positive feedback and/or coupling between ACh and glutamate following DA denervation (Blanchet F. et al., 1997).

Bimodal regulation of synaptic strength has been hypothesized to depend on changes in the number and/or the composition of NMDA and AMPA glutamate receptor subunits. Glutamate receptors' function is regulated by their subunit composition on individual receptors and/or the phosphorylation/dephosphorylation state of individual subunits. Therefore, knowledge of the phosphorylation states of AMPA subunits and NMDA subunits may be a strong predictor of the modality change (increase/decrease) of calcium influx and therefore whether LTD or LTP is induced.

CHAPTER 7

CONCLUDING REMARKS

Among the flaws in the current basal ganglia model are that it does not take into account the anatomical and functional differences between the neurons in the matrix and in the striosomes of the striatum nor the possibility of their influence upon one another. Although most basal ganglia research has independently focused on their control of movement and their role in memory and learning, very few researchers have drawn correlations between these two lines of study. The possibility that the striosomal pathway may influence the direct and indirect striatal pathways of the matrix can be assessed on two levels. First, the striosomal pathway projects to the SNc that gives rise to the nigrostriatal pathway (Starr MS, 1995). Therefore, a cooperative system may exist in the striatum not simply between the direct and indirect pathways, but between the striosomal pathway and the matrix as well. It is possible that the striosomal pathway may serve to regulate or evaluate incoming signals to the nigrostriatal pathway through a heterosynaptic mechanism and once the neurotransmitter systems (i.e. DA and glutamate) are disrupted in PD, the control of input signals into the striatum is reduced. Secondly, lining the border between the striosomes and matrix are ACh interneurons (Pollack AE, 2001; Starr MS, 1995). These ACh interneurons are in the position to relay information from the striosomal pathway to the direct and indirect pathways of the matrix through a heterosynaptic mechanism. The information communicated from the striosomes to the matrix may elicit protein changes in the medium spiny neurons of the cells of the striatal

matrix. These protein changes may contribute to NMDA receptor activation and increased calcium influx. Therefore a bimodality in the heterosynaptic mechanism may explain the differences in responses to continuous versus intermittent levodopa administration eliciting either LTD or LTP in the striatum.

A disruption in the balance between DA and glutamate interaction in the striatum develops following DA denervation and subsequent levodopa treatment. It is not known to what extent ACh functions to modulate the concentrations of DA, glutamate and calcium in the striatum, but clearly ACh plays a role since NMDA and AMPA receptors are found on ACh interneurons in the striatum (Calabresi P. et al., 1995). In addition, following DA denervation, striatal neurons, usually in a hyperpolarized state, transition into an oscillatory state, changing from a hyperpolarized to a depolarized state (Calabresi P. et al., 2000). This oscillatory behavior is not caused by intrinsic membrane properties, but is the result of DA denervation and the release of glutamate from cortical neurons (Calabresi P. et al., 2000). Just as oscillatory neuronal states are not typical for the intact and normal functioning striatum, LTP is not typically the mechanism expressed in the striatum; under normal circumstances, the striatum typically expresses LTD and following DA denervation and levodopa treatment, LTP is produced in the striatum (Calabresi P. et al., 2000).

In many regions of the brain, including the cortex, the magnitude and sign of activity dependent changes in synaptic strength depend on the presynaptic frequency of firing as well as the history of activity at those synapses (Castellani GC et al., 2001). By comparing different dosages of levodopa, ACh and glutamate antagonists and by assessing the relationship of these three neurotransmitters in a bimodal neural network

system, manipulation of LTD over LTP may be achieved. In the model designed for assessing the interactions among ACh, glutamate and DA, each of these neurotransmitters will be used as an input into the system (Figure 19). Various doses of levodopa, ACh and glutamate antagonists for each of these transmitters will be given to act on each variable independently as well as simultaneously. The system will be trained to produce the output LTD and inhibit the output LTP formation by adjustment of the input variables ACh, glutamate and DA.

Necessary elements for building a neural network for therapeutic doing of PD would include a collection of data of the variables described above. The immediate object is to decrease the excitability and thus, the depolarized state of striatal neurons that will further block excitation via NMDA receptor stimulation, thereby, preventing LTP. It is not known which variables: levodopa levels, ACh antagonist levels or glutamate antagonist levels or if a combination of variables will be the most successful in preventing LTP and producing LTD in the neural network model. . The long-term mechanisms (i.e. LTD and LTP) can be directed either way depending on the variables and their nonlinear relationship. An expanded version of a bidirectional/bimodal model is found in Zurada JM (1992) (Figure 20).

A condensed and simplified version of this bi-directional/bimodal model is illustrated (Figure 21). The fundamental structures of Figure 20 and Figure 21 are based upon Zurada's (1992) bidirectional/bimodal associative memory ANN that uses stimulus/response association to produce output. Details are found in the Appendix.

Current and past research has studied the independent effects of anticholinergics and glutamate antagonists on rotational behavior in animal experiments as well as clinical

treatments to decrease LID in PD patients. The reasons why these treatments are effective in decreasing LID have not been understood. If these variables: levodopa levels, ACh antagonist levels and glutamate antagonist levels either acting independently or interacting together control calcium influx in the striatum, this would prevent LTP. No such experiments have been found in recent literature. However, anticholinergics are known to have very severe side effects on memory. Chemical agents that alter phosphorylation of AMPA subunits may be one alternative to anticholinergics and general NMDA antagonist since increased phosphorylation of AMPA receptors has a direct inverse relationship with calcium influx (Castellani GC et al., 2001). Thus, at higher levels of AMPA subunit phosphorylation there is a lowering of intracellular calcium concentration and less chance for LTP to develop (Castellani GC et al., 2001). Although the ANN presented in this thesis does not focus on this issue, potential experiments may want to address this issue.

Many computational models have been designed to study the dynamics of PD. Such idealized models form a solid foundation for laboratory experimentation. Thus, the ultimate goal of designing an ANN model to produce LTD and inhibit LTP would be to test therapeutic dosing regiments in a laboratory environment in hopes of offering novel treatments for PD patients. Since independent variables that will produce the most effective LTP suppression have not been assessed, the computational model will be an efficient tool in unveiling these variables.

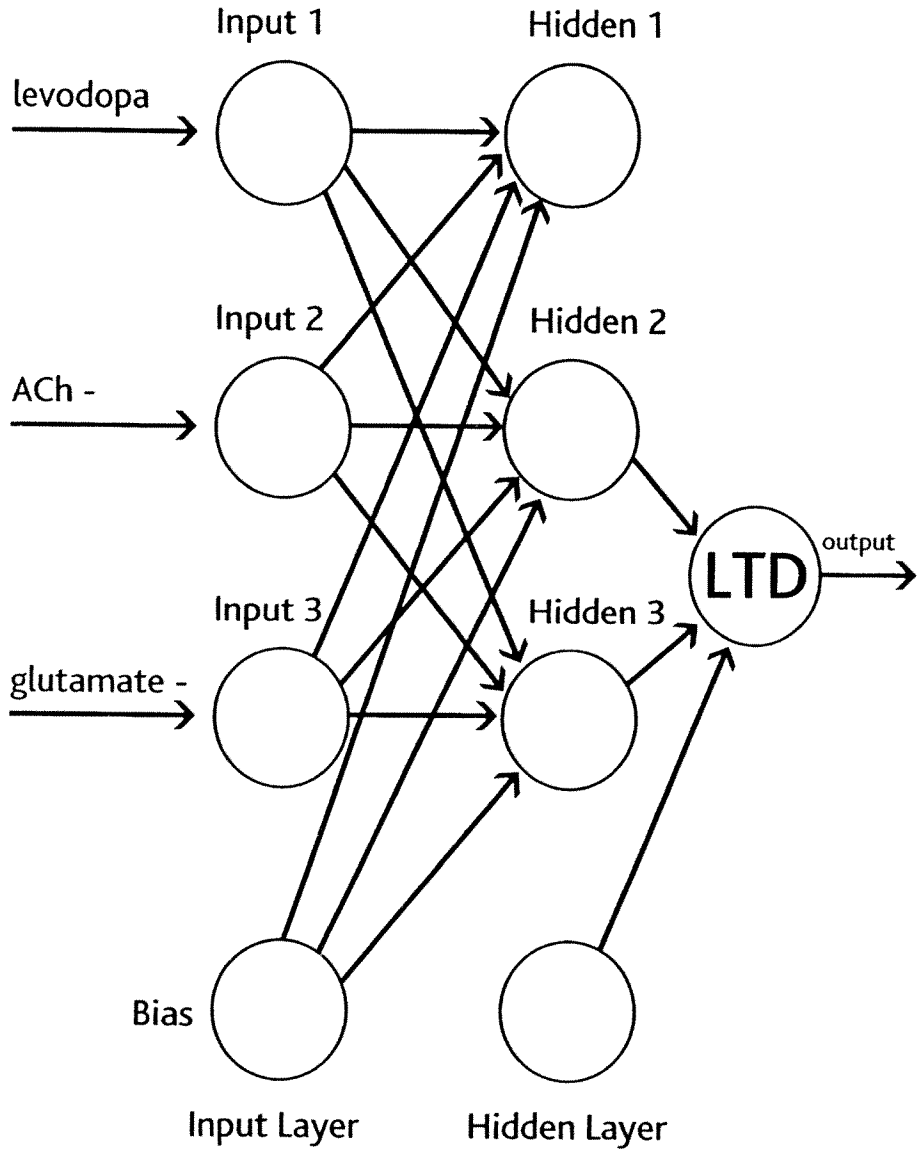

Figure 19: Inputs and Nonlinear Assessment to the LTD Ouput: Interactions amongst the inputs: levodopa, ACh antagonist and glutamate antagonist combine to yield LTD. This is accomplished by minimizing calcium levels and thereby suppressing LTP formation in the striatum. The nonlinear interactions take place in the hidden layer and the combinations that yield LTD will be produced as the output.

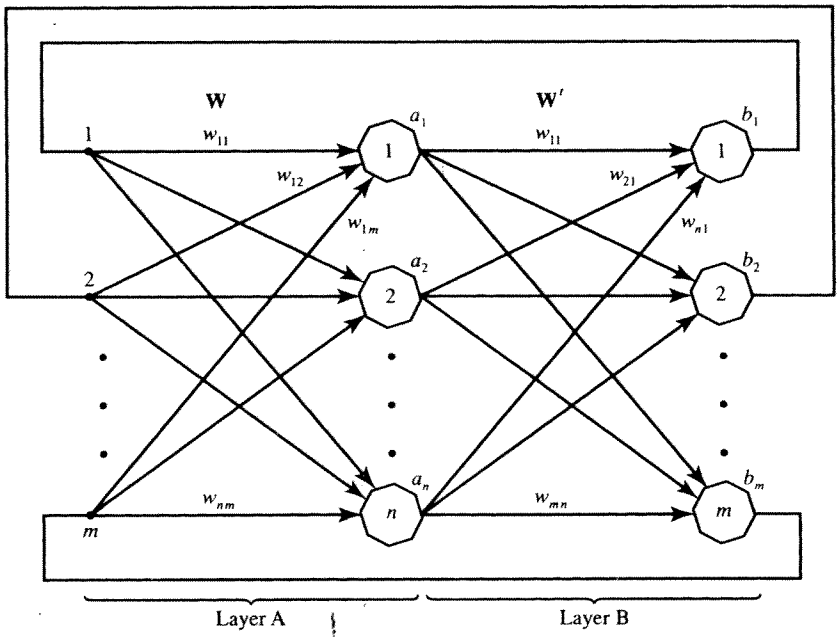

Figure 20: (modified from Zurada, 1992). Expanded Bidirectional ANN Model (from Zurada, 1992). A bidirectional neural network uses the forward and backward information flow to produce an associative search for stored stimulus-response association

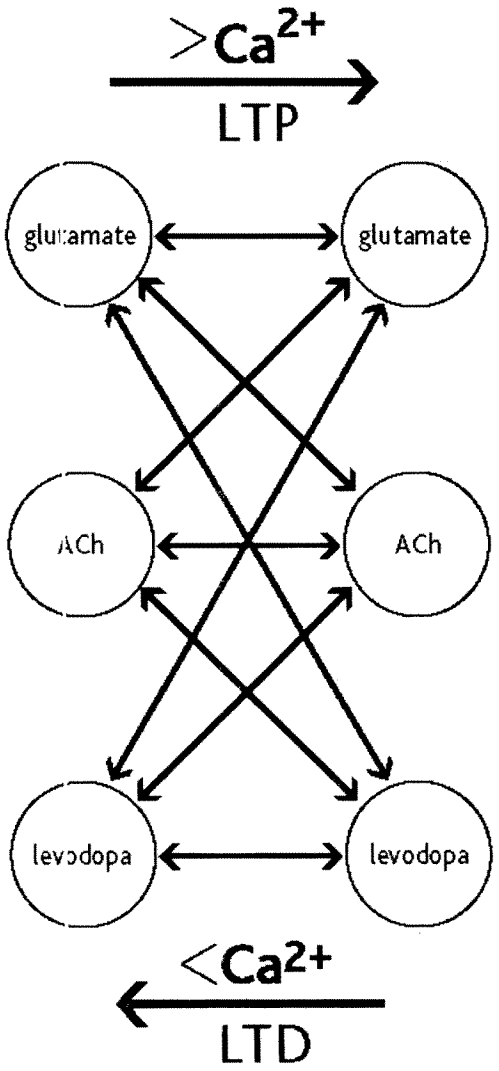

Figure 21: Bimodal ANN Model of LTD-LTP in the Striatum. The appropriate combination of levodopa, ACh antagonist and glutamate antagonist will produce either LTP or LTD in the striatum. An output that generates an elevation in calcium will produce LTP and an output that generates a relative inhibition of calcium will produce LTD.

To date, treatment for PD with levodopa has retained a compromised clinical benefit. The worst and most disabling side effect consists of LID that almost always appears after chronic levodopa treatments. If the hypothesis that LID are a manifestation of LTP in the striatum is correct, then new avenues of treatment for PD will follow. Alternative therapies will be explored that offer less side effects compared to the current standard treatments that produce LID and compromise PD patients' standard of living

APPENDIX

Zurada's (1992) bidirectional/bimodal associative memory ANN that uses stimulus/response association to produce output.

$$\left\{\left(\mathbf{a}^{(1)}, \mathbf{b}^{(1)}\right), \left(\mathbf{a}^{(2)}, \mathbf{b}^{(2)}\right), \ldots, \left(\mathbf{a}^{(p)}, \mathbf{b}^{(p)}\right)\right\}$$

eq. 1

These sets are association pairs that are stored in the memory model. If the memory neurons are activated, the network dynamics correlates two layers of interaction. Refer once again to Figure 16. Assume an initializing vector b at the input to the layer A of neurons. In the assumption, layer A = LTP. This input will be processed through the hidden layer as follows:

$$\mathbf{a}' = \Gamma[\mathbf{Wb}]$$

eq. 2

This equation is a nonlinear operator. It involves matrix multiplication. If layer A = LTP, layer B = LTD

$$a_i' = \text{sgn}\left(\sum_{j=1}^{m} w_{ij} b_j\right), \quad \text{for } i = 1, 2, \ldots, n$$

eq. 3

Vector a′ feeds the layer B, LTD, of neurons. If the transposed matrix is used, W′ of layer B, we find:

$$\mathbf{b}' = \Gamma[\mathbf{W}^t\mathbf{a}']$$

eq. 4

Again, matrix multiplication processes the information:

$$b'_j = \operatorname{sgn}\left(\sum_{i=1}^{n} w_{ij}a'_i\right), \quad \text{for } j = 1, 2, \ldots, m$$

eq. 5

This sequence will be repeated to compute a′, b′, and so on... The process continues as information is received. Once data is no longer being updated, the a & b functions will stop. It is a recursive update mechanism consisting of the following steps:

First Forward Pass:	$\mathbf{a}^1 = \Gamma[\mathbf{W}\mathbf{b}^0]$
First Backward Pass:	$\mathbf{b}^2 = \Gamma[\mathbf{W}^t\mathbf{a}^1]$
Second Forward Pass:	$\mathbf{a}^3 = \Gamma[\mathbf{W}\mathbf{b}^2]$
	$\vdots$
k/2'th Backward Pass:	$\mathbf{b}^k = \Gamma[\mathbf{W}^t\mathbf{a}^{k-1}]$

The back and forth nature of the flow, aims to equilibrate in one of the fixed pairs from eq. 1.

It should be noted, in this particular memory model, that an assumption is made that no state changes are occurring in layers A and B at the same time. Data must flow in a circular fashion. In other words, one cannot simultaneously be at output A and output B. This makes sense in the LTP/LTD model. The direction of flow in the LTP/LTD depends on the level of calcium, the type of glutamate receptor activated and the DA pulsatile activation encountered.

Once data is processed it is coded into the bidirectional/bimodal associative memory usually by the outer product rule, or simply by adding p cross-correlation matrices (Zurada, 1992). The weight matrix formula is:

$$\mathbf{W} = \sum_{i=1}^{p} \mathbf{a}^{(i)}\mathbf{b}^{(i)t} \qquad \text{eq. 6}$$

In eq. 6, a(i) and b(i) are members of the ith pair and are equivalent to the Hebbian learning rule which yields the following weight values:

$$w_{ij} = \sum_{m=1}^{p} a_i^{(m)} b_j^{(m)} \qquad \text{eq. 7}$$

If a stored pattern, a^(m^t) is presented to this memory, the retrieval process follows from eq.5:

$$\mathbf{b} = \Gamma\left[\sum_{m=1}^{p}\left(\mathbf{b}^{(m)}\mathbf{a}^{(m)t}\right)\mathbf{a}^{(m')}\right]$$

eq. 8

This further reduces to:

$$\mathbf{b} = \Gamma\left[n\mathbf{b}^{(m')} + \sum_{m\neq m'}^{p}\mathbf{b}^{(m)}\mathbf{a}^{(m)t}\mathbf{a}^{(m')}\right]$$

eq. 9

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