STRIATAL LTP AND LTD

LONG-TERM POTENTIATION AND LONG-TERM DEPRESSION IN THE CORTICOSTRIATAL MOTOR SYSTEM OF THE NON-ANESTHETIZED RAT

By

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Abstract

Long-term potentiation (LTP) and depression (LTD) are activity-dependent long-lasting changes in synaptic efficacy and have been proposed as mechanisms for learning and memory. Although the exact relationship of LTP and LTD to memory is not known, they do share some properties and mechanisms that relate to memory, such as the strengthening and weakening of synapses. LTP and LTD have been studied extensively in hippocampal brain-slice preparations, due to its relatively organized structure, ease of induction, and its critical function in memory storage. Less work has been done in the neocortex despite the belief that it is heavily involved in the storage of long-term memories. Activity dependent plasticity has also been demonstrated in the basal ganglia in vivo and in vitro, but the results have been somewhat inconsistent. The experiments presented in this thesis explore a novel form of neural plasticity in two excitatory pathways (corticostriatal and thalamocortical) of the basal ganglia motor loop in the intact brain in awake, freely behaving rats.

In thalamocortical slice preparations, simultaneous presynaptic stimulation and postsynaptic depolarization can induce LTP in animals prior to the critical period. However the results presented in this thesis show that applied stimulation to the thalamocortical pathway failed to produce either LTP or LTD in the awake freely moving animal.

Corticostriatal LTD has been shown in slice preparations following direct tetanic stimulation of the striatum. In the current experiment, cortical stimulation failed to induce LTD although there was an observable decrease in the evoked potential following low-frequency stimulation.

Corticostriatal LTP has been shown to depend on the type of stimulation applied. High-frequency and theta burst stimulation produced long-lasting changes in response amplitude in the corticostriatal pathway, with theta burst stimulation appearing to be the more effective stimulation protocol for inducing LTP in both the early and late components. Paired stimulation of the substantia nigra pars compacta and cortex indicated a modulatory action of dopamine on corticostriatal synaptic plasticity. Pairing led to a stable increase in the amplitude of LTP of both early and late components.

We also report that a temporal relationship exists in the striatum with respect to the release of nigral dopamine and cortical glutamate. Simultaneous stimulation produced a more robust LTP compared to the two other conditions in which there was an applied stimulation delay to either the corticostriatal or nigrostriatal pathway.

The results demonstrate the mechanistic differences, not only between the thalamocortical and corticostriatal pathways, but also slice and anesthetized preparations. The results also emphasize the need for further study on mechanisms of LTP and LTD in the various excitatory and inhibitory pathways of the basal ganglia motor loop.

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Lastly, and most importantly, I wish to thank my parents. I am forever indebted for your understanding, endless patience and encouragement when it was most required. You have always supported and encouraged me to do my best in all matters of life. It is to you that I dedicate this work. Thank you.

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Chapter 1

General Introduction

1.1 Introduction

Knowledge, skills, attitudes, and, to some extent, the regulation of emotional states are learned, and through this learning process, our behaviours, beliefs and personalities are shaped. Learning induces changes in our mental constructs that lead to short- or longer-term changes in our behaviour. From a neuroscience perspective, learning is generally believed to be a process leading to a functional reorganization within specific networks of neurons, depending on specific activation patterns within those networks.

Memory is generally measured behaviourally, but other tools are required to explore the physical manifestations of a memory trace. With these tools, we have found that when neurons are repeatedly activated in correlated patterns, the connections between them become stronger and more permanent. It follows then, that the more synchronous and frequent the stimulation, the more likely will be the induction of a long-term change in the strength of neural connections. These changes will likely be more robust when multiple systems within the brain (e.g., visual, auditory, motor) interact during the induction phase.

During the 1820s French physiologist, Pierre Flourens, conducted a number of localized lesion experiments in response to Gall's phrenological approach, which claimed that the brain had 27 areas each corresponding to a specific mental faculty (Simpson, 2005). Flourens' research on vestibular and ontological disorders revealed that a number of brain regions were responsible for balance, posture, and coordination including the cerebellum, brainstem and the cerebral cortex (Clarac, 2008). More recently, several decades of animal research have supported Flourens' assertion that individual sites in the brain are not sufficient for specific behaviours, but rather several regions of the brain participate to perform various functions (Clarac, 2008).

In this thesis, the focus will be on memory mechanisms in one of the brain's motor systems. Before discussing these mechanisms, we will look at the systems themselves and some of what is known about how motor behaviour is controlled.

1.2 Motor Control

The human nervous system is a remarkably effective network structure especially when considering that multiple neural circuits are organized into ensembles that allow specific kinds of information to be processed. This form of organization ultimately provides the basis for sensation, perception and behaviour. The interaction of these systems enables the organism to navigate through ever changing environments. Sensory systems acquire and process

information from the environment, and in turn appropriate movements and other adaptive behaviours (or responses) are generated.

Motor skill and coordinated motor movement involve the proper functioning of various systems within the central nervous system (Fig. 1.1), along with joints and skeletal muscles. A plethora of research has shown that the cerebral cortex, the cerebellum and the basal ganglia are vital structures necessary for normal motor function (Butler et al., 2007; Herrero et al., 2002; Middleton & Strick, 2000; Shimazu et al., 2004; Tsuchiya et al., 2005; Van Elswijk et al., 2008). Motor task directives initiated by the cortex pass along closed motor loops to the cerebellum and basal ganglia where they are evaluated in order to ensure accurate and appropriate motion.

The basic function of the basal ganglia appears to be the maintenance of muscle tone and the initiation and control of, movement, particularly ballistic movements, whereas the cerebellum compares information from the periphery to influence coordination and the organization and timing of precise movements (Baizer et al., 1999; Middleton & Strick, 2000; Turner & Anderson, 1997; Watanabe, 2008). After the motor sequences have been assessed, information from both of these subcortical structures are fed back to the cortex to adjust any incorrect movement (Alexander, 1994; Alexander & Crutcher, 1990; Baizer et al., 1999; Middleton & Strick, 2000; Turner & Anderson, 1997; Watanabe, 2008).

Recent evidence has further demonstrated that the basal ganglia and cerebellum contribute to the motor control and motor learning via a series of

parallel basal ganglia-thalamacortical loops and pontocerebellar connections (Alexander, 1994; Alexander & Crutcher, 1990; Baizer et al., 1999; Jueptner & Weiller, 1998; Middleton & Strick, 2000; Turner & Anderson, 1997; Watanabe, 2008). Basal ganglia and cerebellar projections to brainstem networks are largely involved in postural control, muscle tone and locomotion (Jahn et al., 2007; Mori, 1987).

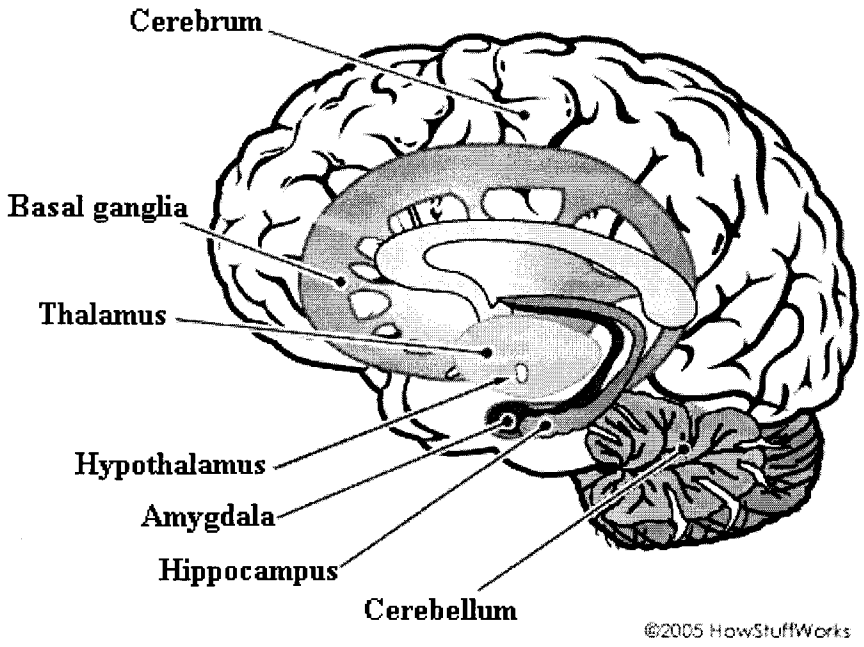


Figure 1.1: Illustrates Brain's Motor Structures.
Adapted from: http://static.howstuffworks.com/gif/swearing-1.gif

Although planning, and directing of voluntary movement is initiated at the cortical level, the cerebellum and basal ganglia are critical for the execution and

control of complex motor patterns. These two structures are often considered to be the primary associational areas involved in the control of voluntary movement, because they regulate the activity of the cortex and do not directly evoke (elicit) motor activity (Alexander & Crutcher, 1990; Jueptner & Weiller, 1998; Kao et al., 2005; Takakusaki et al., 2004; Turner & Anderson, 1997; Van Elswijk et al., 2008).

1.3 Motor Cortex

Fine motor control requires the organism to effectively utilize skeletal muscles in appropriately patterned and timed movements. Two sets of motor neurons are responsible for voluntary and reflexive muscle contraction, upper motor neurons and lower motor neurons. Betz cells, large pyramidal upper motor neurons, that originate in the primary motor cortex, as well as the lateral and medial premotor cortex, project to the spinal cord and brainstem motor circuits, which are essential for voluntary as well as complex forms of movement (Butler et al., 2007; Shimazu et al., 2004; Tsuchiya et al., 2005). Inhibitory multipolar neurons, Martinotti cells, are located throughout the cortex and are responsible for maintaining the balance between excitation and inhibition during cortical activation (Silberberg & Markram, 2007).

Using slice preparations and recordings, Silberberg and Markram (2007) suggest that Martinotti cells are associated with a cortical dampening mechanism. When the pyramidal cells become over-excited, Martinotti cells send inhibitory

feedback to surrounding pyramidal cells as well as to distal pyramidal cell dendrites in layer 1, thereby preventing the prolonged regeneration of calcium spikes, which cause high-frequency bursting of pyramidal cells (Silberberg & Markram, 2007). The upper motor neuron are also responsible for basic navigational movements such as orientating the eyes, head, and body to external stimuli conveyed by vestibular, somatic, auditory, and visual sensory information.

Lower motor neurons are considered to be the final common pathway for transmitting neural information given the fact that they send their axons from the brain stem and the spinal cord to innervate the skeletal muscles of the body and head respectively (Cuccurazzu et al., 2007; Di Lazzaro et al., 2004).

There is a general agreement among researchers that the motor cortex plays a major role in the planning, control, and execution of voluntary motor functions. Typically the motor cortex is divided into three functional regions each having a specific role with respect to motor function. The areas include the primary motor cortex, the pre-motor area, and the supplementary motor area (Fig 1.2) (Haaland et al., 2004; Park et al., 2008; MacNeilage et al., 2001).

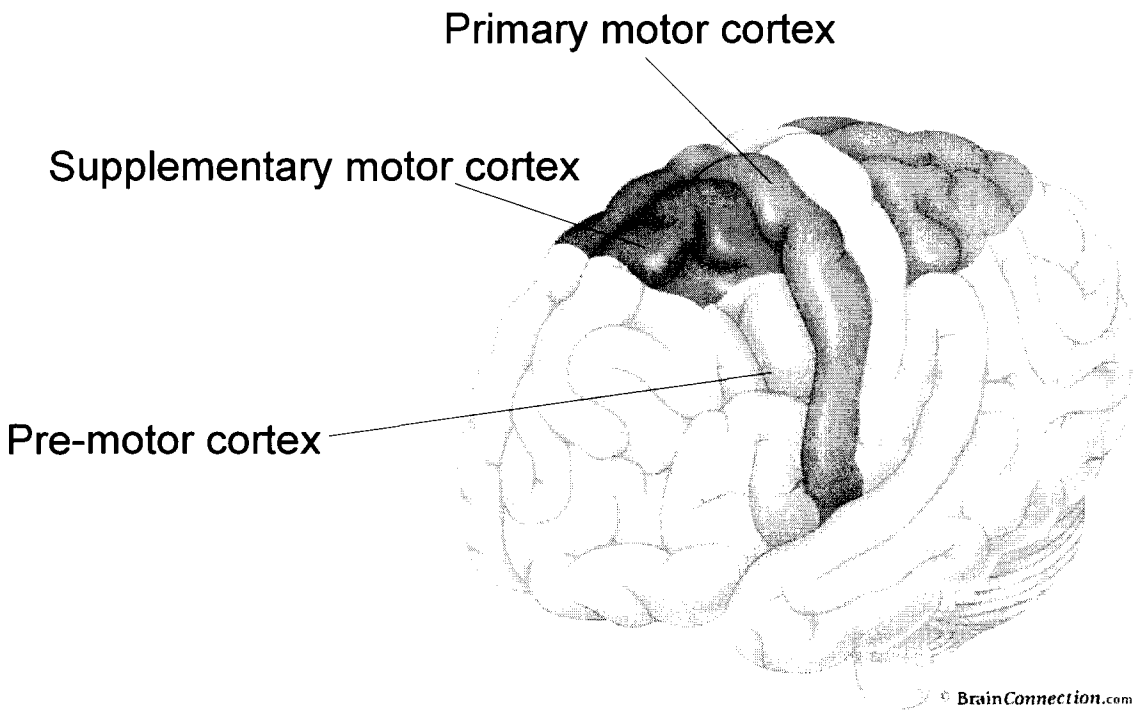


Figure 1.2: Illustrates Cortical Motor Areas.
Adapted from: http://www.brainconnection.com/med/medart/l/motor-cortex.jpg

The primary motor cortex is one of the principal brain areas involved in motor function. Neural impulses that control the execution of movement are generated in the primary motor cortex (Bernard et al., 2002; Matsuzaka et al., 2006; Nowak et al., 2008; Park et al., 2008; Porro et al., 2000). Studies suggest that pre-motor area is responsible for sensory guidance of movement and the activation of proximal muscles as well as trunk muscles that orient the body (Berkowitz &Ansari, 2008; Bernard et al., 2002; Wiese et al., 2004). The supplementary motor area appears to be involved in the planning of complex movements and in coordinating two-handed movements (Bernard et al., 2002;

Kazennikov et al., 1999; MacNeilage et al., 2001; Wiese et al., 2004). Voluntary movement requires the interaction of the various motor areas; the supplementary motor area and the premotor regions both send information to the primary motor cortex as well as to brainstem motor regions.

An interesting study by Park et al., (2008) used fMRI to show the activation of each motor region and hemisphere during simple and complex exercises. For the complex exercise, healthy volunteers were asked to rotate a pair of wooden balls for a specified period of time while brain activation patterns were established using blood oxygen level-dependent (BOLD) fMRI measurement. BOLD measurements were also taken while performing a simple hand grasp.

Park et al., (2008) found several areas of activation for both complex and simple exercises including primary motor cortex, pre-motor area, supplementary motor area, primary sensory cortex, and the cerebellum. A surprising find of this study was significantly stronger ipsilateral activation of cortical regions and bilateral activation of the cerebellum during complex exercises, compared to simple movements (Park et al., 2008). Simple movements only showed contralateral activation of cortical regions (Park et al., 2008). These results suggest that complex movements may require the activation of the ipsilateral motor cortex, perhaps to compensate for the functional demands placed on the contralateral hemisphere, thus allowing the participant to execute the demanding motor task (Park et al., 2008; Pollmann et al., 2003).

Bilateral activation of the motor cortex may occur during normal motor learning of novel or complex tasks, but ipsilateral activity declines rapidly as the task becomes more automatic and less demanding.

The adaptive response of the ipsilateral hemisphere has also been reflected in stroke studies (Seitz et al., 1998). Seitz et al., (1998) observed the recovery of several stroke patients from an infarction of the middle cerebral artery. When the patients were first observed during admission the research team noted that the patients were hemiparetic with complete loss or severe impairment of hand function (Seitz et al., 1998). After a six-month recovery period motor ability improved significantly, on average. Patients were able to perform various activities including individual finger movements, which allowed researchers to measure regional cerebral blood flow while performing finger movement sequences. The results of the PET-scan revealed activation of both cerebral hemispheres after recovery, which likely reflects compensatory mechanisms that served to overcome the residual motor deficit (Seitz et al., 1998).

1.4 Motor learning

Most motor skills are learned and refined through experience and practice during early childhood and rely on the same neural systems that are involved in motor control (Ericsson, 2008; Thelen, 1995; Thelen et al., 1993). Childhood is an important and necessary developmental period in which competence in motor skills are acquired. During this period children develop and learn a variety of

fundamental movement patterns and more specialized motor skills (Thelen, 1995; Thelen et al., 1993). Development of motor competence during infancy and childhood is dependent upon and influenced by the biological growth and maturity of the neuromuscular and central nervous systems (Thelen, 1995; Thelen et al., 1993), which, along with experience, ultimately determine the motor repertoire of the child.

Improvements in the accuracy and smoothness of both simple and complex movements require the development of strength, posture, balance, and perceptual skills (Joyal et al., 1996; Sprague & Chambers, 1953; Thach et al., 1992; Thelen, 1995; Thelen et al., 1993). Gross motor movements such as sitting up, balance, and walking generally precede fine motor acquisition necessary for manipulating small objects, hand-eye coordination task, and writing (Cheung et al., 2001; Laszlo & Sainsbury, 1993; Thelen, 1995; Thelen et al., 1993). Properly calibrated motor activity is essential for voluntary goal directed movement and begins in infancy (Thelen, 1995; Thelen et al., 1993).

Primitive reflexes such as the grasping reflex begin to resemble voluntary movements as cerebral control develops during infancy (Futagi & Yasuhiro, 1999). As the central nervous system gradually matures during infancy and childhood, inhibitory functions of the cerebral cortex begin to operate (Futagi & Yasuhiro, 1999; Thelen, 1995; Thelen et al., 1993). Reflex movements are gradually (cortically) inhibited and integrated into developing movement patterns

resembling controlled and coordinated voluntary movements (Futagi & Yasuhiro, 1999; Oishi et al., 2003).

Studies involving simple behaviours such as eyeblink conditioning, vestibulo-ocular reflex, and birdsong have provided a detailed understanding of the cellular mechanisms of simple forms of learning. It should be noted even simple movement patterns that heavily involve motor reflexes are more complex than they may appear. A walk in the park involves complex coordinated sequence of movements over the whole body to continually adjust posture and balance. These adjustments can change dramatically when simply moving from a sidewalk to walking on a sandy beach. Remaining upright on skis or a surfboard adds several additional levels of complexity. Adjustments in motor patterns change in response to environmental changes and are also altered as the organism ages, requiring a maintained plasticity in the brain's motor systems.

Lesion and conditioning studies have shown that the cerebellum and basal ganglia are critical for continual motor learning over an organism's lifespan. Damage to either structure is correlated with impaired performance on a number of motor tasks, both simple and complex.

1.5 The Cerebellum

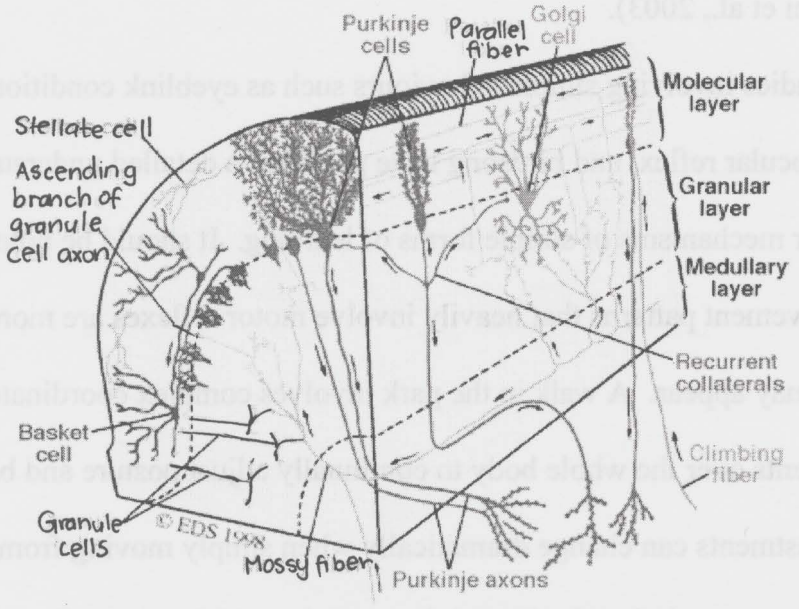


Figure 1.3: Illustrates Cerebellar Circuitry.
http://www.tnb.ua.ac.be/models/index.shtml

In general terms the function of the cerebellum is to compare intent and actual performance with regard to muscle activity and movement by comparing information received from the eyes, muscles, joints, tendons, and the brain (Watanabe, 2008). The cerebellum contains various cell types and circuitry that enables the structure to perform its function, as illustrated in Figure 1.3. The cerebellar cortex consists of a single layer of aligned purkinje cells. The Purkinje cells are the most characteristic type of neurons in the cerebellum. The dendrites of each Purkinje cell have a very distinctive pattern in that their branches all lie in one plane in which they assume the shape of a fan (Ito, 1989). The fan-shaped dendrites of adjacent cells lie parallel to each other (Ito, 1989). The axons of the Purkinje cells synapse on the neurons of the dentate nuclei of the cerebellum. These nuclei relay the information to the thalamus, which then projects to the

cortex and the striatum (Ito, 1989). The dendritic branches of each Purkinje cell receive excitatory synapses from the branch terminations of a single afferent climbing fibre. This fibre is the axon of a neuron in the inferior olive, a nucleus in the medulla oblongata. The inferior olive integrates the information from the muscle proprioceptors. Each climbing fibre winds closely around the dendrites of its corresponding Purkinje cell, so that the activation of this fibre will cause a massive excitation of this cell (Ito, 1989).

In contrast, the second major source of inputs to the cerebellum, the mossy fibres, act in a highly diffuse fashion. These fibres are the axons of neurons in the pontine nuclei that receive information from the cerebral cortex (Ito, 1989). The mossy fibres carry this information to synapses with the small granular cells in the deep layer of the cerebellum (Ito, 1989). There are so many of these granular cells that they are thought to account for half of all the neurons in the brain (Ito, 1989).

The axons of these granular cells ascend into the surface layer of the cerebellum (the cerebellar cortex) where they branch to form the parallel fibres. The parallel fibres run perpendicular to the Purkinje cell dendrites, thus crossing many Purkinje cells and connecting with each in turn. Though each parallel fibre makes only one contact with each Purkinje cell that it crosses, it makes contact with a huge number of such cells along its path, which measures just a few millimeters (Ito, 1989). Likewise, each Purkinje cell receives over 100 000 synapses from 100 000 different parallel fibres (Ito, 1989; Ito & Kano, 1982; Konnerth et al., 1992).

At first, this configuration was believed to be the basis for the internal cerebellar clock (Ivry & Spencer, 2004). Because the incoming message from the parallel fibres takes an increasing amount of time to traverse the succeeding dendritic levels, a brief time lag develops. It was thought that the cerebellum might use this lag to co-ordinate the sequence of movements. Now the most commonly accepted interpretation of this dual afferent system is that it provides the ideal basic structure for an elementary learning mechanism called long-term depression (Hansel et al., 2001; Ito, 2006).

This depression occurs when the dendrite branches of a Purkinje cell are activated by the climbing fibre and the parallel fibres simultaneously (Ekerot & Jörntell, 2001; Ito, 1989). The result is a long-term reduction in the efficiency of the synapse between these parallel fibres and the dendrites of the Purkinje cell (Ekerot & Jörntell, 2001; Ito, 1989; Ito & Kano, 1982). Long-term depression will be more thoroughly addressed in a later section.

In addition to these excitatory inputs, Purkinje cells receive input from two types of intracortical inhibitory interneurons, basket cells and stellate cells (Ramnani, 2006).

1.5.1 Cerebellar learning

In recent years, it has become clear that motor learning, as revealed by associative eyelid conditioning and adaptation of the vestibulo-ocular reflex, can be added to the well-established cerebellar functions of sensorimotor integration

and control. Damage to the cerebellum leads to deficits in fine motor movement, posture, equilibrium, and motor learning (Joyal et al., 1996; Sprague & Chambers, 1953; Thach et al., 1992). Using information about discrepancies between motor intent and outcome, the cerebellum appears to act as a servomechanism to reduce motor error (Ito, 1989). The cerebellum detects differences between the intended movement and the performed movement in order to mediate both real-time and long-term reductions in motor error (Ito, 1989; Ito, 2006).

An example of rapid short-term motor learning can be seen in prism adaptation experiments. Normally, with an intact cerebellum, monkeys have the ability to adapt rapidly to situations where vision is distorted by occluding one eye and placing a laterally displacing prism in front of the other eye. Baizer and Glickstein (1999) used Macaque monkeys to demonstrate cerebellar-dependent prism adaptation. The monkeys were initially trained to press one of two switches located directly beneath lights that were placed on either side of their head, in order to receive a food reward. Following the training period a laterally displacing prism was introduced. This caused a lateral displacement of the visual field. Objects that were previously located on the right now appear on the left and vice versa. As a result of the visual manipulation the monkeys incorrectly reached in the direction opposite the light, and in doing so, pressed the incorrect switch. The incorrect response was not rewarded.

After only a few trials the monkeys learned to adapt and began to reach in the direction of the correct switch. Postadaptation trials (with prism removed) revealed a postadaptation shift where the monkeys incorrectly reached in the direction opposite the light. However, after a few postadaptation trials, the monkeys once again adapted to their situation and correctly reached for the appropriate switch. The ability to adapt to laterally displacing prisms was blocked when the cerebellum was lesioned (Baizer et al., 1999). The work by Baizer et al., (1999) on this associative motor learning task demonstrated a role for the cerebellum in short-term and intermediate-term motor memory.

Classical conditioning of the eyeblink reflex using simple animal models have been used to study cellular mechanisms involved in motor learning (Freeman & Rabinak, 2004). Eyeblink conditioning is perhaps the most thoroughly studied example of a discrete, learned behavioural response (Freeman and Nicholson, 2000; Koekkoek et al., 2003; Lavond, 2002). This form of associative learning involves the pairing of an unconditioned stimulus (UCS), a puff of air to the cornea, with a neutral conditioned stimulus (CS) that does not elicit a response on its own, like a tone or light. Following repeated presentation of paired UCS-CS trials, the presentation of the CS alone eventually elicits an eyeblink reflex. Various structures including the cochlear nuclei, pontine nuclei, cerebellar nuclei and the granule cells of the cerebellar cortex have been identified in the central neural pathway for the auditory conditioned stimulus (Freeman & Rabinak, 2004). Using a pontine stimulation paradigm in rats, Freeman and

Rabinak (2004) demonstrate that cerebellar afferents are critical pathways for associative learning involving conditioned tones. Infusion of Muscimol, a $GABA_A$ agonist, into the deep cerebellar nuclei resulted in the complete loss of conditioned responses (CR).

If delays are imposed between the CS and UCR, the animal learns to delay the initiation of the CR. It has been suggested that the cerebellum is responsible for this temporal shaping of eyeblink conditioning. Koekkoek et al., (2003) present evidence, which shows that synaptic depression in cerebellar pathways (long-term depression or LTD) contributes to the learning-dependent timing of conditioned responses.

Koekkoek et al., (2003) investigated the timing of conditioned responses in L7-PKCi mutant (inhibition of protein kinase C) and wild-type mice using a Pavlovian conditioned eyeblink paradigm. Parallel fiber LTD was blocked in the L7-PKCi mutants. In order to observe timing properties of conditioned responses they varied the inter-stimulus interval between the onset of the CS (tone) and the onset of the US (shock). Wild-type mice robustly increased their average latency to peak amplitude as the inter-stimulus interval was increased. However, latencies to peak were not influenced by the length of the inter-stimulus interval in the L7-PKCi mutants. A follow-up experiment revealed that an intact cerebellum is necessary for well-timed components of eye-blink responses. In the previous experiment the researchers found that after four training sessions the

average peak amplitude in wild-types was 0.68mm/s, whereas the mutants had values of only 0.44mm/s.

In order to demonstrate the contribution of cerebellar LTD to learning-dependent timing, bilateral cerebellar lesions were performed on all groups. The lesions produced trace conditioned responses in both wild-types and mutants. The data from the first experiment show that well-timed eye-blink responses can be learned in intact wild-type mice, but not L7-PKCi mutants. Koekkoek et al., (2003) believe that this form of learning-dependent timing is mediated by PKC-dependent parallel fiber LTD and, as their second experiment demonstrated, is dependent on an intact cerebellum.

Earlier studies have shown that classical conditioning of the eyeblink response is critically dependent upon mature associational systems (Freeman and Nicholson, 2000; Stanton et al., 1998). While recording the evoked neural activity of the cerebellar interpositus nucleus of infant long-evans rats, Freeman and Nicholson (2000) noted an aged-related increase in the rate and magnitude of eye-blink conditioning with an age-related increase in the neuronal activity. Stanton and his group (1998) observed striking differences in the expression of eye-blink conditioning in rats on postnatal day 17 and 24. Postnatal day 24 rats reached peak response amplitudes after 3 training session, whereas, postnatal day 17 rats did not show any conditioned response, even after 600 training sessions (Stanton et al., 1998).

The findings of this study reflect the maturation of this system, which occurs gradually between postnatal day 17 and 24 (Stanton et al., 1998). These results suggest that long-term conditioned learning requires a mature associational system and further suggests an associative process involving the cerebellum facilitates eye-blink conditioning.

Research has also demonstrated the cerebellum's role in producing semi-automatic movement (skills), which can be performed with little or no conscious effort. Human speech, for instance, can be performed without conscious awareness of how the words are selected by the brain during retrieval, freeing the brain to attend to other activities. Nevertheless, speech is a sophisticated voluntary motor activity, which requires phonation (voicing) and articulation (Davis & MacNeilage, 1995; MacNeilage et al., 2001; Rektorova et al., 2007).

Using the Baddeley-Hitch model, which proposes that working memory comprises a limited capacity attentional controller (Baddeley & Della Sala, 1996), and two components of a phonological loop (Baddeley & Gathercole, 1998), Chiricozzi et al., (2008) state that the phonological short-term store is necessary for speech. The limited attentional controller can be thought of as a central executive that allocates attentional resources, whereby allowing the individual to focus on the attended task, (Baddeley & Della Sala, 1996). The first component is a phonological memory store, which can hold traces of acoustic or speech based material. Material in this short-term store lasts about two seconds unless it is maintained through the use of the second subcomponent, articulatory

subvocal rehearsal (Baddeley & Gathercole, 1998). Prevention of articulatory rehearsal results in very rapid forgetting (Baddeley & Gathercole, 1998).

Imaging studies have shown that the cerebellum, along with the inferior frontal lobe, the supplementary motor area, the premotor cortex, and the parietal lobe, is consistently activated during verbal working memory tasks that involve encoding and retrieval. These results suggest that the cerebellum is part of a circuit that sustains articulatory rehearsal mechanisms necessary for phonological short-term storage (Ivry and Justus, 2001).

Lesion studies have also been used to demonstrate the cerebellum's contribution to speech (Chiricozzi et al., 2008; Justus et al., 2005). Ten patients, with various cerebellar damage, were tested for verbal working memory capacity using phonologically similar and dissimilar word lists (Justus et al., 2005). Patients were presented with a 5 or 6 item word lists of similar vowel category (e.g. bead, peace, leaf, tease, deal), and dissimilar vowel category (e.g. bead, pace, ledge, tab, dip), which are selected from 60 monosyllabic English words.

In one condition, the experimenter read the words at a rate of 1 word per 1.5 seconds. In the other, they were presented visually on a computer screen at the same rate. The patient was asked to orally recall the list 5 seconds after it had been presented. The patient was tested with both conditions. The data revealed low average scores for cerebellar patients compared to age-matched controls when asked to repeat phonologically similar words (Justus et al., 2005). Similar sounding words were more difficult to remember when compared to words that

sound different. Presentation of similar sounding words places greater demand on articulatory rehearsal mechanisms, which largely depend on an intact cerebellum for optimal rehearsal processes. The results provide support for the cerebellum's contribution to phonological short-term store and ultimately speech. The cerebellum's connection to various regions of the brain enables this integration of both mental activity (sensory perception) and motor control (Chiricozzi et al., 2008; Ivry and Justus, 2001; Justus et al., 2005).

1.6 Basal Ganglia Anatomy: Structures and Pathways

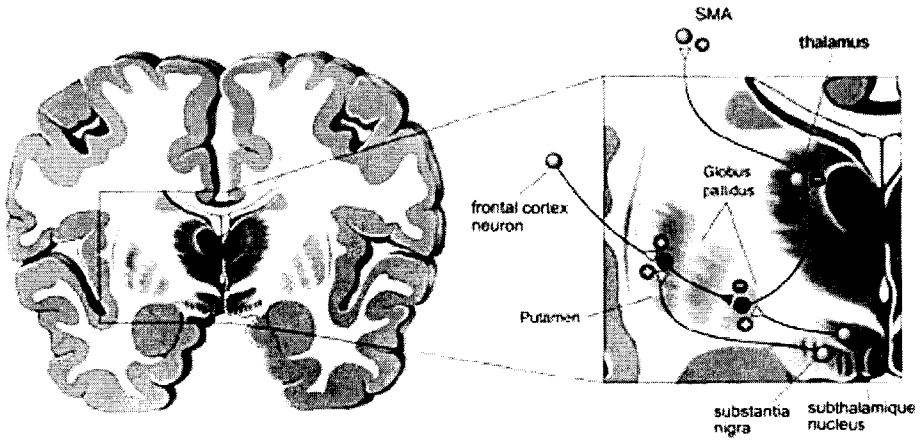


Figure 1.4: Illustrates Basal Ganglia Structures.
http://thebrain.mcgill.ca/flash/i/i_06/i_06_cr/i_06_cr_mou/i_06_cr_mou.html

Voluntary motor behaviour is determined by the interactions of multiple learning and memory systems. For example, understanding limbic system function in motivation and motor behaviour requires an evaluation of the basal ganglia motor loop as a modifiable learning system. Behavioural neuroscientists face the challenge of teasing apart the contributions of these multiple overlapping

neuronal systems in order to fully understand the contributions of the limbic system and other brain systems to voluntary motor behaviour. One approach is to focus on the contributions of component pathways. Of particular interest in the context of motor control are the individual pathways of the basal ganglia motor loop (Fig 1.5).

Alexander and Delong (1986) describe a series of five loops of information, initiating from cortex to basal ganglia and returning to the cortex. Each loop runs through the basal ganglia in parallel direct or indirect pathways. Via these loops, basal ganglia control the overall inhibition of the thalamus, which modulates the final stage of information relayed to the cortex. In addition, the relative activity of the basal ganglia neurons is modulated by dopaminergic signals from the substantia nigra pars compacta (Centonze et al., 1999). These heuristic schemes help account for, and facilitate the investigation of, a variety of movement disorders (Alexander & Delong, 1986; Penney & Young, 1983).

Growing bodies of clinical and experimental evidence support the notion that the basal ganglia motor loops link cortical and subcortical regions involved in the processing of sensorimotor information (Alexander et al., 1986; Alexander & Crutcher, 1990; Smith et al., 1998; Takada et al., 2001). The two main sensorimotor channels connecting the motor areas of the cortex with the basal ganglia are the corticostriatal pathway and the thalamocortical pathway (Fig 1.5).

One of the features of the basal ganglia-cortical loop is that information is segregated according to the anatomic areas of their components (Alexander and

Delong, 1986). Large portions of the cortex project to the striatum through the corticostriatal pathway but the axons remain segregated along the pathway and innervate specific input areas within the striatum (Fig 1.4) (Alexander et al., 1986; Deniau et al., 1996; Flaherty & Graybiel, 1991; Kunzle, 1978). The striatum is comprised of medium spiny cells and interneurons. The striatum is further segregated into dorsal and ventral segments, the caudate nucleus and the putamen respectively.

The primary cortical connection of the *association loop* is the dorsal striatal compartment (Deniau et al., 1996; Selemon & Goldman-Rakic, 1985). Most of the information in the dorsal compartment flows though central striatal regions, such as the head of the caudate. The primary cortical connection of the *limbic loop* is the ventral striatal compartment (Deniau et al., 1996; Flaherty & Graybiel, 1991; Kunzle, 1978). Most of the information in the ventral compartment flows through ventromedial striatal regions. Like other corticostriatal and thalamocortical loops, information travels through parallel indirect and direct pathways, with the output structures being the globus pallidus pars internal (GPi) and the substantia nigra pars reticularis (SNr).

The striatum serves as the input center of the basal ganglia. As mentioned, specific areas of the cortex have been shown to map to specific parts of the striatum (Deniau et al., 1996; Flaherty & Graybiel, 1991; Kunzle, 1978; Selemon & Goldman-Rakic, 1985). Inputs from the somatosensory, motor and premotor cortices project to the putamen (Deniau et al., 1996; Flaherty &

Graybiel, 1991; Kunzle, 1978) and inputs from associative cortices project to the caudate nucleus (Deniau et al., 1996; Selemon & Goldman-Rakic, 1985). The corticostriatal input zone originating from the primary motor cortex is located predominately in the lateral part of the putamen, whereas the input zone originating from the supplementary motor area projects more to the medial and slightly to the dorsal portion of the putamen (Takada et al., 1998).

The putamen is involved in integrating complex motor tasks requiring a combination of inputs from the supplementary motor area, which is mainly involved in memory guided movements, and from the premotor cortex, which plays a role in visually guided movements (Halsband et al., 1994).

Corticostriatal neurons receive complex cortical inputs, showing a context-dependent and or reward-contingent activity (Kimura, 1992; and Aosaki et al., 1994). When the desired motor action is performed a contingent reward signal (dopamine) is delivered via the nigrostriatal pathway, consequently facilitating voluntary behaviour (Henderson et al., 2005). The corticostriatal pathway is characterized by a glutamatergic excitatory connection. Before we get to the processing circuitry with the basal ganglia, we'll look at the major return pathway from the basal ganglia to the cortex, which actually arises in the thalamus.

1.6.1 Thalamocortial Pathway

The ascending thalamocortical pathway is also characterized by a glutamatergic excitatory connection (Fig 1.5). Thalamic relay nuclei form a crucial link between the basal ganglia and cortex by transmitting basal ganglia output to specific frontal cortical areas (Schell & Strick, 1984; Goldman-Rakic & Porrino, 1985; Ilinsky et al., 1985; Matelli & Luppino, 1996). Parallel models of basal ganglia circuitry indicate that these thalamocortical relays maintain functionally distinct corticobasal ganglia loop systems through projections back to the cortical area of origin (Alexander et al., 1986; Parent & Hazrati, 1995).

In most models of basal ganglia function, the thalamocortical projection is treated as a simple relay station back to cortex. However, recent studies show that in addition to relaying subcortical information to cortex, thalamocortical circuits participate in the modulation and regulation of cortical-cortical activity (Sherman & Guillery, 1996, 2002; Castro-Alamancos & Connors, 1997). This regulation can be accomplished in part by projections to different cortical layers, which are in turn, associated with specific cortical and subcortical connections (Jones, 1985). Thus thalamocortical projections to different layers can differentially affect subpopulations of cortical neurons and consequently their output.

Direct and Indirect Pathway

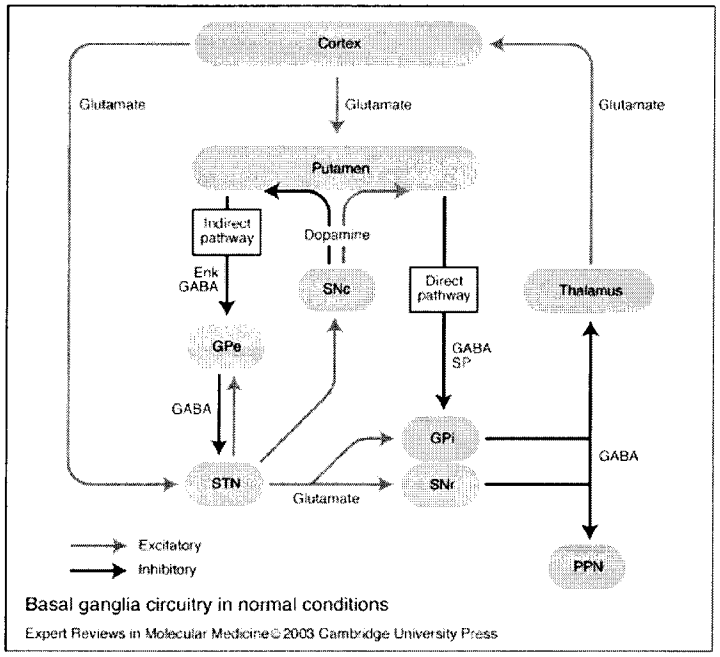


Figure 1.5: Illustrates Basal Ganglia Pathways.
http://www-ermm.cbcu.cam.ac.uk/fig002rbc.gif

Between the input and output pathways, there are two major routes through the basal ganglia, arising from different cellular populations in the putamen. The direct pathway projects monosynaptically from the putamen to the motor regions of the internal globus pallidus and the substantia nigra pars reticulata (Wichmann & DeLong, 1993). The indirect pathway is inhibitory from the putamen to the external globus pallidus, inhibitory from there to the subthalamic nucleus, and excitatory to the external globus pallidus and substantia nigra pars reticulata.

Dopamine released from the terminals of substantia nigra neurons excites the direct pathway and inhibits the indirect pathway. The net affect of the direct pathway is to facilitate voluntary motor behaviour by exciting the supplementary motor area while the indirect pathway operates by inhibiting activity in the supplementary motor area (Kandel et al., 1991). In effect, the dopamine terminals modulate the efficacy of corticostriatal inputs (Steg & Jonels, 1993; Centonze et al., 2001; Picconi et al., 2003).

The substantia nigra is the source of the dopaminergic neurons that terminate in the caudate nucleus (Henderson et al., 2005). The substantia nigra, along with the internal globus pallidus, is one of the two major output structures of the basal ganglia (Sabatino et al., 1986; Goldberg et al., 2003). The neurons from the substantia nigra fire tonically rather than in response to specific sensory input (Venton et al., 2004). Hence the substantia nigra system appears to serve as a tonic sustaining influence on motor activity rather than participating in specific movements.

The globus pallidus is segregated into internal and external segments. The internal section is one of two main output nuclei of the basal ganglia, along with the substantia nigra pars reticulata (Wichmann & DeLong, 1993). The pallidal output is passed by the thalamus primarily to the supplementary motor area, and possibly also to the premotor cortex (Brotchie et al., 1991). The supplementary motor area also provides a major input to the basal ganglia, therefore there is a loop between the supplementary motor area and the basal ganglia. The external

section of the globus pallidus passes information within the basal ganglia, in particular to the subthalamic nucleus (Brotchie et al., 1991).

The subthalamic nucleus receives projections from the motor and premotor cortices, and from the external segment of the globus pallidus. The subthalamic nucleus projects to both segments of the globus pallidus and to the substantia nigra pars reticulata.

The dopamine that is released by the inputs from the substantia nigra has excitatory effects on D1 receptors and inhibitory effects on D2 receptors (Centonze et al., 2001; Picconi et al., 2003). Hence, both excitatory and inhibitory signals are received by the caudate, which innervates different areas of the putamen. The resulting signals can be traced through both the direct and indirect pathways. In both pathways it can be seen that the net result is either cortical excitation or inhibition by the ventral lateral nucleus of the thalamus, which conveys signals to the cortex and the rest of the body via the brain stem and spinal cord (Houk & Wise, 1995).

The excitatory dopaminergic signal targets the direct pathway, where medium spiny neurons cells make inhibitory gamma-amino butyric acid (GABA) connections onto neurons in the internal globus pallidus, which in turn make inhibitory GABA connections with cells of the ventral lateral thalamic nucleus (Picconi et al., 2003. The final thalamocortical connection with the cortex is excitatory. Hence, the excitatory dopaminergic signal causes more inhibition of

the globus pallidus, causing less inhibition of the ventral lateral nucleus, allowing more excitation of the cortex (Centonze et al., 2001; Picconi et al., 2003).

The alternative signaling route is the indirect pathway, which is modulated by the inhibitory dopaminergic signal. The putamen cells (medium spiny neurons) make inhibitory GABA connections onto neurons in the external globus pallidus, which make inhibitory connections with cells in the subthalamic nucleus. These make excitatory glutamatergic connections with the internal globus pallidus. The further connections to the ventral lateral nucleus and supplementary motor area are the same as in the direct pathway.

In the indirect pathway the inhibitory dopaminergic signal causes less inhibition of the external globus pallidus, allowing more inhibition of the subthalamic nucleus, causing less excitation of the internal globus pallidus, causing less inhibition of the ventral lateral nucleus thalamic nucleus, allowing more excitation of the cortex (Centonze et al., 2001; Picconi et al., 2003).

The basal ganglia do not generate movement directly, but rather take input from the cortex, modify it in some way, and pass it back to the cortex via the thalamus. The control of movement by the central nervous system is further complicated in that multiple brain regions are involved. While specific regions have been designated as "motor cortex," almost all of the neocortex becomes engaged during the control of voluntary movement.

1.6.2 The basal ganglia and motor learning

Similar to the cerebellum, the basal ganglia modulates cortical activity. The basal ganglia, which is comprised of the caudate nucleus, putamen, and the globus palladus, helps to regulate the voluntary initiation and termination of movements through reinforcement learning.

Subconscious contractions of skeletal muscles are also regulated by the basal ganglia. Studies have shown that neurons in the putamen are heavily involved in anticipatory movements of the body (Cromwell and Shultz, 2008), while activity of neurons in caudate nucleus occur prior to eye movement (Watanabe and Hikosaka, 2005). Muscle tone required for voluntary movement is regulated by the globus pallidus (Haaxma et al., 1995; Kuoppamäki et al., 2005).

With respect to motor learning, reinforcement can be thought of as the animal's responsiveness or motivational drive state associated with engaging in self-generated naturally rewarding behaviours (van de Laar et al., 2004). Researchers have attempted to link the brain's reinforcement system (one which facilitates information storage) to a particular set of chemical mechanisms involving dopamine, which has been shown to be involved in the mechanisms of pleasure and reward (Acquas et al., 1989; Aosaki et al., 2004; Berridge, 2007; Berridge & Robinson, 1998; Schultz, 1998; van de Laar et al., 2004; Voruganti &Awad, 2007).

The mechanics of how dopamine might signal pleasure in the brain is still controversial, however there appears to be a complex temporal relationship between pleasure and dopamine (Aosaki et al., 2004; Floresco et al., 2003; Goto & Grace, 2005; Goto et al., 2007; Lapish et al., 2007). The data on the timing of dopamine release are somewhat contradictory, but the prevalent theory is that the dopamine reward signal acts as a kind of learning reinforcer. The release of dopamine motivates and compels us to engage in behaviours and activities that are pleasurable (Acquas et al., 1989; Aosaki et al., 2004; Berridge, 2007; Berridge & Robinson, 1998; Schultz, 1998; van de Laar et al., 2004; Voruganti &Awad, 2007). This suggests that reward in general, and the dopamine system in particular, may play a central role in learning.

Kao, Doupe, and Brainard (2005) used microstimulation of the basal ganglia-forebrain circuit (the anterior forebrain pathway) to demonstrate song learning and adult vocal plasticity in songbirds. Vocal plasticity refers to the experience-dependent physical development of vocal and respiratory musculature necessary for producing highly stereotyped renditions of a memorized song (Kao et al., 2005). Stimulation of the anterior forebrain pathway did not elicit vocalizations, whereas stimulation of the motor pathway produced immediate vocalizations (Vicario and Simpson, 1995). Kao, Doupe and Brainard (2005) found that applying various stimulation patterns to the basal ganglia during natural vocalization induced acute changes in the structure of individual song elements.

Undirected singing of the male zebra finch occurs when the bird sings alone, in the absence of a female. Directed song production occurs when he is singing to a female during courtship. Variability in the song structure of undirected singing is associated with neural activity in the output nucleus of the striatum, the lateral magnocellular nucleus of the anterior nidopallium, and is necessary for normal song development. During song learning, juvenile zebra finch memorize the sound of adult birds and begin to use various vocalizations and feedback to gradually refine song production until it closely resembles that of the adult bird.

Electrolytic lesions of the lateral magnocellular nucleus of the anterior nidopallium prevented song variability during undirected song production (Kao and Brainard, 2006). Lesions of the lateral magnocellular nucleus of the anterior nidopallium results in stereotypy with incorrect repetitive and simple song production. The results of the two studies indicate that anterior forebrain pathway activity is associated with variability in song output and further suggests that the basal ganglia may provide a general mechanism for enabling experience-dependent plasticity in sensorimotor circuits (Kao and Brainard, 2006).

1.7 Motor system pathology

The precise orchestration of actions across participating systems is necessary for correct muscle control involving dedicated motor systems in both the central and peripheral nervous systems. Disturbances of motor control can

result from developmental abnormalities (mutations), injury, and/or environmental factors such as drug and alcohol abuse, disease and toxins.

The two more commonly known motor dysfunctions associated with cerebellum and basal ganglia damage are cerebellar ataxia (Bastian, 2002) and Parkinson's disease, respectively.

1.7.1 Cerebellar Ataxia

Cerebellar ataxia, a gross incoordination of muscle movements associated with the cerebellum, can cause a variety of neurological deficits. The type of deficit that a person experiences depends on the area of cerebellar damage, although there may be some overlap between areas damaged and observed symptoms (Blaney & Hewlett, 2007; Christova et al., 2008; Diener et al., 1984; Ishikawa et al., 2001; Sears et al., 2000; Tokuda et al., 1991). Vestibulo-cerebellar dysfunction, cerebro-cerebellar dysfunction, and spino-cerebellar dysfunction are forms of ataxia that result from cerebellar dysfunction, each presenting with a specific, manifested symptom (Blaney & Hewlett, 2007; Diener et al., 1984; Ishikawa et al., 2001; Sears et al., 2000; Strupp, Zwergal. 2007; Tokuda et al., 1991).

Damage to the cerebellar midline likely results in vestibulo- cerebellar ataxia (Brandt & Strupp, 2005; Strupp et al., 2007). Vestibulo-cerebellar ataxia, as its name suggests is a dysfunction associated with vestibular system and disrupts balance (Diener et al., 1984). Individuals afflicted with this disorder feel

unsteady on their feet (vertigo) even when their eyes open and tend to stand with their feet apart in an attempt to gain a wider base (Diener et al., 1984; Strupp et al., 2007). Some people are likely to experience nystagmus and ocular dysmetria (Brandt & Strupp, 2005; Christova et al., 2008; Marti et al., 2008). Commonly, nystagmus is characterized by saccadic eye movement in the horizontal plane represented by an uncontrolled up and down oscillation, as well as alternating smooth pursuit in the other direction (Brandt & Strupp, 2005; Wagner et al., 2007). Ocular dysmetria is an error in ocular fixation consisting of over or undershooting of the focus of the eye followed by oscillations of focus until fixation is achieved (Hubner et al., 2007).

Cerebro-cerebellar dysfunction, which is caused by damage to the cerebellar hemisphere, is often associated with disturbances in the planning and execution of normal voluntary motor functions (Blaney & Hewlett, 2007; Gonzalez-Islas et al., 2005). Intention tremor is likely to be observed with this type of ataxia as well (Blaney & Hewlett, 2007; Gonzalez et al., 2005).

Cognitive impairments as well as incoordination (uncoordinated gait), is often found among patients with spinocerebellar ataxias, which likely results from damage to the vermis and paravermis (Diener et al., 1984; Gotoda et al., 1995; Ishikawa et al., 2001; Tokuda et al., 1991). The two hemispheres of the cerebellum are connected by the vermis, a wormlike structure involved in proprioception. Damage to this structure causes disturbances in balance and posture. Damage to the paravermis, the intermediate zone, produces limb rigidity.

1.7.2 Parkinson's Disease

The importance of the basal ganglia in the control of movement is apparent in people with Parkinson's disease. Parkinson's disease is a chronic and progressive degenerative disorder of the central nervous system characterized by the deterioration of dopaminergic neurons that project from the substantia nigra of the midbrain to the striatum (Blandini et al., 2000; Braak & Tredici, 2008; Henderson et al., 2005; Noble, 2000; Sung et al., 2008). The lose of dopamine within the basal ganglia system ultimately leads to hypo-activity of the motor cortex (Centonze et al., 1999; Centonze et al., 2001; Henderson et al., 2005; Ridley et al., 2006).

Symptoms of Parkinson's disease are manifested by muscular rigidity, slowness of movement, a shuffling wide-based gait, and tremor that is particularly pronounced during periods of inactivity (Blandini et al., 2000, Braak & Tredici, 2008).

A neuron usually receives synapses from thousands of other neurons, and many of those inputs may be synchronously active. The way in which postsynaptic cells integrate these numerous inputs and alters its own electrical activity is the fundamental basis of neuronal integration, information processing, and storage in the brain. The laboratory phenomenon that best captures the integration and storage process at the synaptic level, in a form that is amenable to experimental analysis, is long-term potentiation (LTP). LTP is the focus of the next section.

1.8 Properties of long-term potentiation (LTP)

Use-dependent long-lasting changes in synaptic efficacy have been proposed as a mechanism for learning and memory. In the laboratory environment one of the most popular models of neural plasticity and memory is LTP. The exact relationship of LTP to memory is not known. Real learning involves complex and diffuse patterns of activation (Milgram et al., 1987) unlike LTP, in which enhancement is restricted to an experimentally-activated pathway. However, long-term potentiation does share some properties and mechanisms that relate to memory, such as the strengthening of synapses.

In 1949 Donald Hebb proposed a theory to guide the investigation of how the brain stores memories by changing synaptic strengths. Like Lashley (1950), Hebb (1950) believed that memories are stored as distributed and redundant neural traces, and he also suggested biophysical mechanisms of synaptic modification. Hebb (1950) postulated that when there was correlated activity between pre- and post-synaptic neuron, the strength of the synaptic connection between the two neurons would increase. Three fundamental features of Hebbian synaptic modification have been described by Brown, Kairiss, and Keenan (1990).

First, there must be a precise temporal occurrence of pre- and post-synaptic activity. Second, there must be a spatial component where modifications

are restricted to synapses where correlated activity occurs. Third, both the pre- and post-synaptic neuron must reach a certain threshold of activity before modification can occur. As a model, LTP satisfied these criteria by providing a highly localized change in synaptic strength in response to correlated conditioning.

LTP, discovered by Timothy Bliss and Terje Lomo (1973), is an enduring increase in synaptic efficacy that lasts for hours in acute preparations and days to months in chronic preparations. This enduring synaptic change results from high-frequency stimulation of an afferent pathway (Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973). Over time these synapses become increasingly sensitive so that a constant level of presynaptic stimulation becomes converted into a larger postsynaptic output.

Bliss and Lomo (1973) discovered that a brief high-frequency train of stimuli to any of the three major intrahippocampal synaptic pathways, the perforant pathway, the mossy fibre pathway, and the Schaffer collateral pathway, increased the amplitude of the excitatory postsynaptic potentials evoked in the target hippocampal neurons of these pathways. For example, to monitor the strength of synaptic connections, they recorded the amplitudes of the excitatory post-synaptic potentials (EPSPs) evoked in the dentate gyrus after delivering electrical pulses to the perforant path in the rabbit. They were able to record both individual cell and population responses by using both *in vivo* and *in vitro*

preparations. They recorded the EPSP amplitude both prior to, and following the high-frequency stimulation protocol and found that the evoked potentials had increased in size following stimulation (Bliss & Lomo, 1973). They further noted that the responses of the unstimulated control pathway in the same animal remained unchanged, thus demonstrating that the response enhancement was specific to the activated pathway.

Responses recorded at excitatory synapses are due primarily to activation of another ionotropic glutamate receptor, the alpha-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptor. However most forms of LTP are critically dependent on N-methyl D-aspartate (NMDA) receptor activation (Malenka & Nichol, 1999).The criteria for activation of the NMDA receptors might be expected to relate to their involvement in synaptic plasticity.

LTP-inducing stimulation triggers a surge of glutamate release. The binding of glutamate to the NMDA receptor permits the corresponding ion channel to open. If there is enough background depolarization (contributed in large part by the activation of AMPA receptors), then a Mg^{2+} block of NMDA receptor channel is released, leaving the channel open for traffic. Opening of the NMDA receptor ion channel allows Ca^{2+} ions to flow into the cell and bind to a variety of calcium binding proteins, including calmodulin. NMDA receptors are considered to be "coincidence detectors" because they require that glutamate

release be coupled with depolarizations of the pre- and post-synaptic neuron (Coan & Collingridge, 1985).

Calmodulin mediates many calcium-driven metabolic reactions, and is found in all eukaryotes and its sequence is remarkably conserved (Persechini and Stemmer, 2002). Calmodulin activates calcium-calmodulin-dependent kinase II (CaMKII), which phosphorylates AMPA receptors making them more permeable to the inflow of Na^+ ions and thus increasing the sensitivity of the cell to depolarization (Malenka & Nicholl, 1999). Increased gene expression and protein synthesis also occurs during the development of LTP.

Evidence has shown that LTP in rat brains coincides with the formation of additional synapses between the presynaptic axon terminal and its dendritic target (Toni et al., 1999). Input specificity has typically been studied in the hippocampal slice because of the experimental control this preparation affords. Anderson et al., (1980) placed stimulating electrodes into two independent pathways both of which converge on CA1 pyramidal neurons. Pulses applied to both pathways produced a response in the target neuron. Tetanus was delivered to one of the two pathways, but not both. Following stimulation, only the stimulated pathway showed potentiation (Andersen et al., 1980), showing that this synapse, like the perforant path synapse, shows LTP specificity. The input specificity of LTP has proven to be advantageous to researchers. By activating a specific

pathway researchers have been able to confirm that the response enhancement is, in fact, due to a synaptic mechanism.

It is also known that LTP-inducing stimulation needs to reach a certain threshold in order to produce potentiation. In a 1983 study, Lee used hippocampal slice preparations to demonstrate cooperative interactions between afferents for the induction and maintenance of LTP in CA1. Cooperative or associative interactions are necessary because it is thought that sufficient numbers of fibers need to be active before a system will reach a certain threshold and potentiate (Lee, 1983). The term associativity is used to describe an interaction between multiple inputs to a particular post-synaptic target.

The demonstration of a similar plasticity phenomenon, LTD, has shown that synaptic strength can also be reduced by the appropriate pattern of activation. LTD is the topic of the next section.

1.9 Properties of long-term depression (LTD)

While a significant portion of synaptic plasticity research has focused on the strengthening of synapses, recent research has also been directed to the weakening of synapses. Synapses can be experimentally weakened through the induction of long-term depression (LTD). LTD is a lasting activity-dependent decrease in synaptic efficacy, which ultimately leads to a reduction in receptor sensitivity. Similar to LTP, LTD may be NMDA receptor dependent or independent (Martin et al., 2000). Zhang et al., (2006) monitored LTD in slice

preparations using dual-photon laser-scanning microscopy to visualize presynaptic vesicular release from the rapidly releasing vesicle pools in CA1 of the hippocampus. They were interested in determining whether long-term presynaptic changes underlie a component of either activity dependent NMDA-LTD or mGluR1-LTD (Zhang et al., 2000). LTD had been associated with long-term changes in transmitter release (Stanton et al., 2001). Brief applications of NMDA or mGluR1 antagonists were applied in order to induce each form of LTD, respectively. Zhang et al., (2000) found that the induction of NMDA-LTD but not mGluR1-LTD led to a reduction in presynaptic vesicle release (Zhang et al., 2006). This study demonstrates both NMDA-dependent and NMDA-independent LTD, as well as presynaptic properties associated with the NMDA-dependent LTD.

Unlike LTP, LTD produces a smaller amplitude change (although its impact on information flow may not be small). It can also be more difficult to induce, both in slice and in chronic preparations with the procedures used thus far (Errington et al., 1995; Abraham et al., 1996; Manahan-Vaughan, 1997; Staubli and Lynch, 1990; Doyle et al., 1997; Thiels et al., 1994; Heynen et al., 1996). Researchers have experienced difficulty in establishing a consistent stimulation protocol that could be used to induce LTD both in slice or chronic preparations.

Errington et al., (1995) failed to induce LTD in the hippocampus of both the awake and anesthetized adult rat using low frequency trains (1-5Hz). They administered a low frequency protocol similar to that used by Stanton and

Sejnowski (1989), hoping to find similar effects in vivo. Barrionuevo, Schottler, and Lynch (1980) also failed to induce LTD in the Schaffer commissural pathway in the anesthetized rat using low-frequency stimulation. Staubli and Lynch (1990) also encountered difficulty in their attempts to induce LTD in the dentate gyrus of the anesthetized rat.

Fortunately, Froc et al., (2000) were able to successfully induce LTD in the sensorimotor cortex of the freely moving rat. They used prolonged multiple, spaced, low-frequency stimulation (1 Hz for 15 min) to induce LTD. They also found that LTD was more reliably induced by stimulation of the ipsilateral white matter compared to stimulating the contralateral motor cortex.

Researchers argue that the weakening of synapses by means of activity-dependant reductions in synaptic efficacy, are necessary for learning and memory (Froc et al., 2000; Martin et al., 2000). If circuits were shaped only by increases in synaptic efficacy, saturation of synaptic plasticity in a network could destroy the pattern of trace strengths corresponding to established memories and occlude new memory encoding (Martin et al., 2000). In other words, if the synaptic connection weights become saturated, no further learning can occur.

1.10 Interactions between LTP and LTD and relevance to motor learning

It appears that networks utilize shifting relative thresholds for long-term potentiation (LTP) and long-term depression (LTD) to help maintain a reasonably

normalized state with a balance between weak and strong connection strengths. For example, Rioult-Pedotti, Friedman, and Donoghue (2000) demonstrated this point in their skill-learning experiments. After training rats in a unilateral reaching task, which involves reaching through a small hole to retrieve small food pellets, they found that the untrained hemisphere expressed much more LTP, in response to stimulation, compared to the trained hemisphere, whereas the trained hemisphere expressed more LTD compared to the untrained hemisphere (Rioult-Pedotti et al., 2000).

The idea that learning may require the interaction of both LTP and LTD, the strengthening and the weakening of synaptic connection weights, led to the introduction of the term bi-directional modification. Recent work has shown that the relative thresholds for LTP and LTD induction are themselves modifiable based on previous neural activation. The notion of sliding thresholds for LTP and LTD is sometimes referred to as metaplasticity (Abraham, 1996; Abraham and Bear, 1996). Essentially metaplasticity describes one means by which a kind of renormalization process can shape changes in synaptic connection weights. If LTP is first induced, then the subsequent presentation of LTD should be able to depress the response more easily. This effect may underlie the depotentiation effects reported in the Froc et al. (2000) experiment. Froc et al., (2000) were able to depotentiate the LTP response using massed and spaced patterns of low-frequency stimulation.

Bi-directional synaptic plasticity was also observed in the rat basolateral amygdala (Li et al., 1998). Li et al., (1998) examined forms of activity-dependent synaptic plasticity in the basolateral amygdala *in vitro* and found that brief high frequency stimulus trains could induce a switch in the direction of the enduring change in synaptic strength induced by earlier low-frequency stimulation.

1.10.1 Corticostriatal Plasticity

Several investigators have explored LTP-induction at corticostriatal synapses, because this form of synaptic plasticity might represent the cellular correlates of sensorimotor learning. Repetitive activation of the corticostriatal pathway has been shown to induce both LTD and LTP (Calabresi et al., 1992a; Calabresi et al., 1992b; Spencer & Murphy, 2000). Calabresi et al., (1992b) used corticostriatal rat slice preparations to record the amplitude of both extracellular and intracellular evoked potentials following tetanic stimulation. They found that in a Mg^{2+} free medium, tetanic stimulation induced LTP whereas LTD was induced when the slices were placed in a medium with external Mg^{2+}.

Spencer and Murphy (2000) investigated bidirectional plasticity induced at corticostriatal synapses in vitro. They also found that corticostriatal synapses expressed both LTD and LTP. However, their study revealed that LTD induction required direct stimulation within the striatum, unlike LTP, which can be induced from tetanic stimulation of the white matter (Spencer and Murphy, 2000). They

suggest that corticostriatal depression likely involves the recruitment of local striatal circuits and dopaminergic inputs.

LTP has also been demonstrated in vivo following low-frequency stimulation. Charpier, Mahon, and Deniau (1999) used barbiturate-anesthetized rats to test the ability of corticostriatal connections to express use-dependent modifications after cortical stimulation. Intracellular recordings of medium spiny neurons revealed that 5 Hz Low-frequency stimulation increased the amplitude of the excitatory postsynaptic potential (EPSP). Charpier et al., (1999) believe that the potassium current at hyperpolarized levels exerts a strong inhibitory shunting effect on weak and asynchronous excitatory synaptic potentials. However, when low-frequency stimulation is applied, the medium spiny neurons are subjected to a synchronized synaptic barrage arising from a large number of slow-wave excitatory afferents (Kasanetz et al., 2002). The synergistic effects of increasing synaptic depolarization and subsequent potassium deactivation lead to the removal of the inhibitory shunt, resulting in an amplified synaptic potential (Charpier et al., 1999).

D1 dopamine receptors are also involved in LTP induction in the striatum (Centonze et al., 2001; Centonze et al., 1999). Centonze et al., (1999) lesioned the nigro-striatal dopaminergic pathway in the rat using 6-hydroxydopamine (6-OHDA) to demonstrate that D1 dopamine receptors are crucial for the striatal plasticity. They found that unilateral dopamine denervation blocks corticostriatal LTP (Centonze et al., 1999).

The direction of the type of synaptic plasticity expressed (LTP or LTD) is largely dependent on the level of membrane depolarization and on the ionotropic glutamate receptor subtype involved. Medium spiny neurons have been shown to undergo spontaneous membrane depolarizations in coincidence with phasic release of glutamate from corticostriatal terminals, defined as the "up state" (Kasanetz et al., 2008; Mahon et al., 2003; Plenz & Kitai, 1998). Striatal neurons in the "up state" display subthreshold depolarizations from –71 to –40 mV (Wilson & Kawaguchi, 1996). Striatal neurons also experience a "down state" (-61 to –94 mV) where they are silent, with a negative resting potential due to intrinsic non-linear membrane conductances (Kasanetz et al., 2008; Mahon et al., 2003; Plenz & Kitai, 1998; Spencer and Murphy, 2000).

NMDA does not appear necessary for the induction of LTD (Kasanetz et al., 2002). LTD does require depolarization of the postsynaptic cell, coactivation of D1 and D2 dopamine receptors, and activation of AMPA and metabotropic glutamate receptors (Calabresi et al., 1994; Gubellini et al., 2001; Gubellini et al., 2003). An increase in the concentration of calcium in the postsynaptic neuron and the activation of calcium-dependent protein kinases are required to induce striatal LTD (Calabresi et al., 1994).

Intracellular in vitro (rat) recordings of cortical projecting neurons, striatal medium spiny neurons, and striatal fast-spiking interneurons revealed that unlike the medium spiny neurons, striatal fast-spiking interneurons do not show an "up

state" (Plenz & Kitai, 1998). Both the medium spiny neurons and striatal fast-spiking interneuron receive low frequency inputs from the cortex (Charpier et al., 1999; Goldberg et al., 2003). The fact that only medium spiny neurons show an "up state" suggests that the cortex provides feedforward inhibition of medium spiny neurons activity during the up state via striatal fast-spiking interneurons.

1.10.2 Microcircuitry

The original models of corticostriatal plasticity neglected the role of intrinsic circuits. However, current models suggest that striatal interneurons are heavily involved in the modulation of striatal signals and the plasticity of striatal circuitry (Centzone et al., 2003; Pisani et al., 2003). Intrinsic circuits provide the striatum with acetylcholine, GABA, nitric oxide, and adenosine (Centzone et al., 1999). Electrophysiological, morphological, and pharmacological studies have revealed the existence of four distinct subtypes of interneurons (Wilson et al., 1990; Kawaguchi, 1993; & Kawaguchi et al., 1995): large aspiny interneurons identified by the presence of choline acetyltransferase; nitric-oxide positive interneurons containing somatostatin, neuropeptide Y, and nitric-oxide synthase; and finally, two GABAergic interneurons, one containing calretinin and the other parvabumin.

Functionally, interneurons regulate local circuitry by changing the excitability of striatal projection neurons (medium spiny neurons) thus mediating

the efficacy of synaptic transmission via feedforward mechanisms (Centonze et al., 1999).

1.10.3 The Nitric-oxide positive interneuron

The nitric-oxide positive interneuron is believed to be involved in the induction of LTD within the striatum (Centzone et al., 1999; Calabresi et al., 1999). As mentioned previously this interneuron contains several transmitters and peptides. Transmitter release from this neuron is dependent on the pattern of firing activity from corticostriatal neurons, with the release of nitric-oxide only during prolonged depolarizations (Kawaguchi et al., 1995; Manzoni et al., 1992).

Calabresi et al., (1999) used fast-spiking of corticostriatal glutamatergic fibers (rat) to induce LTD of excitatory synaptic potentials recorded from striatal spiny neurons in vitro. A nitric-oxide antagonist (7-NINA) was used to confirm that LTD induction was the result of nitric-oxide release. When the slices were placed in a bath application of 7-NINA, the fast-spiking inputs failed to induce LTD. Calabresi et al., (1999) make the assertion, based on electron microscopy immunocytochemistry, that nitric-oxide positive terminals of striatal interneurons form synaptic contacts with dendrites of spiny neurons and exert feed-forward control on corticostriatal synaptic plasticity.

1.10.4 Cholinergic Interneurons

Cholinergic interneurons appear to be crucial in triggering the intracellular changes associated with corticostriatal LTP. During repetitive stimulation of corticostriatal fibers, endogenously released Ach causes a long-lasting facilitation of corticostriatal EPSPs recorded in magnesium-free solution (Calabresi et al., 1998, 2000). The muscarinic receptor antagonist scopolamine completely block LTP when bath-applied before and during fast-spiking input, but does not affect corticostriatal synaptic transmission when applied after the induction of LTP (Miller & Blaha, 2005).

1.10.5 GABAergic Interneurons

In vivo and in vitro studies have revealed that GABAergic interneurons are responsible for collateral inhibition of medium spiny neurons (Galaretta & Hestrin, 2001; Nisenbaum et al., 1992). Nisenbaum, Grace, and Berger (1992) found that spontaneous activity of medium spiny neurons increased fourfold after the disruption of intrastriatal GABAergic circuitry by intrastriatal injection of bicuculline in vitro. The parvalbumin-containing interneuron is the best-characterized striatal GABAergic interneuron and is often classified as a striatal fast-spiking interneuron (Jones & Buhl, 1993). Each GABAergic interneuron has multiple contacts on single medium spiny neurons and exerts powerful feedforward inhibition of medium spiny neurons via large inhibitory postsynaptic potentials (IPSPs) (Jones & Buhl, 1993).

1.11 Relative merits of in vivo and in vitro protocols

In accordance with Flourens' findings, research over the last century has shown that multiple regions within the central nervous system are collectively responsible for motor learning and motor behaviour. Therefore, one of the goals for electrophysiolgists should be to develop methods, models, preparations, and concepts that enhance our understanding of the interactions among these regions. A variety of laboratory preparations and phenomena have been utilized in the search for these interactions and their underlying mechanisms.

In vitro preparations permit a variety of controlled manipulations that can lead to a deeper understanding of basic cell and membrane physiology. In vitro methods allow the measurement of single cell events, including the membrane potential, using slice preparations and sharp microelectrodes. There are various techniques for removal and incubation of slice tissue, but generally speaking, the brain is quickly removed from the skull and placed in a cooling medium, usually ice-cold artificial cerebrospinal fluid. Slices are then cut using a vibrating microtome and placed in a recording chamber containing an artificial medium. Recordings can then be taken for several hours or as long as the tissue remains healthy.

Current-clamp is a popular method used to measure the voltage difference across the membrane, while holding the current constant by injecting a current into the cell as necessary. Conversely, current can be measured by using the

voltage-clamp technique, which involves holding a neuron at a constant voltage and allowing the current to vary. These, and a variety of other techniques allow the experimenter to answer quite specific questions about cellular mechanisms. The disadvantages are that 1) the system may have been compromised and may no longer function the same as in the normal intact state, and 2) this preparation does not lend itself to the investigation of slowly developing and long lasting changes in neural function.

In vivo electrophysiology, on the other hand, allows us to study interactions among brain regions in their intact state. The cells and circuits in the in vivo preparation have not been compromised, as is the case with in vitro studies. Another advantage of in vivo, and particularly chronic, preparations is that they enable researchers to directly correlate neural activity with behaviour. This can be especially powerful when the behaviour being measured is itself a reflection of the cellular processes under investigation. Another advantage of the chronic preparation is that there is no confounding effect of anesthesia on neural activity recorded. The downside of this approach is that the researcher is more limited in the options available to investigate cellular mechanisms.

1.12 Objectives:

The goal of the thesis project is to explore neural plasticity in networks known to be involved in motor control and motor learning. The experiments outlined in the thesis investigate a form of LTP in cortical-striatal pathways and

explore the effects of dopaminergic modulation on this form of LTP. This was done by monitoring LTP (and LTD) induction in the primary ascending (thalamocortical) and descending (corticostriatal) components of the cortical striatal circuitry in awake, freely moving animals.

The phenomena described in the following chapters satisfy most of the criteria for LTP or LTD. There was insufficient time to run one key test (that the potentiation effect is restricted to the activated line) to allow for a full confirmation, but the arguments for using the terms LTP and LTD are presented in the Discussion section along with the additional cautionary note that additional experiments will be required for a more complete characterization of these phenomena.

The pathways of interest are glutamatergic, and the LTP (or LTD) will be induced by application of repeated stimulation trains to those monosynaptic pathways. The exploration of the modulatory effects of dopaminergic input will be done by pairing activation of the glutamatergic and dopaminergic inputs to the target site. Several researchers have observed LTP using slice preparations and in intact systems using anesthetized preparations. Prior to the experiments reported here, it was not known if LTP occurs in the corticostriatal system in the intact brain in awake, freely moving animals, or if it can be modulated by dopamine.

In summary, the experiments reported in this thesis were designed to investigate a novel form of corticostriatal LTP/LTD in the intact freely moving

rat, and to explore the effects of dopaminergic modulation on this form of plasticity.

Chapter 2

Thalamocortical Plasticity

2.1 Introduction

The basal ganglia and related circuitry appear to play a major role in the learning of new movement patterns. Changes in the synaptic efficacy of basal ganglia connections are believed to be among the various factors contributing to sensorimotor learning, and similar changes can be studied using the phenomena of long-term potentiation and long-term depression.

The basal ganglia motor circuit modulates cortical output necessary for normal movement (Smith et al., 1998; Takada et al., 2001). Signals from the cerebral cortex are processed through the basal ganglia-thalamocortical motor circuit and return to the same cortical area via a feedback pathway (Castro-Alamancos & Conners, 1996). The corticostriatal and thalamocortical pathways are part of the "direct pathway" and are characterized by glutamatergic excitatory connections. Although we clearly understand that thalamic relay nuclei form a necessary link between the basal ganglia and the cortex, little is known about thalamocortical plasticity, especially in the intact animal.

In the past, the thalamus was often described as a relay station that simply forwards sensory information to the brain, having little to do with processing or integration (Glees & Le Gros Clark, 1941; Zeki & Shipp, 1988).

Glees and Le Gros Clark's (1941) anatomical study utilized a transneuronal degeneration technique to investigate retinal projections in the lateral geniculate body, which receives information directly from the retina. This technique involved silver-impregnation of retinal nerve fibres and terminals (of 5 monkeys) followed by a lesion of one eye or a section of the optic nerve. The animals were euthanized several days afterwards (days 3, 7, 17, 18, and 46) and silver sections were collected and examined under a microscope. Lesions of the optic nerve resulted in massive degeneration of axons and terminal boutons of the optic tract in the laminae of the geniculate body.

In discussing their results, Glees and Le Gros Clark (1941) were among those to conclude that the thalamus was primarily a relay structure, stating that "... cells of the lateral geniculate body have one function only to perform – to serve as simple relays for retinal impulses on their way to the visual cortex". Despite the fact that Glees and Le Gros Clark (1941) were merely observing anatomical differences between lesioned and non-lesioned sites in the lateral geniculate nucleus, they were confident the structure's function was a simple one. That view was not quite correct.

For example, in addition to its role as a first-order relay station, transferring sensory information from the periphery and subcortical areas to the

cortex, the thalamus is now known to be involved in complex higher-order relays, modulating and regulating cortico-cortical activity (Ramcharan et al., 2005; Sherman & Guillery, 1996, 2002). The highly recurrent connectivity of the thalamocortical system, along with projections from the basal ganglia, is but one of the challenges to the seemingly simple belief that the thalamus is a straightforward relay from the senses to cortex.

The central location of the thalamus (Fig. 1.1) and the diverse converging inputs, make this system a key component in the integrative functions of the CNS. Researchers have experienced difficulty in demonstrating plasticity of thalamocortical synapses. It has been suggested by a number of researchers that long-term changes of thalamocortical synapses is only possible during the critical period early in life (Feldman et al., 1999; Fagiolini et al., 1994; Hubel and Wiesel, 1970; Woolsey and Wann, 1976; Fagiolini et al., 1994). A study by Crair and Malenka (1995) examined LTP in the thalamocortical synapse that form whisker barrels in rat somatosensory cortex. The barrels of somatosensory cortex are a somatotopic representation of sensory vibrissae. Using thalamocortical slice preparation of rat pups (Sprague-Dawley) from postnatal day 0 (24 hrs after birth) to postnatal day 14, they found that LTP was limited to the critical period, which ended on postnatal day 7 (Crair & Malenka, 1995). Crair and Malenka (1995) report that it was extremely difficult to elicit any LTP in the older animals (postnatal days 8 – 14).

In addition to demonstrating that thalamocortical LTP can only be induced during the critical period, they also showed that this form of LTP is NMDA dependent. After pharmacologically isolating NMDA receptor-mediated currents, they found a profound decrease in the relative contribution of NMDA receptors as a function of age, and this reduction presumably made it more difficult to induce LTP at thalamocortical synapses (Crair& Malenka, 1995).

Iwasato et al., (1997) also found reduced NMDA receptor activation (Iwasato et al., 1997) and reduced expression of AMPA receptors in thalamocortical synapses of adults, adding weight to the argument that thalamocortical plasticity is limited to a critical period. Thus, it should not be possible to alter the synaptic efficacy of thalamocortical synapses in adult rats.

However, the bulk of previous research on thalamocortical plasticity has been done in anesthetized or in vitro preparations, which may alter plasticity properties of the tested networks. Further, most of the earlier work has focused on thalamocortical plasticity within pathways from thalamic sensory nuclei, which may not be the best model for pathways to or from thalamic motor nuclei. .

Thus, it remains to be seen how thalamocortical plasticity expresses itself in *motor* pathways in the *awake* animal. The purpose of the present study was to examine properties of LTP and/or LTD at thalamocortical synapses in awake, freely moving rats in a major component pathway of the striatal-cortical motor system. Either high frequency stimulation or theta burst stimulation was applied to the thalamocortical pathway to determine if the response could be altered. The

recording electrode was located in the primary motor cortex and was activated via the thalamocortical pathway. The results show that neither stimulation protocol induced LTP or LTD in the thalamocortical pathway in the awake, freely behaving rats.

2.2 Materials and Methods

2.2.1 Animals

A total of 20 male Long-Evans rats were used in this experiment. They weighed between 350 g and 500 g. The animals were housed individually on a 12 h on/ 12 h off light cycle and maintained on an ad libitum feeding schedule. Testing occurred during the lights-on phase.

2.2.2 Surgery

Prior to surgery the surgical area was shaved (handheld clippers) and cleaned with savlodil and betadine to reduce contamination during surgery. After the incision was made, 0.04 ml of a local anesthetic, xylocane, was injected into the tissue surrounding the incision site. Haemostatic cotton pellets were used to apply epinephrine, a basal constrictor used to control local bleeders. Two stainless steel anchor screws and a grounding screw were screwed into the skull after the periocium was removed. The grounding screw consisted of a stainless screw with a Teflon-coated stainless steel wire attached to a gold plated male pin.

Bipolar electrodes were prepared from twisted Teflon-coated stainless steel wire, 125 μm in diameter. In each electrode, one of the poles was approximately 1800 μm longer then the other. This allowed for differential recording across the dorsal/ventral axis of the major pyramidal cells of the motor cortex. The stimulating electrode was placed in the thalamus. The poles of the stimulating electrode had a separation of 500 μm. Both the recording and the stimulating electrodes were implanted while the animal was under anesthesia. Somnitol, an anesthetic, was administered during the surgery. The rats received 60 mg/kg i.p. of the barbiturate drug Somnitol.

The bipolar stimulating electrode was placed in the left ventrolateral nucleus (Vlo) of the thalamus and was situated 2.3 mm posterior to Bregma, -1.8 mm lateral to the midline and approximately 6mm ventral from the skull surface (Fig 2.1). The recording electrode was inserted into the ipsilateral primary motor cortex, placed 1.7 mm anterior to Bregma, -3.0 mm lateral to the midline and approximately 2.2 mm ventral from the surface of the skull (Fig 2.1).

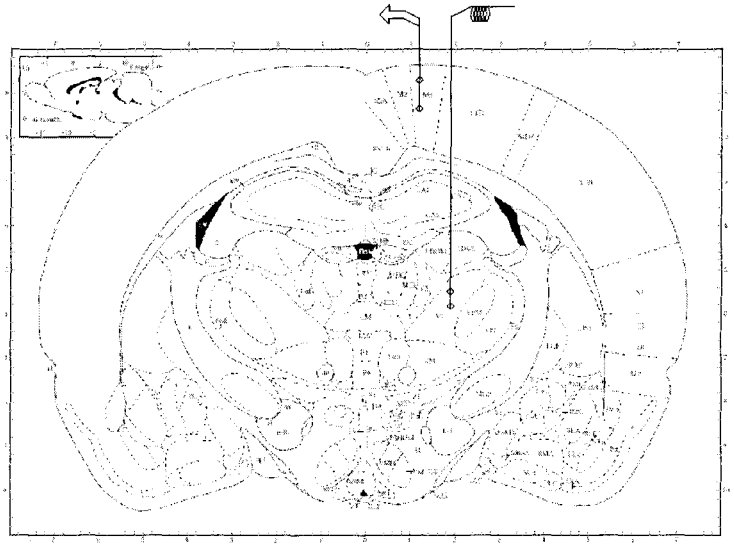


Figure 2.1: Stimulating and recording electrode placements. Both thalamic and primary motor cortical sites are shown. Diagram courtesy of Paxinos and Watson (1998).

2.2.3 Stimulation and Recording.

All testing was done in a chamber (30 cm x 30 cm x 40 cm) with plywood walls, a clear plastic front and a mirrored rear wall. The leads were inserted into a nine-pin plug and connected to the amplifiers via a slip ring commutator. The responses were high and low pass filtered at (0.3 Hz to 3 kHz) and amplified using a Grass model 12 amplifier. The amplified responses were digitized (10 kHz, 12 bit) and stored on a computer for off-line analysis. During input-output (I-O) tests, stimulation pulses were delivered at varying intensities to the ventrolateral nucleus, and the evoked field potentials were monitored in the

cortex. Single 0.1 msec biphasic square wave pulses were delivered through constant current isolation units at frequency of 0.1 Hz. Monopolar recordings were taken from each tip of the bipolar electrode, in addition to the bipolar recording across the tips. An example of a field potential evoked by thalamic stimulation is shown in Fig. 2.2.

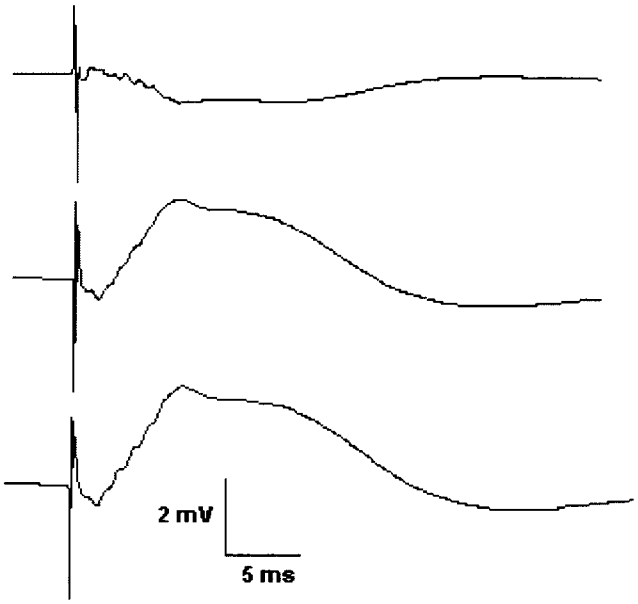


Figure 2.2: Example of evoked potential. The upper trace shows the monopolar response from the superficial tip. The middle trace shows the monopolar response from the deep tip. The bottom trace shows the bipolar response.

The I-O tests included ten responses evoked at each of seven intensities (30, 80, 160, 400, 630, 1000, 1260 μA). An example input/output function is shown in Fig. 2.3.

Input/Output Function

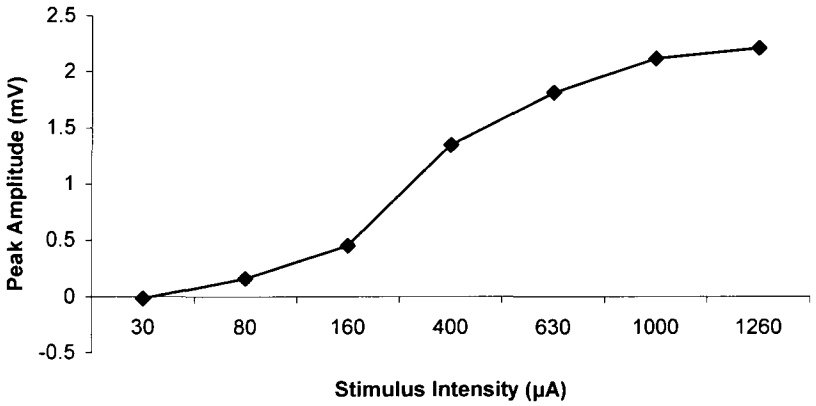


Figure 2.3: Example input/output function. The amplitude of the field potential is plotted as a function of the intensity of the test pulse.

Three baseline I-O tests, with a 48 hr separation between tests, were taken to confirm the stability of the evoked responses. After confirming response stability, the three baseline I-O's were averaged over each intensity in order to produce a single baseline I-O measure.

The animals were randomly assigned to one of three groups, an experimental high frequency stimulation (HFS) group (n= 7), an experimental theta burst stimulation (TBS) group (n= 7) and a control group (n= 6). The control group did not receive any stimulation.

2.2.4 Tests for Long-term Potentiation

HFS and TBS sessions commenced one day after baseline I-O tests were completed. For the HFS group, 60 brief high frequency trains were delivered once per day for 10 consecutive days. The duration of each train was 24 msec and consisted of 8 pulses at 100 Hz with a 10 sec delay between each train. The trains were delivered at a pulse intensity of 1000 μA and pulse duration of 0.1 msec. The TBS group received 60 trains of 5 Hz bursts involving 4 pulses at 100 Hz delivered once per day for 10 consecutive days.

Input-output tests were taken on the third, seventh, and tenth day during train sessions. The experiment did not include a subsequent tracking of LTP decay, because there were no observable potentiation effects following the stimulation period.

2.2.5 Data Analysis

The primary measure used throughout most of the experiments was the amplitude of the evoked potential. The bipolar recordings were used for the data analysis, in most cases, because they provided large amplitude and stable responses with low noise. The amplitude of the evoked potentials for the thalamocortical pathway was measured at two latencies. The first latency, known as the early component, and the second latency, known as the late component,

peaked at approximately 9.1 ms and 23.6 ms respectively. An example of a corticostriatal response is shown in Fig. 2.4.

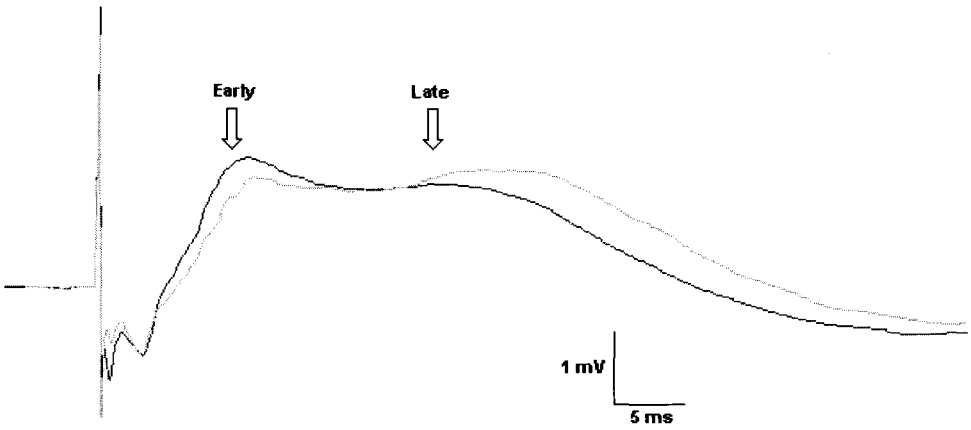


Figure 2.4: Latencies of early and late components of the corticostriatal pathway.

2.3 Results

2.3.1 Session Effects

All the animals in the sample exhibited the characteristic morphological properties of neocortical recordings (Fig. 2.4). Measurements of amplitude were done at a test pulse intensity of 630 μA, an intensity near the middle of the input/output function. An ANOVA revealed that thalamic stimulation failed to induce activity-dependent changes in the amplitude of either the early or late components of the cortical EPSPs (Fig.2.5 & 2.6). Results further indicated that both early and late components failed to show any observable main effect for group ($F_{(2,17)} = 0.998$, $p>0.05$ and $F_{(2,17)} = 0.998$, $p>0.05$ respectively) (Fig.2.5 & 2.6).

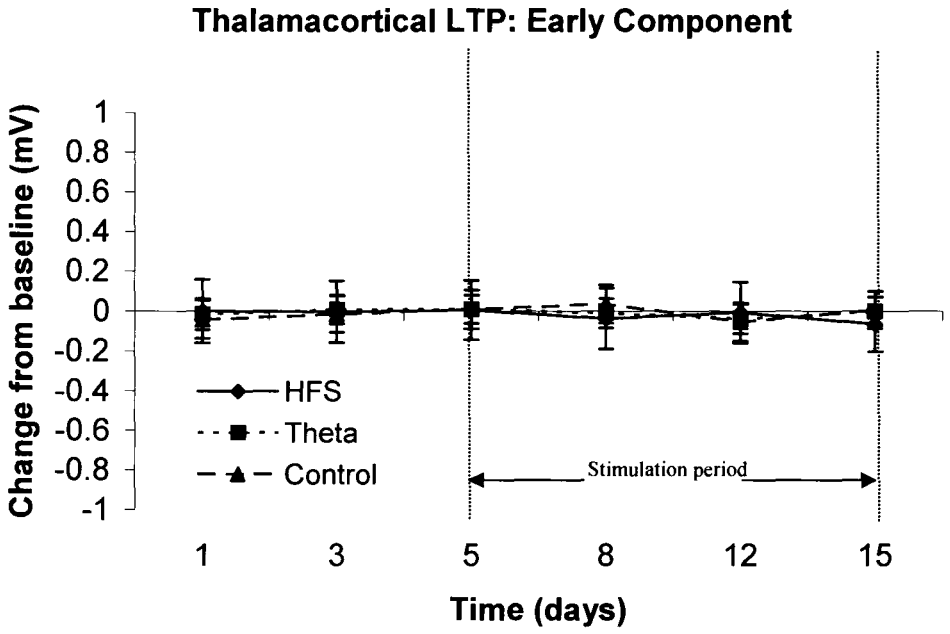


Figure 2.5: Change from baseline amplitude. The averaged peak amplitude changes from baseline of the early component are shown above for both experimental and control groups.

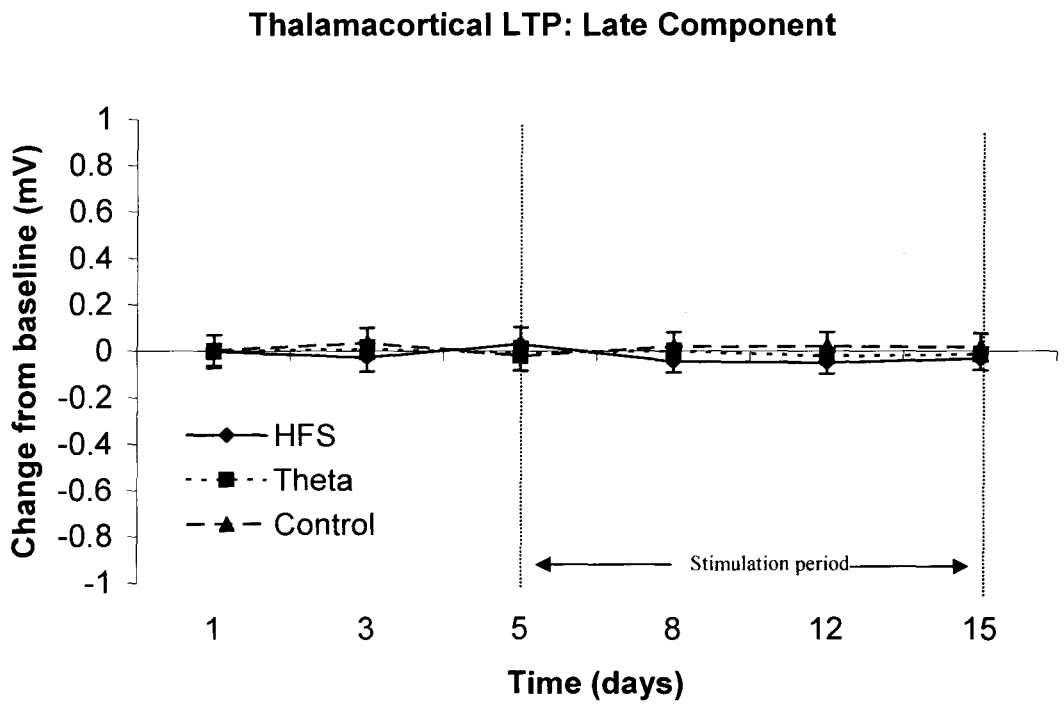


Figure 2.6: Thalamocortical Pathway Input/Output Functions. The averaged peak amplitude changes from baseline are shown for the late component of both experimental and control groups.

2.3.2 Thalamocortical input/output functions

Figures 2.7 – 2.8 show the data from the thalamocortical pathway for all intensities of stimulation. The control measures showed the response amplitudes to be stable over time. The plotted input/output curves are from the last baseline measure and 24 hours following the last day of stimulation trains. There was no evidence of potentiation for either standard or theta burst stimulation groups at the end of the stimulation period for any of the latency components (Fig. 2.7). The slight reduction in the amplitude of the early component following tetanization

(Fig. 2.7) is clearly not significant, but it is reminiscent of the larger, significant effects seen in cortico-cortical pathways (Chapman et al., 1998). Those effects appeared to be due to population spike potentiation. The stimulation effects reported here are clearly non-significant, so if LTP exists in this system, a different induction protocol would be required to reveal it.

Similar results were found for the late component. Neither tetanization nor theta burst stimulation were able to potentiate the late component of the thalamocortical pathway (Fig. 2.8). The input/output functions for the tetanization and theta burst condition are virtually identical before and after stimulation. Thus, there was no effect of either stimulation pattern on either early or late components in the thalamocortical pathway. Again, the input/output functions for the control group show that the amplitudes remained stable over the course of the experiment. Neither a main effect of group nor interaction effects were significant for either the early or late components ($F_{(2,17)}= 0.998$, $p>0.05$ and $F_{(2,17)}= 0.998$, $p>0.05$ respectively).

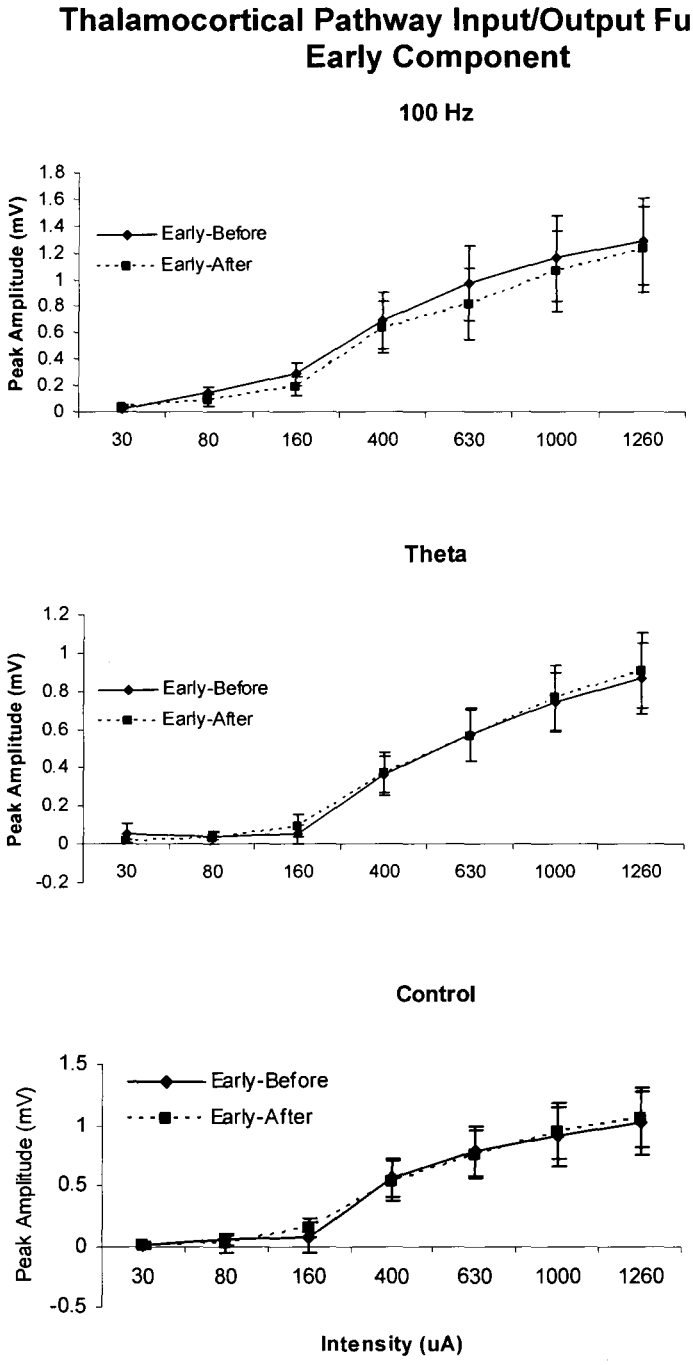


Figure 2.7: Input/Output function for the early components. Early component input/output functions from the thalamocortical pathway are shown before and after stimulation (the figure title indicates stimulation condition). Solid lines are baseline measures, dashed lines are 24 hours after last day of tetanization.

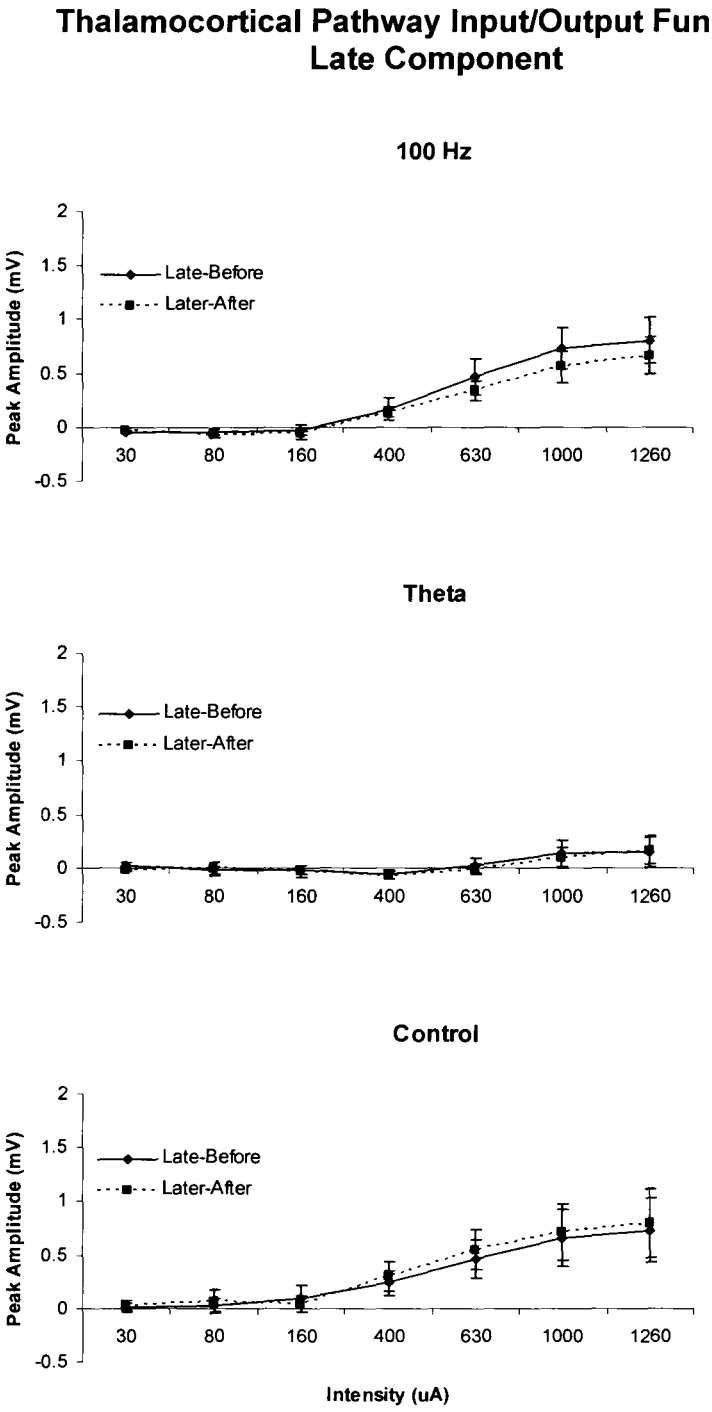


Figure 2.8: Input/Output functions for the late component. Late component input/output functions from the thalamocortical pathway are shown before and after stimulation (figure title indicates stimulation condition). Solid lines are baseline measures, dashed lines are 24 hours after last day of tetanization.

Representative Sweeps

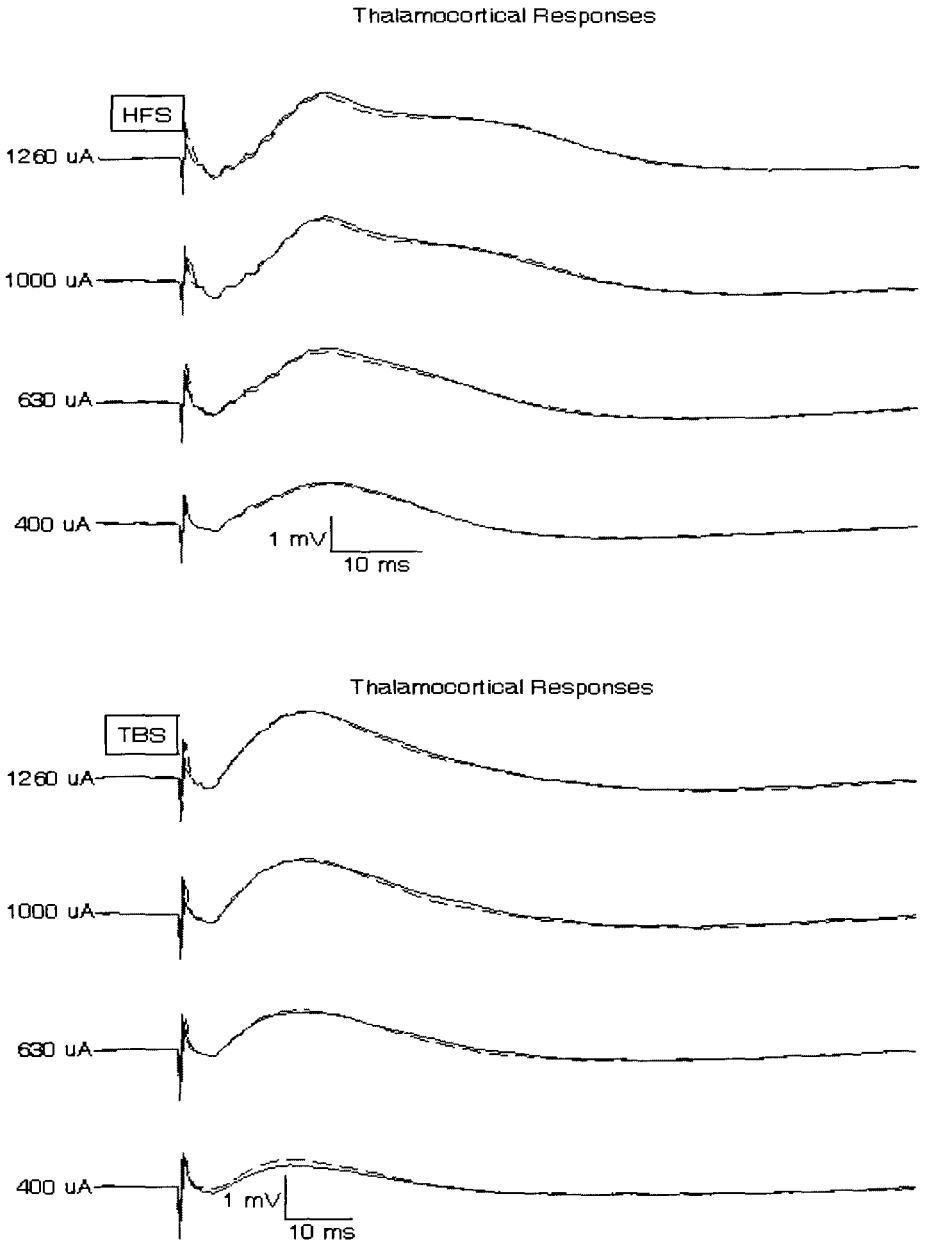


Figure 2.9: Representative field potentials evoked by thalamic stimulation before and after high frequency and theta burst stimulation of the thalamic pathway. Solid lines represent baseline responses and dashed lines indicate responses measured 24 hours after the last train session.

2.4 Discussion

The main question addressed in this experiment was whether the thalamocortical pathway could express either LTP or LTD following either high-frequency or theta burst stimulation. The answer appears to be no, as both forms of stimulation failed to induce any change in this particular pathway. Following ten days of stimulation, there was no observable change in the amplitude in either the early or late components. Recordings during the stimulation period revealed no change from baseline for either experimental group. The results of the experiment appear to be consistent with a non-plastic role for the thalamocortical pathway in the awake, adult animal.

It is possible that the current stimulation protocols failed to induce a change because a non-optimal pattern of stimulation had been selected. However, the failure of other researchers (Hubel and Wiesel, 1970; Woolsey and Wann, 1976; Fagiolini et al., 1994) to find a reliable plasticity in thalamocortical pathways suggests that the mature pathways have lost their ability to be modified (e.g., Crair & Malenka, 1995). As can readily be seen in the figures (e.g., 2.5 and 2.6), neither the early nor the late component showed any changes from baseline field potentials.

A histological analysis was not performed due to ongoing use of the animals, therefore electrode placement could not be illustrated anatomically. However, the electrophysiological response morphology was reliably indicative

of accurate placements. In addition, stimulus-dependent myoclonic jerks were consistently observed in all the animals, which is also consistent with accurate electrode placements.

Chapter 3

Corticostriatal LTD

3.1 Introduction

Very little is known about the mechanisms by which memories are encoded and maintained in the basal ganglia motor-loop system. Recent work has provided insights into some of the synaptic and neurochemical processes thought to mediate experience-dependent plasticity and memory in the corticostriatal circuit. Acute and anesthetized studies have shown that corticostriatal circuits can express both long-term depression and long-term potentiation. Researchers believe that bi-directional synaptic modification is necessary for maintaining a normalized state and for learning and memory (Froc et al., 2000, 2005; Martin et al., 2000).

Unfortunately, researchers have yet to establish a consistent stimulation protocol for inducing long-term depression and long-term potentiation in both acute and chronic preparations. Given that long-term potentiation is easier to induce in many systems than long-term depression, and easier to relate to circuit building, researchers have generally concentrated on the properties and mechanisms related to synaptic strengthening rather than synaptic weakening.

However, I decided to begin my investigation into corticostriatal plasticity by focusing on long-term depression. This decision was based on an earlier pilot study, in which I applied high-frequency stimulation (300 Hz) to the corticostriatal pathway in an attempt to induce a potentiation effect. I found that application of a 300 Hz tetanus to the primary motor cortex appeared to decrease the amplitude of the field potential, although the effect was minimal. The results of the pilot study raised the possibility that the corticostriatal pathway of the awake freely moving animal might demonstrate long-term depression, if more appropriate patterns of stimulation (e.g., low frequency trains) were applied.

Previous studies from our lab have shown that long-term depression could be reliably induced in corticocortical pathways in the freely moving rat following low-frequency stimulation (1 Hz) (Froc et al., 2000). Thus, I chose to investigate long-term depression in the corticostriatal pathway using the same low-frequency stimulation protocol as Froc et al., (2000).

The evidence indicates that levels of glutamate transmission play a critical role in the initiation and maintenance of long-term depression. At most synapses, induction of long-term depression is achieved when presynaptic glutamatergic release is uncorrelated with postsynaptic depolarization. Experimentally, long-term depression can be achieved by applying low frequency stimulation to afferent pathways (Froc et al., 2000). The experiment presented in this chapter investigates corticostriatal long-term depression in freely behaving animals. The number of stimuli applied to the afferent pathway can affect the probability of

inducing long-term depression, hence I have chosen to use a prolonged low-frequency (1 Hz) stimulation session that has worked well in corticocortical systems.

3.2 Materials and Methods

3.2.1 Animals

A total of 30 male Long-Evans rats were used in this experiment. They weighed between 350 g and 500 g. Animals were prepared using the same procedures as those in the thalamocortical plasticity experiment (Chapter 2), with the exception of electrode coordinates and stimulation parameters.

3.2.2 Electrodes and Surgery

The stimulating electrode was placed in the primary motor cortex, which projects ipsilaterally to the striatum. This is the descending pathway or entry pathway to the basal ganglia motor-loop. The poles of the stimulating electrode had a separation of 500 μm. Both the recording and the stimulating electrodes were implanted while the animal was under anesthesia.

The bipolar recording electrode was placed in the putamen (left side) and was situated 2.3 mm posterior to Bregma, -3.5 mm lateral to the midline and approximately 3.5 mm ventral from the skull surface (Fig 3.1). The stimulating electrode was inserted into the ipsilateral primary motor cortex, placed 1.7 mm

anterior to Bregma, -2.0 mm lateral to the midline and approximately 2.8 mm ventral from the surface of the skull (Fig 3.1).

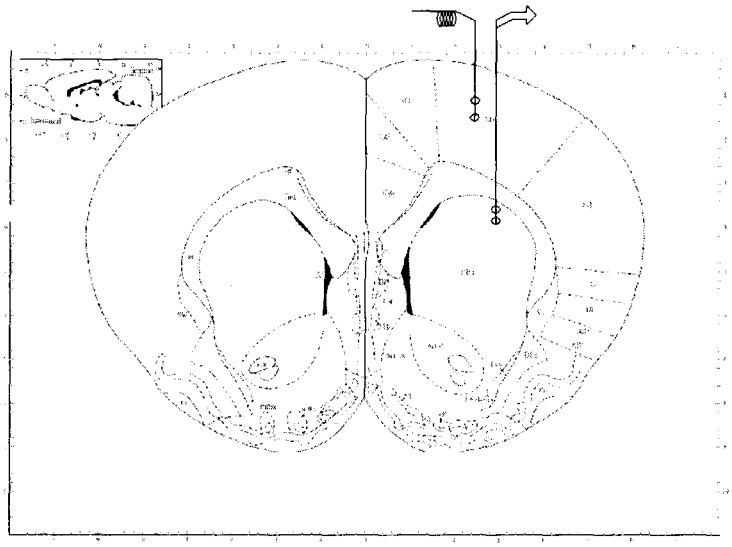


Figure 3.1: The desired placements of putamen and the primary motor cortex electrodes. Diagram courtesy of Paxinos and Watson (1998).

After both electrodes had been set to the appropriate target sites, the dorsal ventral setting of the electrodes was finely adjusted to obtain maximum field response amplitudes. Once the desired signal had been achieved, the gold plated electrode pins were inserted into a connector plug, which was mounted to the skull of the animal using anchor screws and dental cement.

3.2.3 Stimulation and Recording

All testing was done in a chamber (30 cm x 30 cm x 40 cm) with plywood walls, a clear plastic front and a mirrored rear wall. The leads were inserted into the nine-pin plug and connected to the amplifiers via a slip ring commutator. The responses were high and low pass filtered at (0.3 Hz to 3 kHz) and amplified using a Grass model 12 amplifier. The amplified responses were digitized (10 kHz, 12 bit) and stored on a computer for off-line analysis. During input-output (I-O) tests, stimulation pulses were delivered at varying intensities to the contralateral white matter, and the evoked field potentials were monitored in the putamen. Single 0.1 msec biphasic square wave pulses were delivered through constant current isolation units at a frequency of 0.1 Hz. An example of a field potential evoked by cortical stimulation is shown in Fig. 3.2.

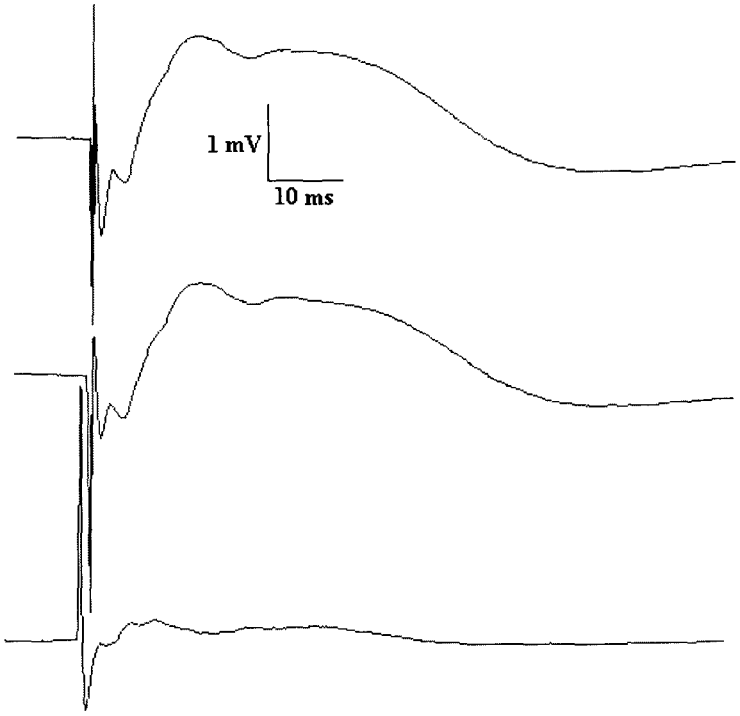


Figure 3.2: Example of corticostriatal evoked potential. The upper trace shows the monopolar response from the superficial tip. The middle trace shows the monopolar response from the deep tip. The bottom trace shows the bipolar response.

The I-O tests included ten responses evoked at each of seven intensities (30, 80, 160, 400, 600, 1000, 1260 μA). An example input/output function is shown in Fig. 3.3.

Input/Output Function

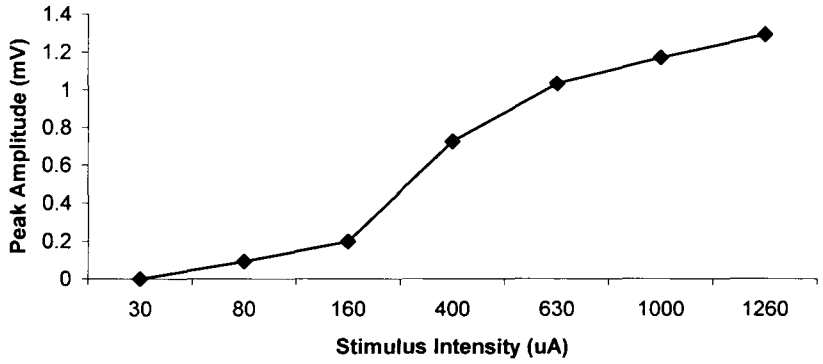


Figure 3.3: Example input/output function. The amplitude of the field potential is plotted as a function of the intensity of the test pulse.

Three baseline I-O tests, separated by 48 hr, were taken to confirm the stability of the evoked responses. The three baseline I-O's were then averaged together in order to produce a single baseline measure for each intensity.

The animals were randomly assigned to one of three groups, an experimental low frequency stimulation (LFS) group (n= 9) receiving a total of ten trains, 1 train per hour for 10 hours; an experimental LFS group (n= 9) receiving a total of ten trains, 1 train every half hour for 5 hours; and a control group (n = 12). The control group did not receive low-frequency stimulations.

3.2.4 Test for Long-term Depression

LFS sessions commenced one day after baseline I-O tests were taken. For the LFS groups, a 900 pulse train (1 Hz) was delivered either every hour or half hour depending on group assignment for a total of 10 trains. The duration of each train was 15 mins. The trains were delivered at a pulse intensity of 1000 μA. Input-output tests were taken every half-hour or hour during the stimulation period depending on group assignment. In addition to input/output tests during the stimulation period, test responses were acquired on post-stimulation days 1, 3, 6, and 9 in order to observe the changes in induction effects over sessions.

3.2.5 Data Analysis

The primary measure was the amplitude of the evoked potential. Monopolar evoked potentials were used for the purposes of data analysis because responses recorded in the striatum did not show strong bipolar potentials across the poles of the recording electrodes. This is presumably due to the anatomical organization of the putamen, which lacks a clear laminar structure. The amplitude of the evoked potentials for the corticostriatal pathway was tracked at two latencies. The early component appeared to peak at a latency of approximately 9.1 ms and the late component peaked at about 23.6 ms. An example is shown in Fig. 3.4. The late component is clearly polysynaptic. The short latency onset of the early component indicates that it is likely to be a monosynaptic response, although that cannot be stated definitively until the response properties are more

thoroughly characterized. Appendix A outlines my efforts to run the first stage of tests to differentiate these components. The results are fully consistent with an identification of the early component as a monosynaptic event. However, until a more thorough battery of tests have been run, including single unit recordings, I will continue to use the terms early and late component to identify these responses.

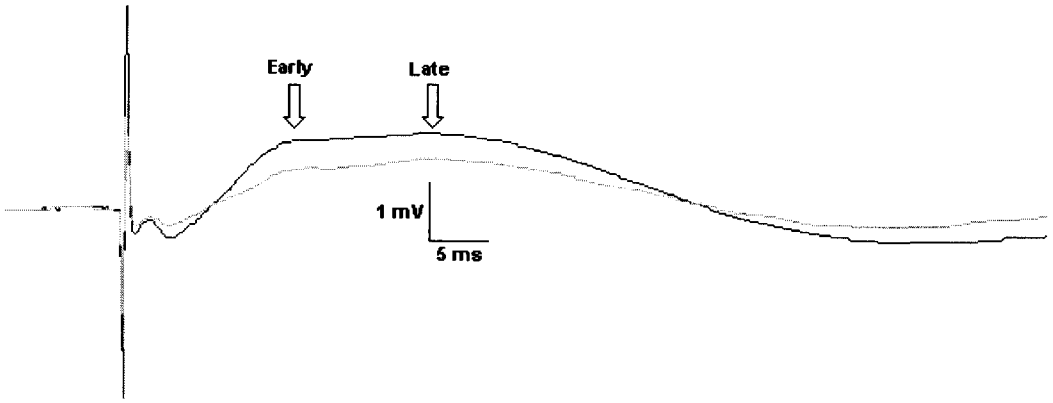


Figure 3.4: Latencies of early and late components of the corticostriatal pathway.

3.2.6 Histology

Following the recording of the final input/output function, an anodal DC current was passed through the tip of each electrode for approximately 20 s using a 10 μA current. This caused an accumulation of Fe^{2+} atoms to be deposited around the stainless steel electrode tip allowing for later visualization using a Neutral Red stain.

After marking the electrode placements, the animals were sacrificed and perfused through the heart with phosphate-buffered saline (PBS, pH 7.4) immediately followed by cold 10% formalin in PBS. Once the brains were removed, they were post-fixed over-night and cryoprotected for several more days using 30% sucrose in PBS. They were sectioned on a cryostat at 50 μm, mounted on to gelatin-subbed slides and allowed to air-dry overnight.

The tissue was gradually hydrated through a series of graded alcohols and then stained with 2% Ferrocyanide and Neutral Red, a biological stain, which stains cell bodies. Following staining, the tissue was dehydrated, cleared with Xylenes, and coverslipped. Representative diagram of electrode placements are shown in Figure 3.5.

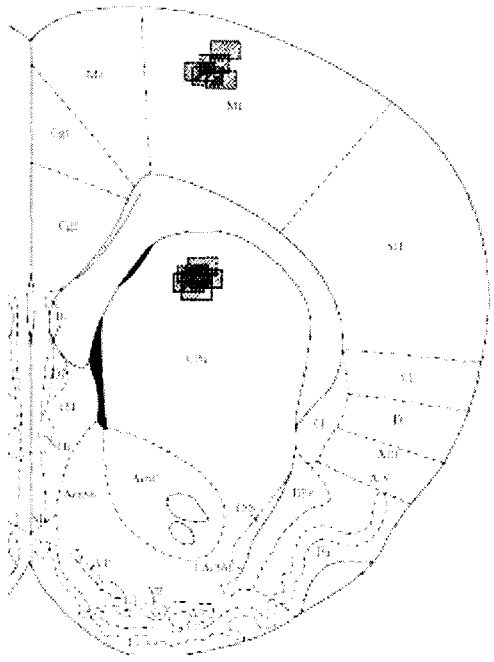


Figure 3.5: Representative diagram showing placement of electrodes. The cross-hatched areas show the regions within which electrode tips were located. Stimulating electrode is in the motor cortex and the recording electrode is in the putamen.

3.3 Results

3.3.1 Session Effects

All the animals in the sample exhibited similar corticostriatal response morphologies (Fig. 3.4 and 3.10). The trend for the change in response amplitude over sessions was similar to that seen for LTD induction in other pathways of cortical origin thus far tested in chronic preparations (Froc et al., 2000). However, the magnitude of amplitude change in the corticostriatal response in this

experiment did not reach statistical significance (ANOVA for the early component interaction effect: $F_{12,102} = 1.225$, $p = .277$; ANOVA for the late component interaction effect: $F_{12,102} = 1.434$, $p = .240$). For both early and late components, there was a tendency for the post-stimulation responses to be more reduced in amplitude in the animals receiving low frequency stimulation every hour as apposed to every half-hour (Figs. 3.6 and 3.7).

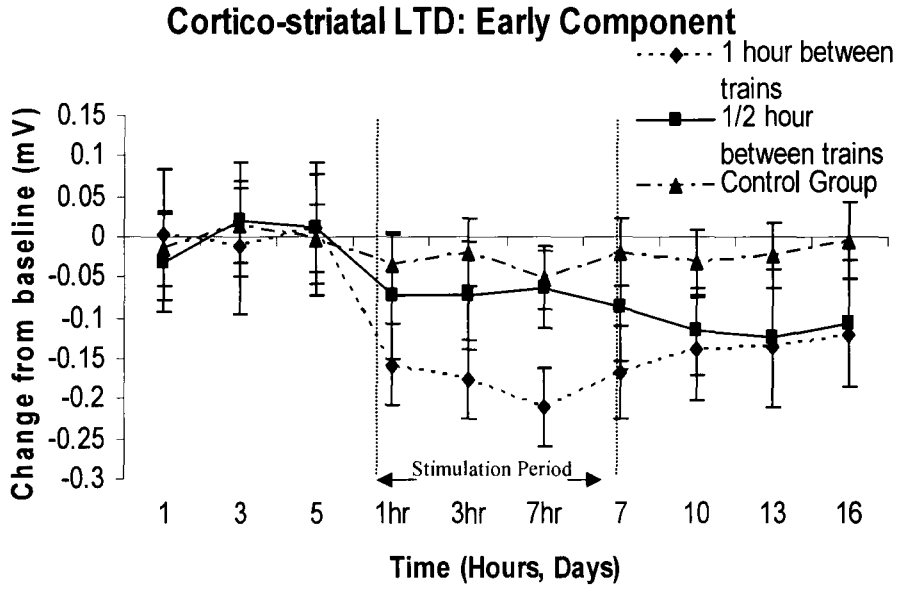


Figure 3.6: Average peak amplitude change from baseline of the early component of both experimental and control groups. (Note: 3 test pulses were taken during the stimulation period at 1, 3, and 7 hours).

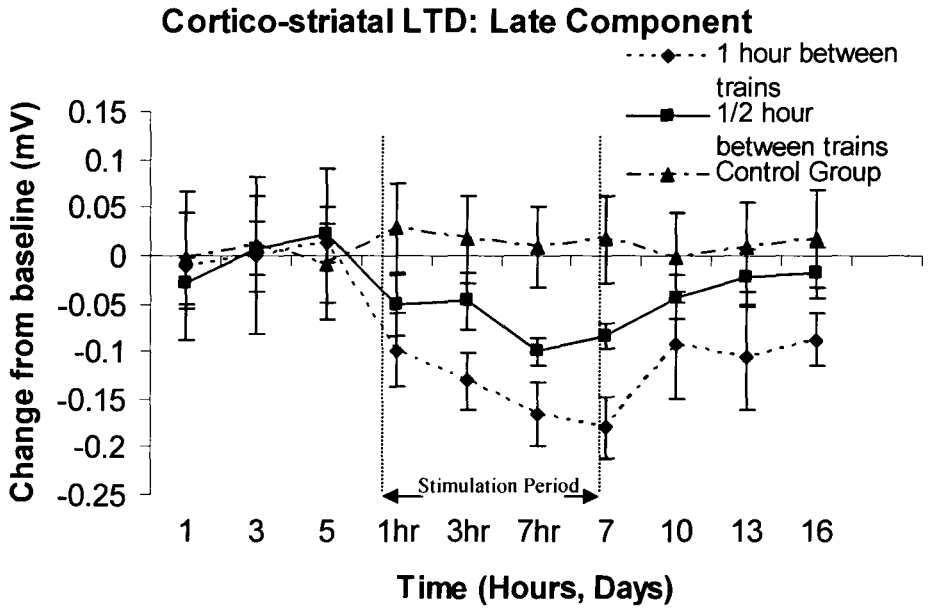


Figure 3.7: Average peak amplitude change from baseline of the early component of both experimental and control groups. (Note: 4 test pulses were taken during the stimulation period at 1, 3, and 7 hours).

3.3.2 Corticostriatal input/output functions

Despite the lack of significance for the session effects, when the changes were compared before (baseline measure taken on day 5) and after the LTD induction phase (24 hours after the last stimulation session) over all intensities (Figures 3.8 & 3.9), a difference was found between the 30 min and 60 min groups. When the amplitude changes were compared between these two groups, an ANOVA revealed a significant difference, with the 60 min group showing a larger depression effect ($F_{2,17} = 4.880$, $p = .021$), but only for the late component. The change in the early component was not significant.

These mixed analysis results are not encouraging for the protocol used here. Of course, further exploration of electrode placement and stimulation pattern in this pathway might reveal a more robust LTD effect than found with the present protocol. Also, it should be kept in mind that LTD effects in intact systems tend to be small, while variability in response measurement tend to be relatively large. In any case, further research is clearly necessary to characterize responses to repeated low-frequency stimulation in this pathway.

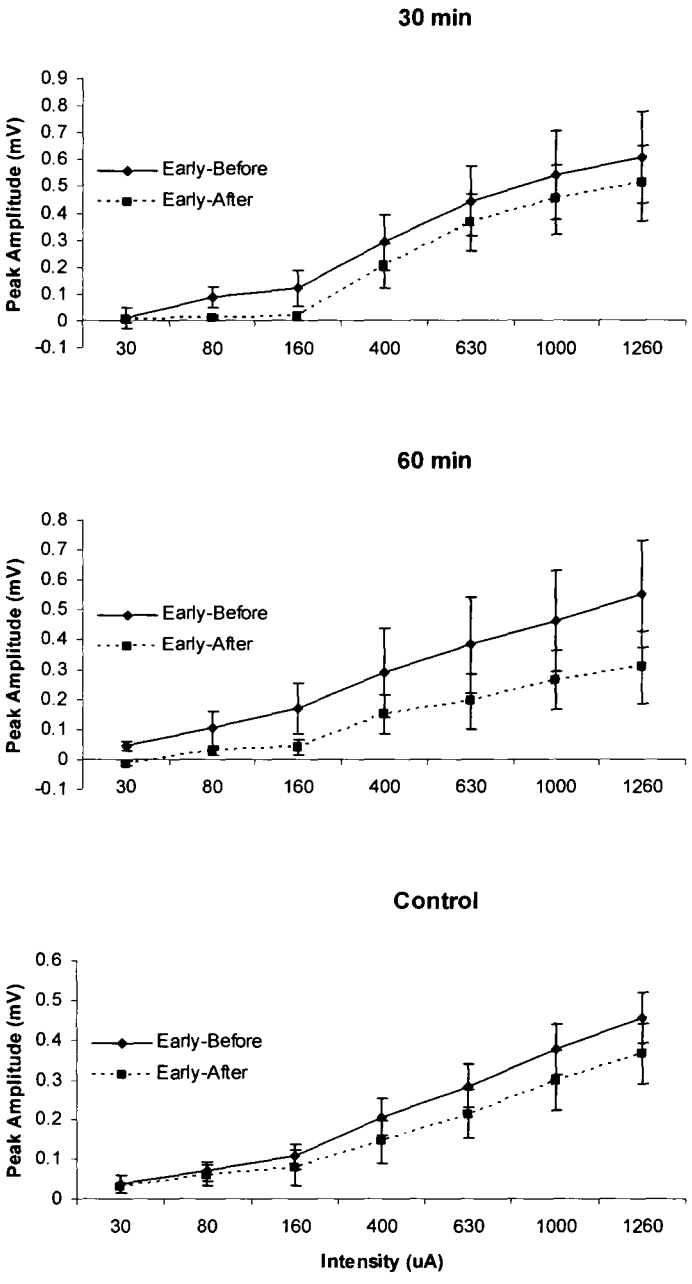


Figure 3.8: Early component input/output functions. Measures from the Corticostriatal responses are shown before and after stimulation (figure title indicates stimulation condition). Solid lines are baseline measures, dashed lines are 24 hours after last day of tetanization.

Corticostriatal Pathway Input/Output Functions;
Late Component

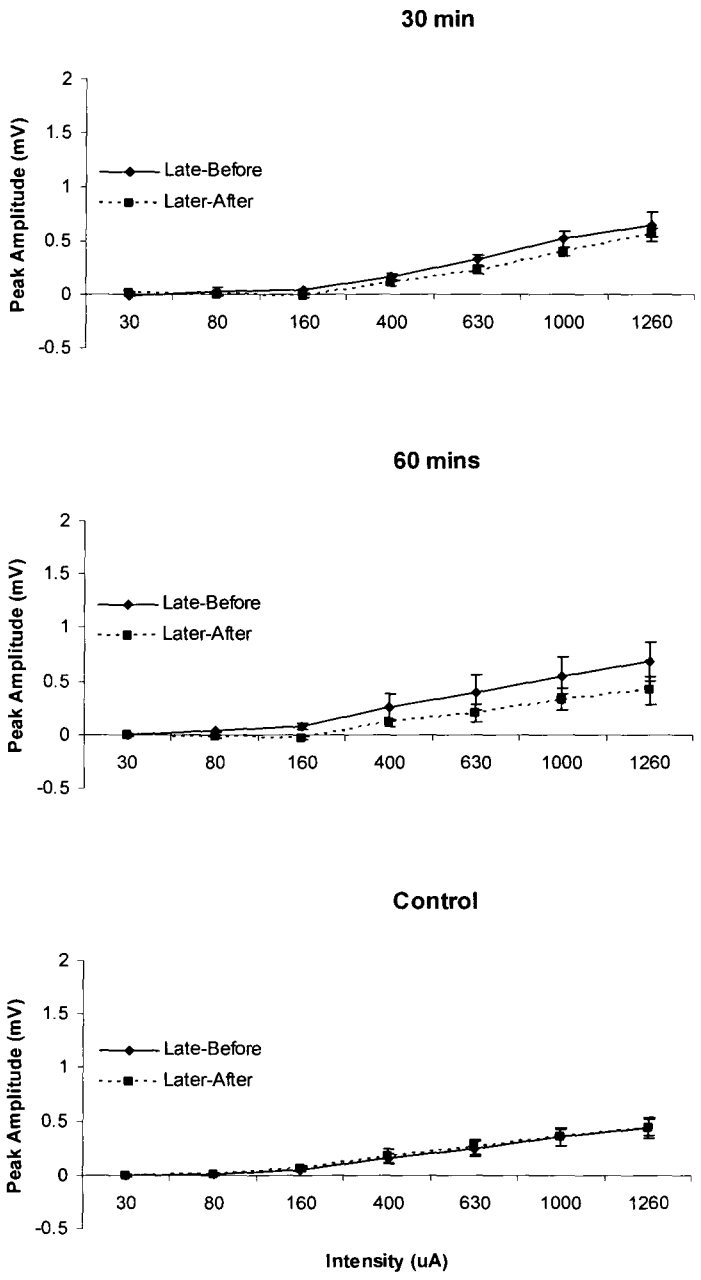


Figure 3.9: Late component input/output functions from the corticostriatal pathway before and after stimulation (figure title indicates stimulation condition). Solid lines are baseline measures, dashed lines are 24 hours after last day of tetanization.

Representative Sweeps

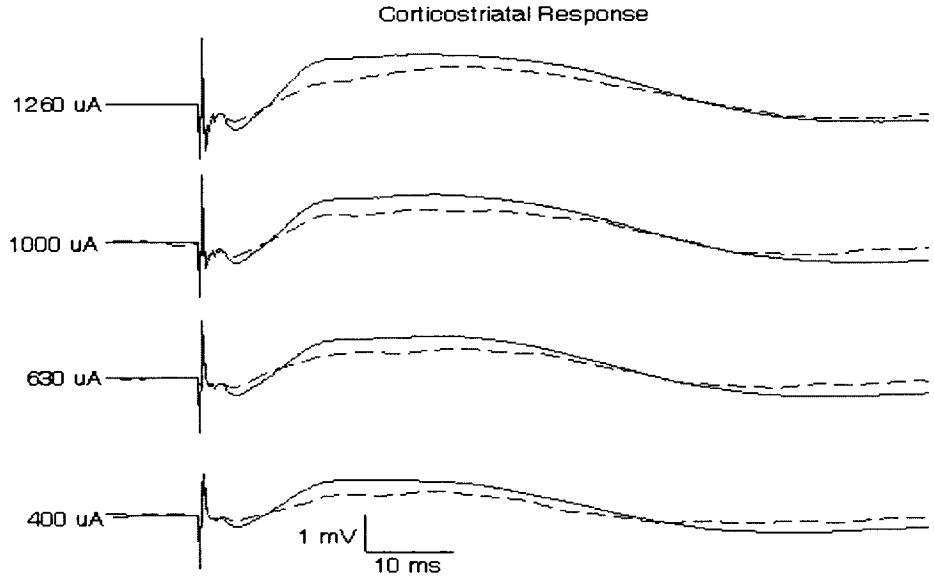


Figure 3.10: Field Potentials evoked by cortical stimulation before and after stimulation of the corticostriatal pathway. Solid lines represent baseline responses and dashed lines are 24 hours after the last stimulation train session.

3.4 Discussion

This experiment was designed to examine the possibility of inducing homosynaptic LTD in the corticostriatal pathway following low-frequency stimulation. In general, an ANOVA did not reveal any clear effects of low-frequency stimulation on the induction of striatal LTD over stimulation sessions. Although there were no clear effects over sessions, an analysis of response amplitude changes over intensities for the late component showed a significant difference between the 30 min and 60 min groups, with a greater depression effect in the 60 min group. There was no significant result for the early component.

It is not too surprising that the effects reported here were marginal. LTD effects tend to be small and less reliably seen than LTP effects (Errington et al., 1995; Abraham et al., 1996; Manahan-Vaughan, 1997; Staubli and Lynch, 1990; Doyle et al., 1997; Thiels et al., 1994; Heynen et al., 1996). In addition, there are a variety of reasons why the present experiment failed to induce LTD in the striatum. The pattern of low-frequency stimulation chosen for this experiment was derived from a previous experiment in which LTD was induced in a corticocortical pathway (Froc et al., 2000), and it may not be optimal for the induction of LTP in the corticostriatal pathway. For example, the selected intervals (30 and 60 min) between low-frequency stimulation trains may have been too brief. The larger effect seen in the 60 min ISI group suggests that future experiments should explore this, and perhaps longer intervals.

In addition, the pulse-frequency within the stimulation trains may have been too low, the number of sessions too low, or the electrode placements may have been slightly off the optimal target. Whatever the optimal conditions, they have to trigger the necessary rise in intracellular calcium (Mulkey and Malenka, 1992), without reaching the levels that lead to potentiation effects. This may be one of the primary reasons why LTD effects are less reliably induced than LTP effects: the effective range for calcium levels may be quite narrow for LTD compared to LTP.

Rather than pursue a potentially fruitless search for more robust LTD effects in the corticostriatal pathway, I decided to move on to a planned experiment designed to test for LTP in this pathway in the chronic preparation. The results of that experiment will be reported in the next Chapter.

Chapter 4

Corticostriatal LTP: High Frequency versus Theta Burst Stimulation

4.1 Introduction

Long-lasting changes in synaptic efficacy represent one of the cellular models for information storage in the brain and behavioral learning. One form of activity-dependent plasticity is long-term potentiation (LTP). This type of synaptic modification has been found in several regions of the central nervous system, including the striatum.

The bulk of previous research on corticostriatal LTP has been done in anesthetized or in vitro preparations. Thus it remains to be seen how LTP induction operates in the striatum of the awake animal. The purpose of the present study was to examine properties of LTP at striatal synapses in awake, freely behaving rats following either high-frequency or theta burst stimulation. The design of the experiment is similar to that in the previous experiment with the exception of the type of stimulation applied. In the previous experiment we were unable to induce robust LTD effect at corticostriatal synapses, although the pre-post input/output measure showed a significant change in the late component. The current experiment stimulates the same corticostriatal pathway, using high-frequency stimulation, and tests for an LTP effect.

4.2 Materials and Methods

4.2.1 Animals

A total of 23 male Long-Evans rats were used in this experiment. They weighed between 350g and 500g. Except for stimulation parameters, the animals were prepared using the same procedures as those in the previous corticostriatal LTD experiment.

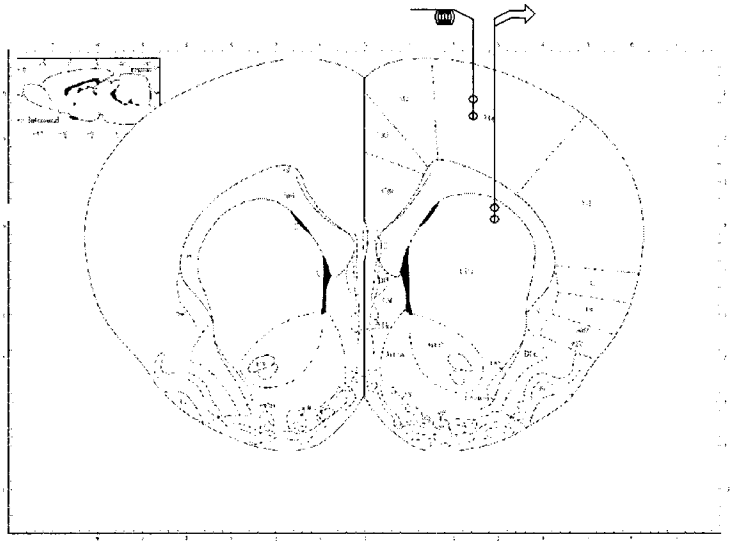


Figure 4.1: The target placements of the recording and stimulating electrodes in both the putamen and the primary motor cortex respectively. Diagram courtesy of Paxinos and Watson (1998).

4.2.2 Stimulation and Recording

All testing was performed in the same manner as the previous corticostriatal LTD experiment. An example input/output function is shown in Fig. 4.2.

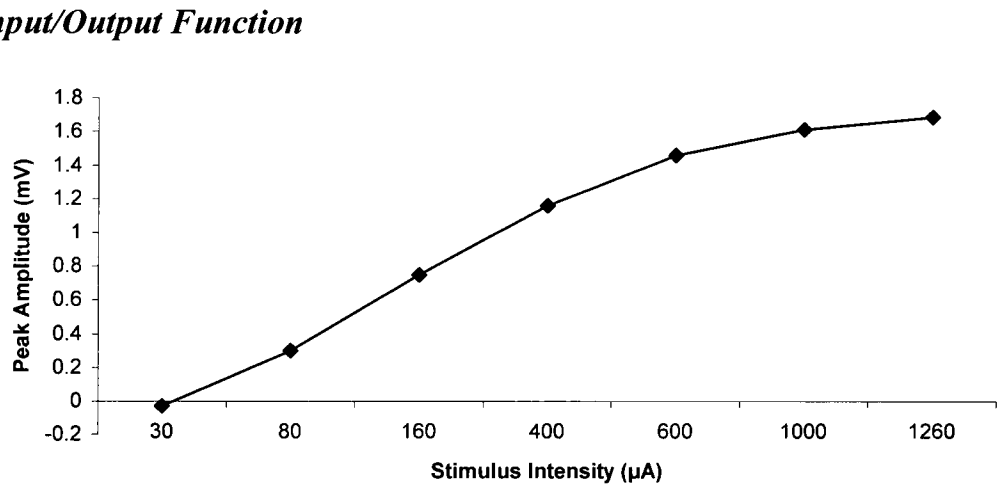


Figure 4.2: Representative input/output function. The amplitude of the field potential is plotted as a function of the intensity of the test pulse.

Three baseline I-O tests, separated by 48 hr, were taken to confirm the stability of the evoked responses. The three baseline I-O's were then averaged together in order to produce a single baseline measure.

The animals were randomly assigned to one of three groups, an experimental high frequency stimulation (HFS) group, an experimental theta burst stimulation (TBS) group and a control group. The control group received the

same test pulse stimulation as the experimental groups, but did not receive high frequency train stimulation.

4.2.3 Test for Long-term Potentiation

HFS and TBS sessions commenced one day after baseline I-O tests were taken. For the HFS group, 60 high frequency trains were delivered once per day for 12 consecutive days. The duration of each train was 70 msec and consisted of 8 pulses at 100 Hz with a 10 sec delay between each train (Fig. 4.3). The trains were delivered at a pulse intensity of 1000 μA and a pulse duration of 0.1 msec.

The TBS group received 60 trains of 4-pulse bursts at a pulse frequency of 100 Hz and a burst frequency of 5 Hz. These 60 train sets were delivered once per day for 12 consecutive days. Previous research has shown theta burst stimulation to be more effective in inducing LTP in some pathways, because it more effectively suppresses inhibition during the stimulation.

Input-output tests were taken every 3 days during train sessions. Both stimulation patterns contain high-frequency stimulation. To distinguish the groups, those receiving the battery of spaced single trains will be referred to as the HFS group, while those receiving the grouped 5 Hz burst will be referred to as the theta burst group.

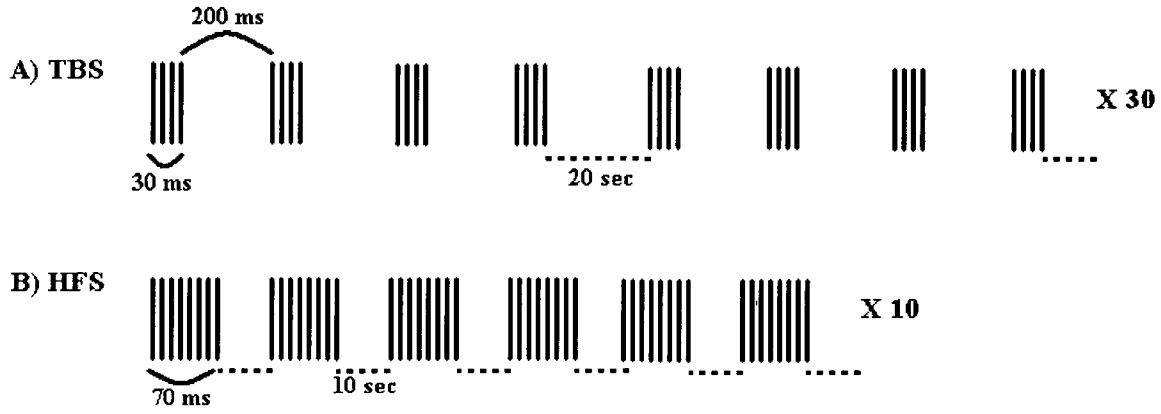


Figure 4.3: Illustrates stimulation timing patterns of a) the TBS train and b) the HFS train.

4.2.4 Data Analysis

The primary measure was the amplitude of the evoked potential. Monopolar evoked potentials were used for most of the data analysis, because responses recorded in the striatum showed little difference between the two poles of the bipolar electrode. As previously noted, the putamen lacks the clear laminar structure required to generate local polarized field responses. The amplitude of the evoked potentials for the thalamocortical pathway was tracked at two latencies. The first early component emerged at approximately 9.1 ms and the late component at 23.6 ms respectively. An example is shown in Fig. 4.4.

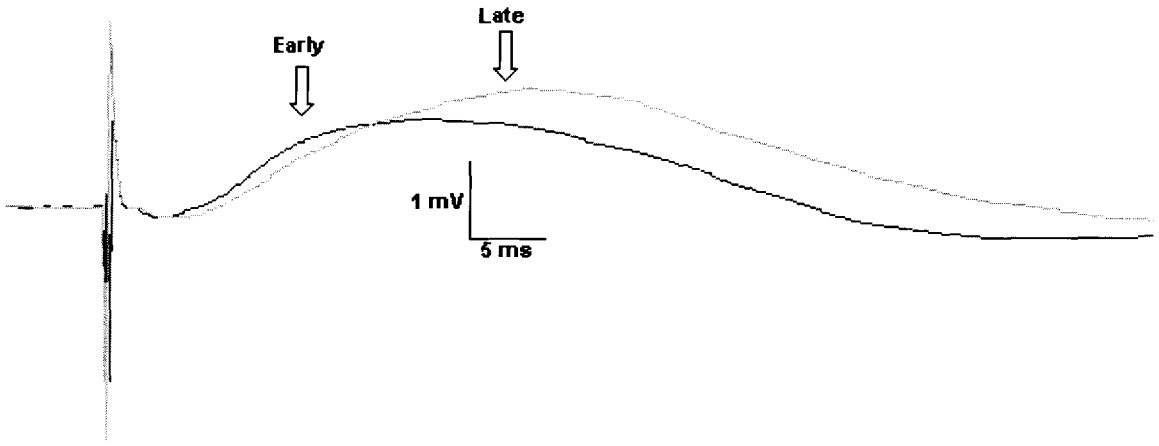


Figure 4.4: Latencies of early and late components of the corticostriatal pathway.

4.2.5 Histology

Upon completion of the experiment the animals were perfused, as in the previous corticostriatal LTD experiment. The brains were stained with 2% Ferrocyanide and Neutral Red to confirm location of the electrode tips.

4.3 Results

4.3.1 Session Effects

All evoked responses exhibited the same characteristic morphology as seen in the previous experiment (Fig. 4.3). Electrical stimulations applied to the ipsilateral primary motor cortex evoked short-latency field EPSPs lasting about 45 ms (Fig. 4.9). Increasing the intensity of the stimulation resulted in a progressive increase in the amplitude of the population EPSPs, without any clear change in onset latency (Fig. 4.9). The time-to-peak of the cortically evoked monosynaptic EPSPs, measured from the onset of the rising phase, averaged 12 ms. Peaks for

the late components were not well delineated prior to LTP induction. Consequently, the latency for taking measures of the late (and presumably polysynaptic) component was at 23 ms (based on post-stimulation measures).

Activity-dependent changes in corticostriatal synaptic efficacy were assessed by measuring the changes in the amplitude of cortically-evoked EPSPs after stimulating the primary motor cortex. An ANOVA revealed that cortical stimulation induced significant changes in the amplitude over stimulation sessions for both early (Group by Session Interaction: $F_{12, 120} = 7.197$, $p < .0001$) and late (Group by Session Interaction: $F_{12, 120} = 14.724$, $p < .0001$) components (Fig 4.5 & 4.6). As in other experiments involving pathways of cortical origin, the changes in average peak amplitude developed over days. Follow-up input/output tests revealed that the observed amplitude changes persisted for at least 6 weeks in the late component measures (Fig 4.6).

4.3.2 Corticostriatal input/output functions

The pre-/post-stimulation comparisons for the input/output data also showed significant interaction effects between the pre-post measures and the stimulation pattern used (high frequency stimulation versus theta burst stimulation). These comparisons were significant for both early (Pre-Post/HFS-Theta: $F_{12, 120} = 4.022$, $p <.0001$) and late ($F_{12, 120} = 5.124$, $p <.0001$) components. These results indicate that the theta pattern was more effective than the high-frequency trains (Figures 4.5 to 4.8). The theta burst stimulation produced an

amplitude shift in the early component (measured at 630 μA) that was about 37.6% larger than that induced by the high-frequency stimulation. Theta burst stimulation also produced a larger amplitude change than the high-frequency stimulation (by about 18%) in the late component.

The downward shift in amplitude of the early component is similar to the downward shift seen following LTP induction in corticocortical responses (Fig 4.5). In the cortex, these effects appear to be due to an increase in cell discharge in the target cell population, which sets up field potential shifts in opposition to those of the field EPSP. However, in the cortex, there is a clear correlate of the population discharge in the field response. In the putamen responses recorded here, there were no such correlates. Consequently, the downward shift in the amplitude of the early component could represent either an LTP or LTD effect. Further physiological works needs to be done at the cellular level in order to answer this question.

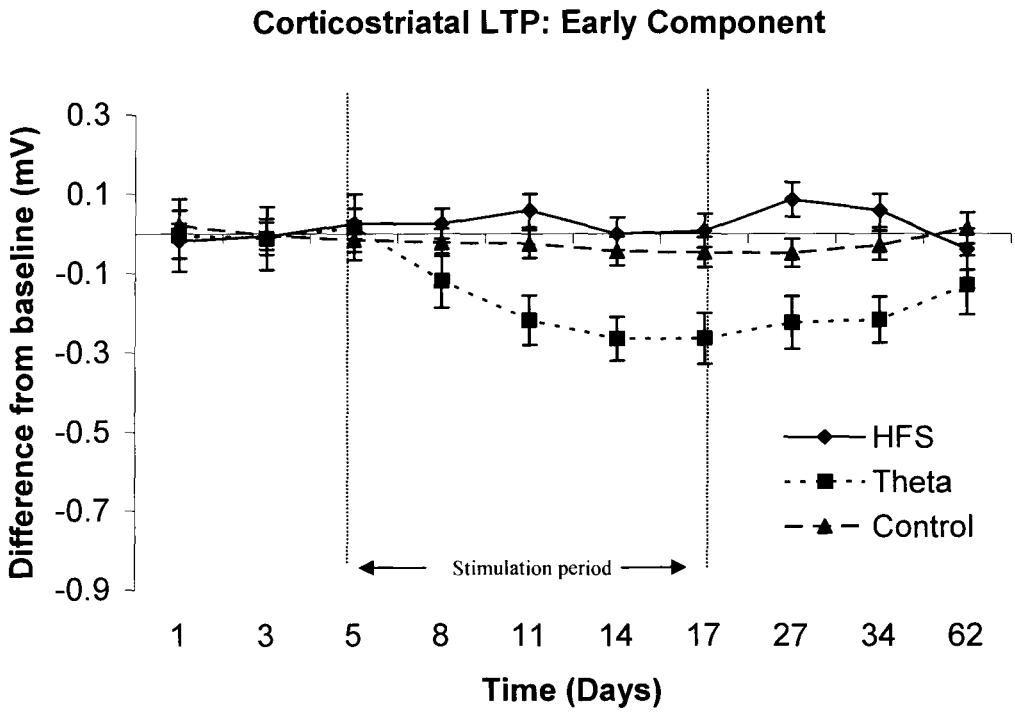


Figure 4.5: Average peak amplitude change from baseline of the early component of both experimental and control groups.

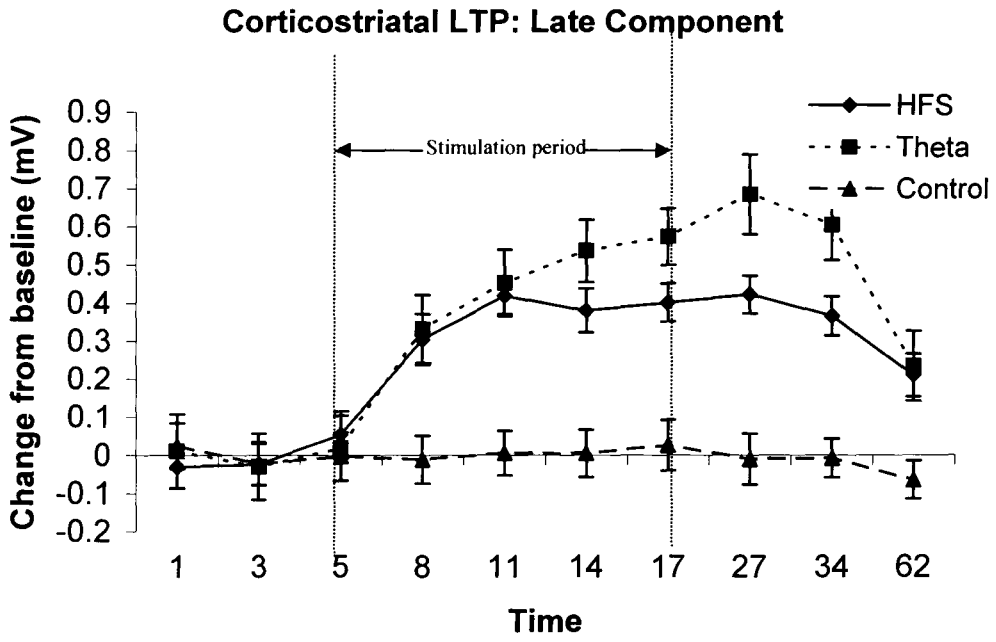


Figure 4.6: Average peak amplitude change from baseline of the late component of both experimental and control groups.

Corticostriatal Pathway Input/Output Functions: Early Component

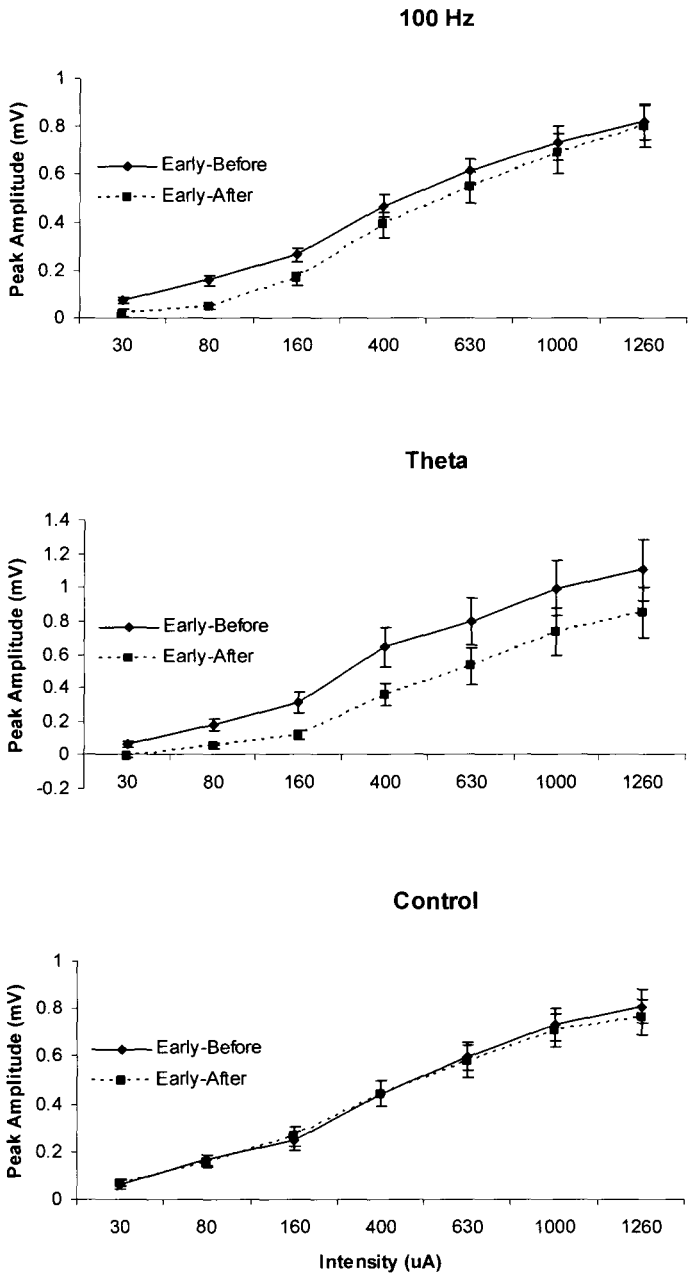


Figure 4.7: Input/output functions for the early component of the corticostriatal response. Solid lines represent baseline measures, while the dashed lines show responses 24 hours after last day of stimulation. Results are shown for both 100 Hz and theta burst groups as well as control groups.

Corticostriatal Pathway Input/Output Functions: Late Component

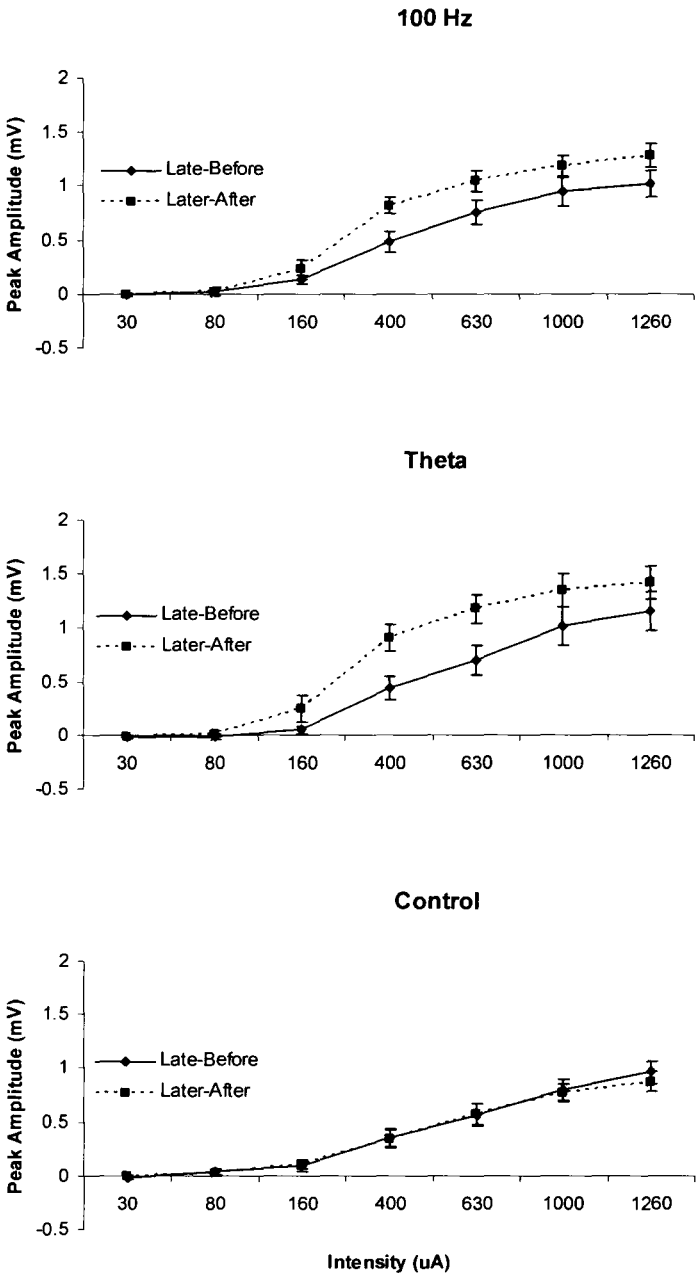


Figure 4.8: Input/output functions for the late component of the corticostriatal response. Solid lines represent baseline measures, while the dashed lines show responses 24 hours after last day of stimulation. Results are shown for both 100 Hz and theta burst groups as well as control groups.

Representative Sweeps

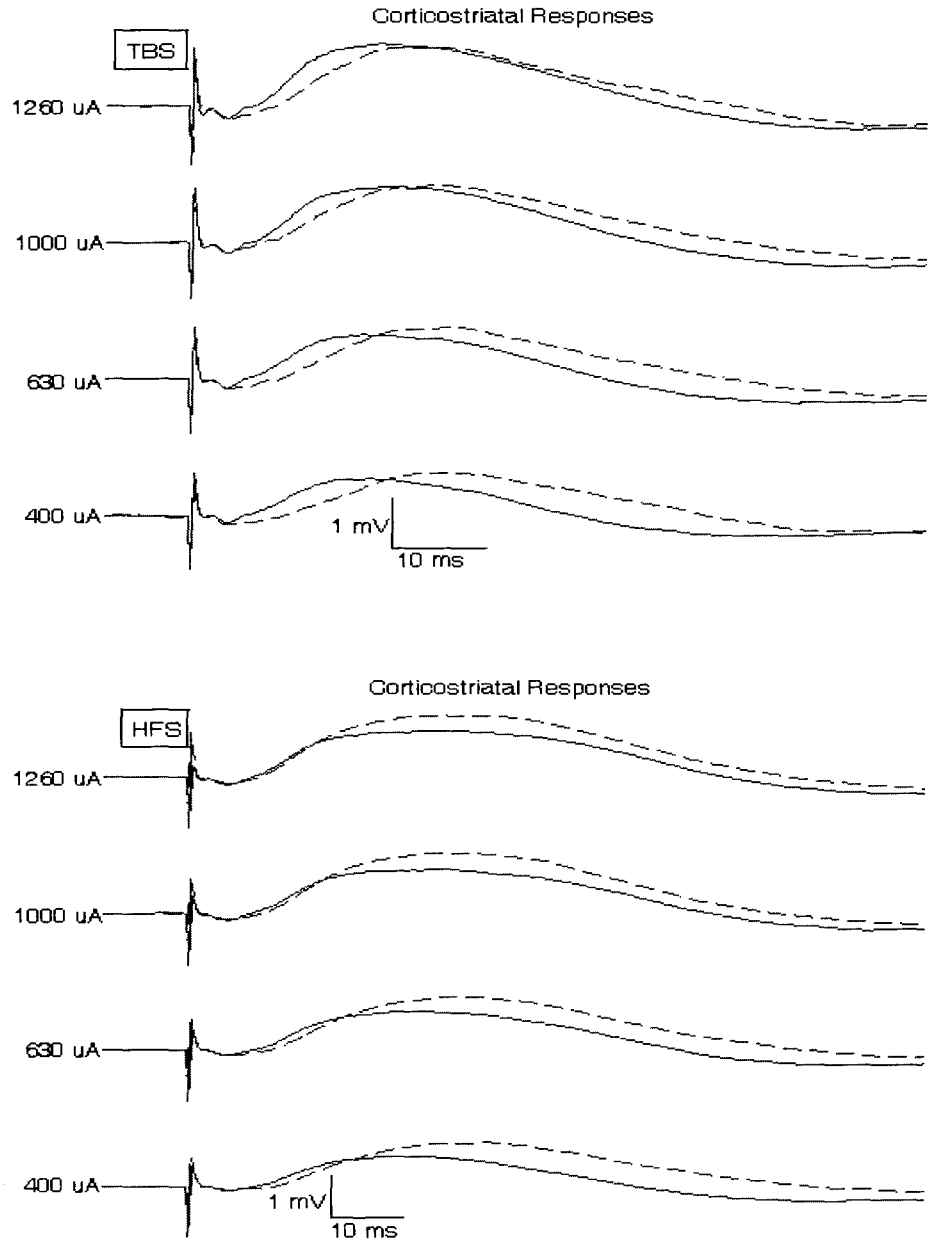


Figure 4.9: Field Potentials evoked in putamen by cortical stimulation. The solid lines represent the baseline responses, while the dashed lines show the responses 24 hr after the last stimulation train session.

4.4 Discussion

Although it is well established that striatal LTP can be induced by cortical stimulation using in vitro and anesthetized preparations, it was not clear if LTP could be induced in the intact, awake-freely moving rat. This study demonstrated that cortical stimulation (HFS or TBS) could produce a stable and long-lasting enhancement of synaptic transmission in the corticostriatal pathway in the awake-freely moving rat. The results suggest that striatal cells have a propensity for potentiation following stimulation of cortical afferents following either HFS or TBS (Fig 4.5 & 4.6).

The early component was greatly decreased for the TBS group following cortical stimulation, while little change was observed for the HFS group. It should be noted that the observed decrease might have been due to competing field currents generated by enhanced population spike activity (Froc et al., 2000). Therefore more work will be required before we can confidently interpret the change in the early component.

Despite the fact that both HFS and TBS reliably induced LTP in the rat striatum, TBS appears to be a more effective protocol for inducing robust and persistent LTP in vivo. TBS more closely mimics the firing pattern found in the deep-layer cortical pyramidal neurons that innervate the striatum. As a result, TBS may more effectively initiate down-stream molecular events necessary for LTP when compared to events caused by HFS.

Charpier and colleague's (1999) in vivo study revealed a synchronous firing pattern between cortical neurons and the medium spiny neurons of the striatum. Membrane potentials oscillated at a frequency of 5 Hz, suggesting that an activation pattern in the theta range may be optimal for information flow between cortical pyramidal and striatal spiny neurons (Stern et al., 1998).

The in vivo striatal LTP described is this report shares common properties with the excitatory LTP described in other brain regions (Bliss & Lomo, 1973; Bliss & Gardner-Medwin, 1973). The stable potentiation we observed in this experiment lasted for more than 8 weeks, which is a characteristic feature of LTP in the awake freely behaving animal (Eckert & Racine, 2004; Froc & Racine, 2005; Hodgson et al., 2005).

Chapter 5

Corticostriatal LTP:
Paired Stimulation of Motor Cortex and Substantia Nigra

5.1 Introduction

The previous experiment provided convincing evidence that LTP can be induced in the corticostriatal pathway using both TBS and HFS, with TBS proving to be the more effective method for LTP induction. The current experiment is designed to investigate dopaminergic modulation of striatal LTP in the awake-behaving rats.

Glutamate is the primary neurotransmitter in the corticostriatal pathway, and the release of glutamate may be modulated by dopamine receptors located on corticostriatal terminals (Henderson et al., 2005). Over the last several years, research reports have indicated an interaction between nigrostriatal dopaminergic inputs and corticostriatal glutamatergic afferents. Dopamine, released from the nigrostriatal terminals is thought to facilitate transmission along the direct pathway, and to reduce transmission along the indirect pathway (Wichmann & DeLong, 1993). The dual actions of dopamine likely leads to increased disinhibition of thalamocortical activity, which may facilitate an increase in cortical activity.

No studies to date have investigated the effects of direct cortical stimulation paired with direct substantia nigra pars compacta stimulation in the freely behaving animal. The present study uses TBS applied to both regions to investigate, in vivo, the electrophysiological effect of a dopamine-glutamate interaction within the striatum.

5.2 Materials and Methods

5.2.1 Animals

A total of 22 male Long-Evans rats were used in this experiment. They weighed between 350 g and 500 g. Animals were prepared using the same procedures as those in the previous corticostriatal LTP experiment, except for the addition of a second stimulating electrode placement. The usual bipolar stimulating electrodes were placed in the primary motor cortex, and an additional stimulating electrode was placed in the ipsilateral substantia nigra pars compacta (coordinates: 5.3 mm posterior to Bregma, -2.2 mm lateral to the midline and approximately 6.6 mm ventral from the skull surface – see Fig 5.1).

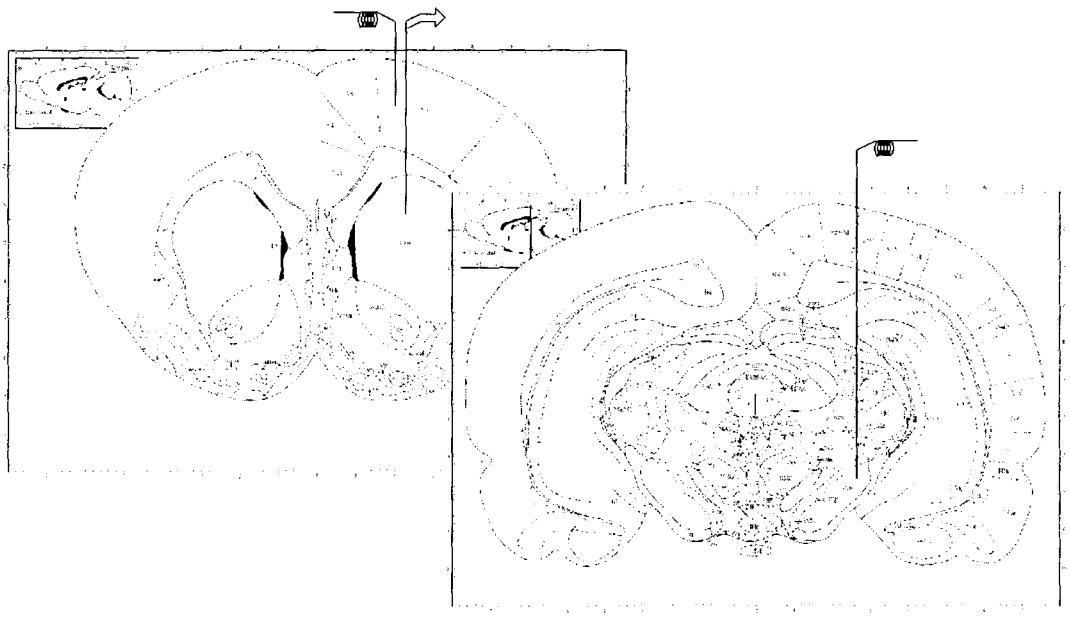


Figure 5.1: The target sites for placement of the recording electrode in the putamen and the stimulating electrodes in the primary motor cortex and SNc. Diagram courtesy of Paxinos and Watson (1998).

5.2.2 Stimulation and Recording

With two exceptions, testing was done in the same manner as the previous corticostriatal LTP experiment. In the previous experiment both 100 Hz high-frequency stimulation and theta burst stimulation were used to induce LTP. In this experiment, only theta burst stimulation was used to induce LTP, and substantia nigra stimulation was added in the combined stimulation group. The I-O tests included ten responses evoked at each of seven intensities (30, 80, 160, 400, 630, 1000, 1260 μA). An example input/output function is shown in Fig. 5.2.

Input/Output Function

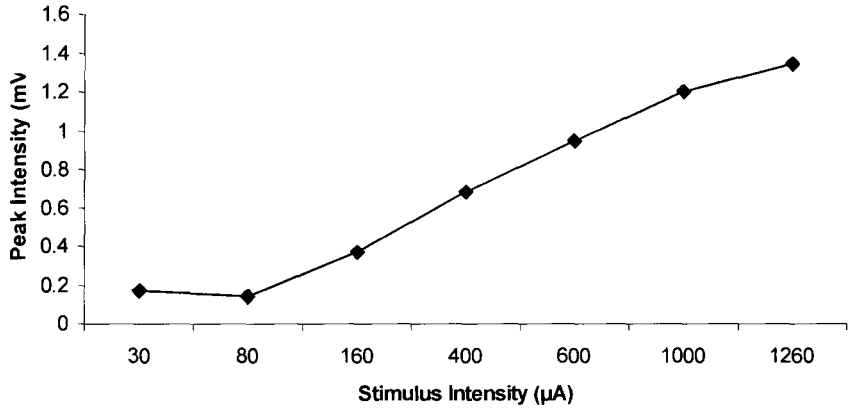


Figure 5.2: Representative input/output function. The amplitude of the field potential is plotted as a function of the intensity of the test pulse.

Three baseline I-O tests, separated by 48 hr, were taken to confirm the stability of the evoked responses. The three baseline I-O's were then averaged together in order to produce a single baseline measure.

The animals were randomly assigned to one of three groups: 1) a cortical stimulation group; 2) a paired cortical and SNc stimulation group; and 3) a control group receiving only test pulses.

5.2.3 Test for Long-term Potentiation

TBS sessions commenced one day after baseline I-O tests were taken. For the paired cortical/SNc group, trains were delivered simultaneously to the primary motor cortex and the SNc. Pulse intensity was set at 500 μA and 160 μA, respectively, and the pulse duration was 0.1 msec. To prevent ceiling effects from the paired stimulations, the stimulus intensities were lowered from those used in

the previous experiments. For the corticostriatal pathway, the stimulus intensity was reduced from 1000 μA to 500 μA. To reduce the amount of current spread to the internal capsule, a structure adjacent to the substantia nigra and known to cause strong stimulus dependent myoclonic jerks, a low stimulus intensity of 160 μA was delivered to the nigrostriatal pathway. The cortical stimulation group also received stimulation at a pulse intensity of 500 μA.

The TBS train consisted of 2 4-pulse bursts separated by 200 ms. The pulse frequency within each burst was 100 Hz. One set of 60 trains was delivered each day for 12 consecutive days. Field potentials were recorded in the striatum every 3 days during the induction phase.

5.2.4 Data Analysis

The procedures for data analysis were the same as in the previous corticostriatal LTP experiment (chapter 4). An example of a typical response morphology, and the latencies used to measure early (9.1 ms) and late (23.6 ms), components is shown in Fig. 5.3.

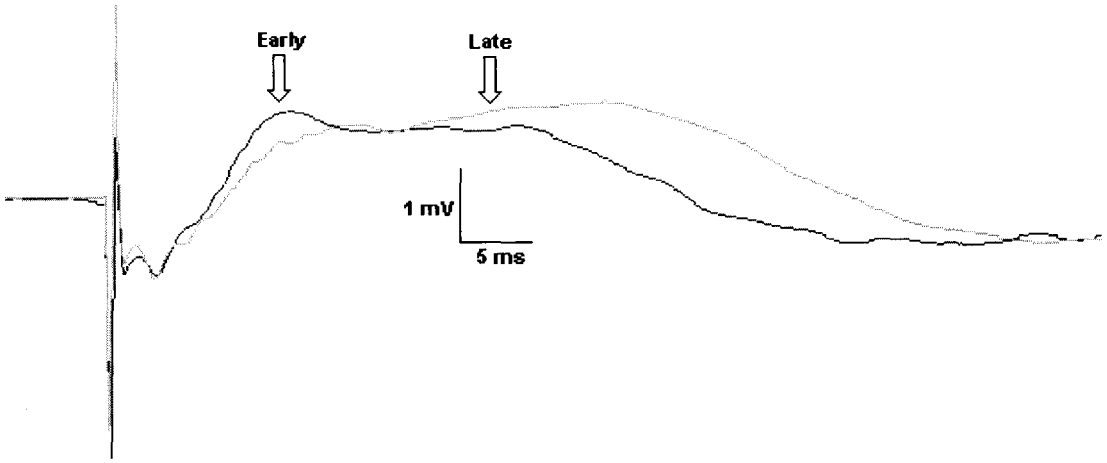


Figure 5.3: Latencies of early and late components of the corticostriatal pathway.

5.2.5 Histology

Following the recording of the final input/output series, an anodal DC current was passed through the tip of each electrode for approximately 20 s using a 10 μA current. This caused an accumulation of Fe^{2+} atoms to be deposited around the stainless steel electrode tip allowing for later visualization using a Neutral Red stain.

After marking the electrode placements using an anodal DC current, the animals were sacrificed and perfused through the heart with phosphate-buffered saline (PBS, pH 7.4), and immediately followed by cold 10% formalin in PBS. Once the brains were removed, they were post-fixed over-night and cryoprotected for several more days using 30% sucrose in PBS. They were sectioned on a cryostat at 50 μm, mounted on to gelatin-subbed slides and allowed to air-dry overnight.

The tissue was gradually hydrated through a series of graded alcohols and then stained with 2% Ferrocyanide and Neutral Red, a cell body stain. Following staining, the tissue was dehydrated, cleared with Xylene, and coverslipped. Representative photomicrographs of electrode placements are shown in Figure 5.4.

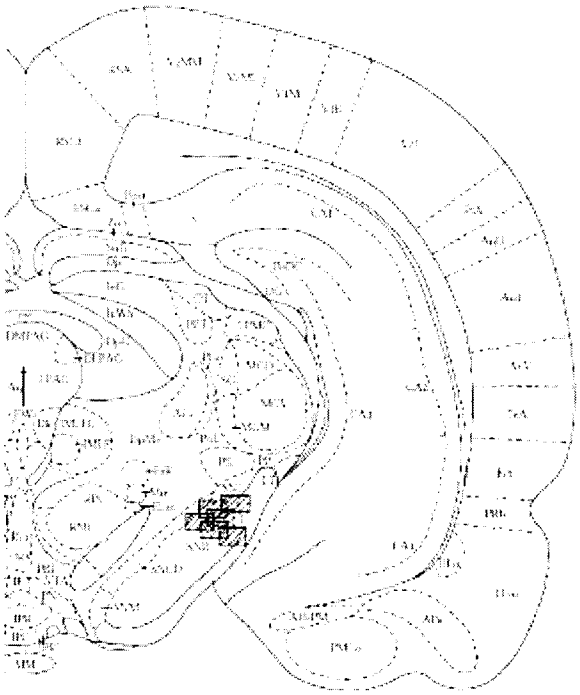


Figure 5.4: Representative diagram showing placement of electrodes. The crosshatched areas show the regions within which electrode tips were located. The recording electrode is in the substantia nigra.

5.3 Results

5.3.1 Session Effects

Extracellular recordings were obtained from the prepared animals. Figure 5.3 shows the typical morphology that we have seen in corticostriatal responses. Activity-dependent changes in corticostriatal synaptic efficacy were assessed by measuring the changes in the amplitude of cortically-evoked population EPSPs after stimulation of the cortex alone or after pairing stimulation of the primary motor cortex and the substantia nigra pars compacta. EPSPs were averaged to reduce the effects of natural amplitude fluctuations.

An ANOVA revealed a nearly significant interaction effect between group and session for the early component ($F_{12, 114} = 1.079$, $p = .074$) and a significant effect for the late component ($F_{12, 114} = 8.776$, $p < .0001$). For the early component, the amplitude changes were greater in the group receiving for paired stimulation of the motor cortex and the substantia nigra pars compacta compared to cortical stimulation alone (Fig 5.5 & 5.6). Although there was a significant interaction effect in the late component, due to the LTP induction, there was very little difference between the two LTP-induction protocols. Follow-up decay tests showed that amplitude changes persisted for both stimulation conditions for at least 4 weeks after the last train session (Fig 5.5 & 5.6).

5.3.2 Corticostriatal input/output functions

These effects can also be seen in the input/output functions (Fig 5.7 & 5.8). When the amplitude changes for the combined stimulation group were compared to those in group receiving only cortical stimulation, the changes were found to be significantly greater in the combined stimulation group for the early component (Ctx+SNc vs. Ctx Group by Session Interaction Effect: $F_{12, 132}$ = 2.217, p = .014). Paired stimulation resulted in a 33% greater reduction of the average peak amplitude of the early component versus the change observed in the situation where only the cortex is stimulated (Fig 5.7). The differences between the two stimulation regimens did not reach significance for the late component ($F_{12, 132}$ = 1.365, p = .190), but the trend was in the same direction.

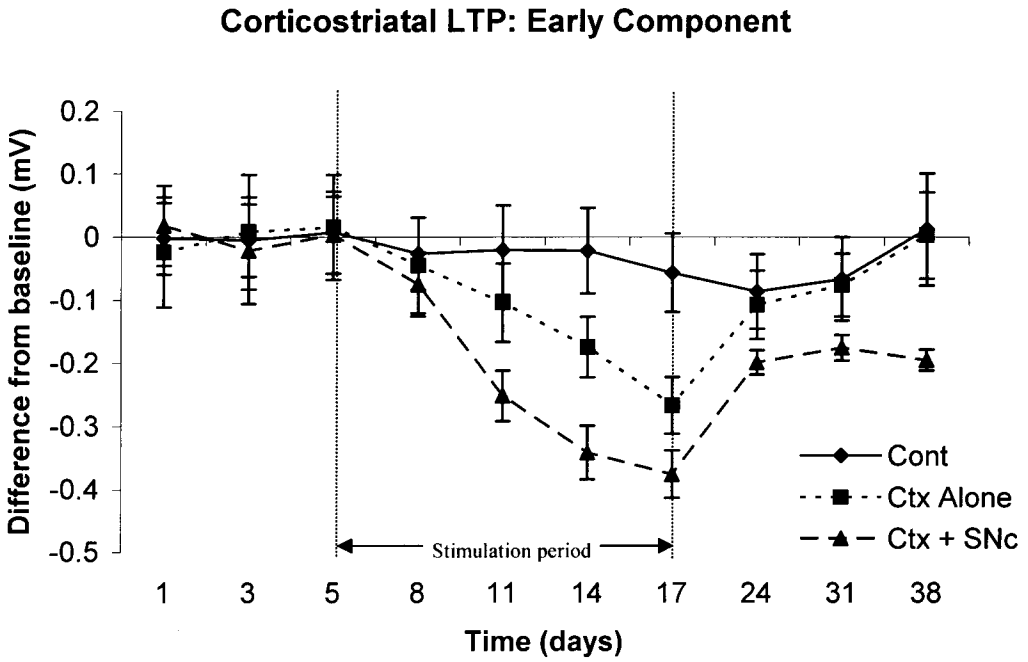


Figure 5.5: Average peak amplitude change from baseline. The early component effects are shown for the control and both experimental groups.

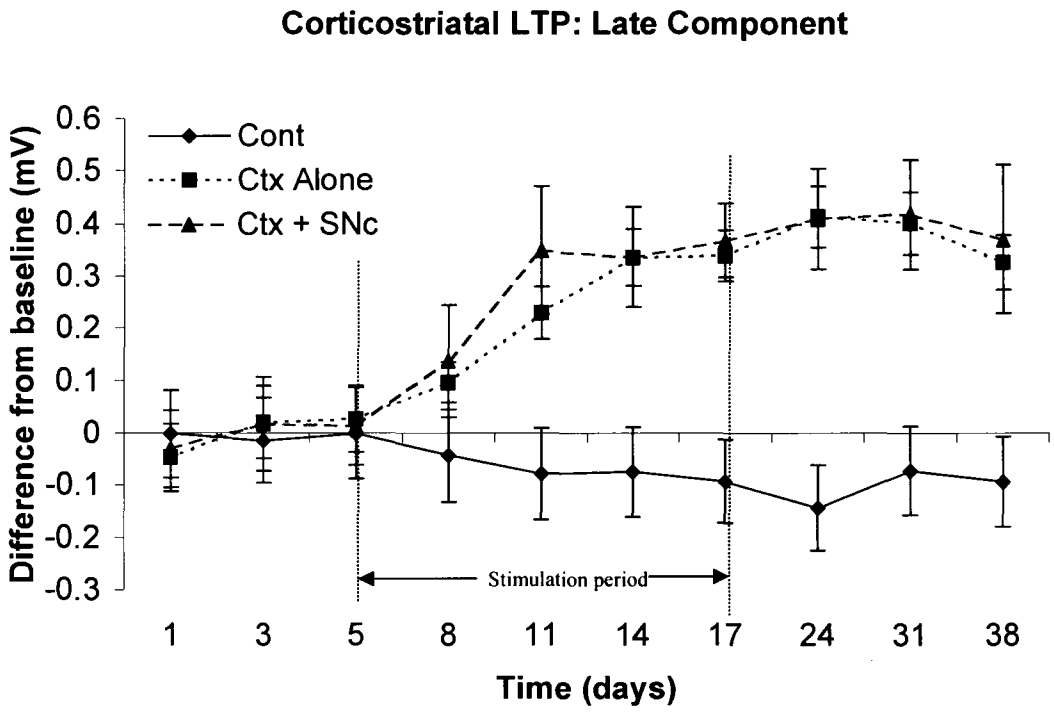


Figure 5.6: Average peak amplitude change from baseline. The late component effects are shown for the control and both experimental groups.

Corticostriatal Pathway Input/Output Functions:
Early Component

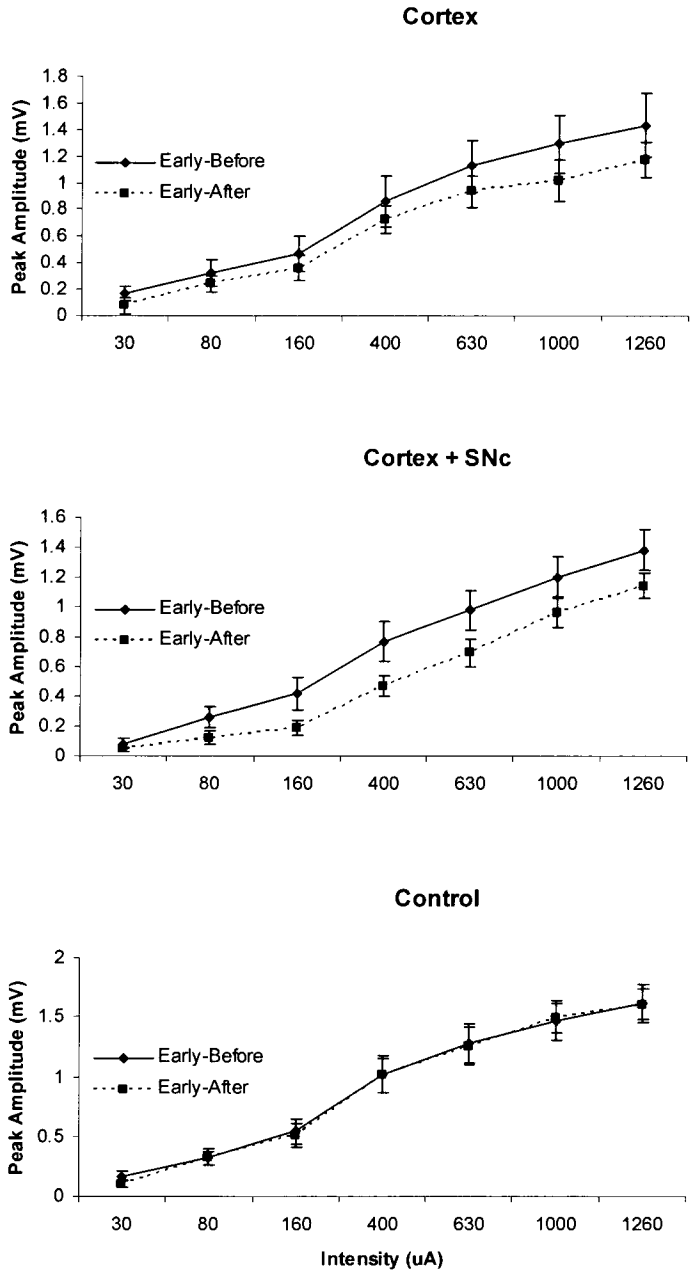


Figure 5.7: Early component input/output functions from the corticostriatal pathway. I/O functions are shown both before onset of stimulation and 24 hours after last day of tetanization for the control and both stimulation groups. Solid lines are baseline measures, dashed lines represent post-stimulation (for experimental groups) measures.

Corticostriatal Pathway Input/Output Functions: Late Component

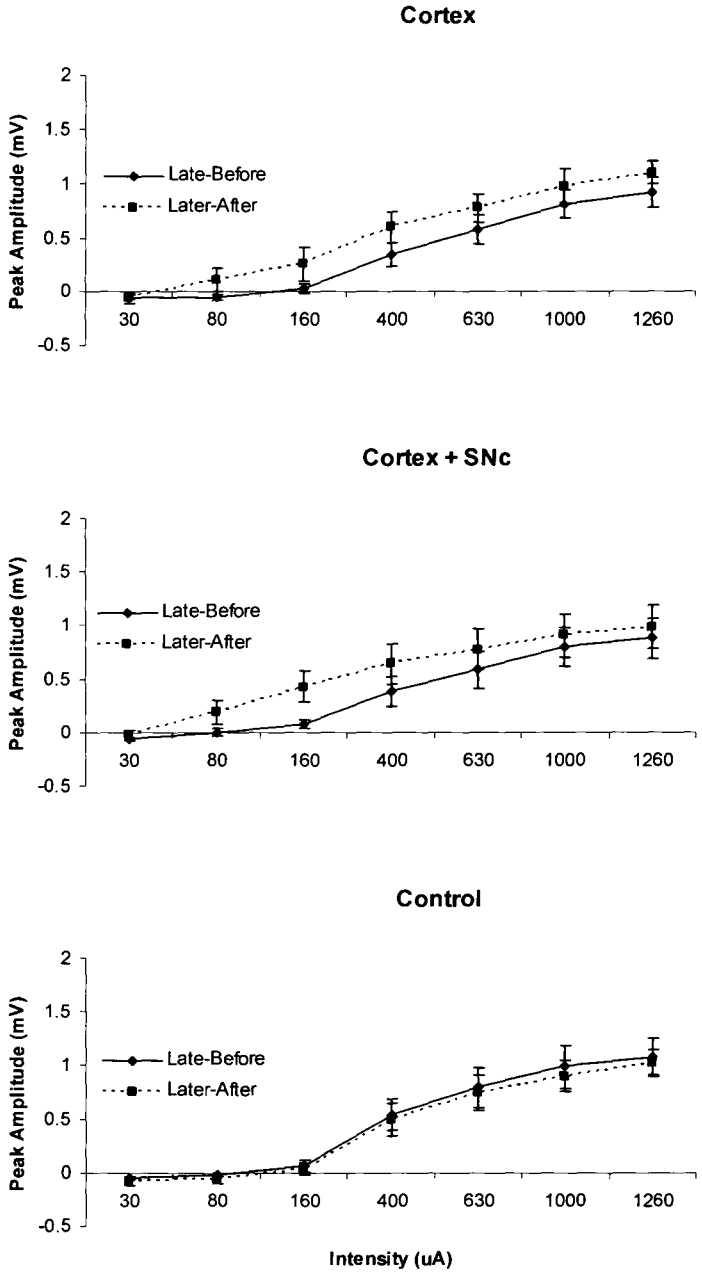


Figure 5.8: Late component input/output functions from the corticostriatal pathway. I/O functions are shown both before onset of stimulation and 24 hours after last day of tetanization for the control and both stimulation groups. Solid lines are baseline measures, dashed lines represent post-stimulation (for experimental groups) measures.

Representative Sweeps

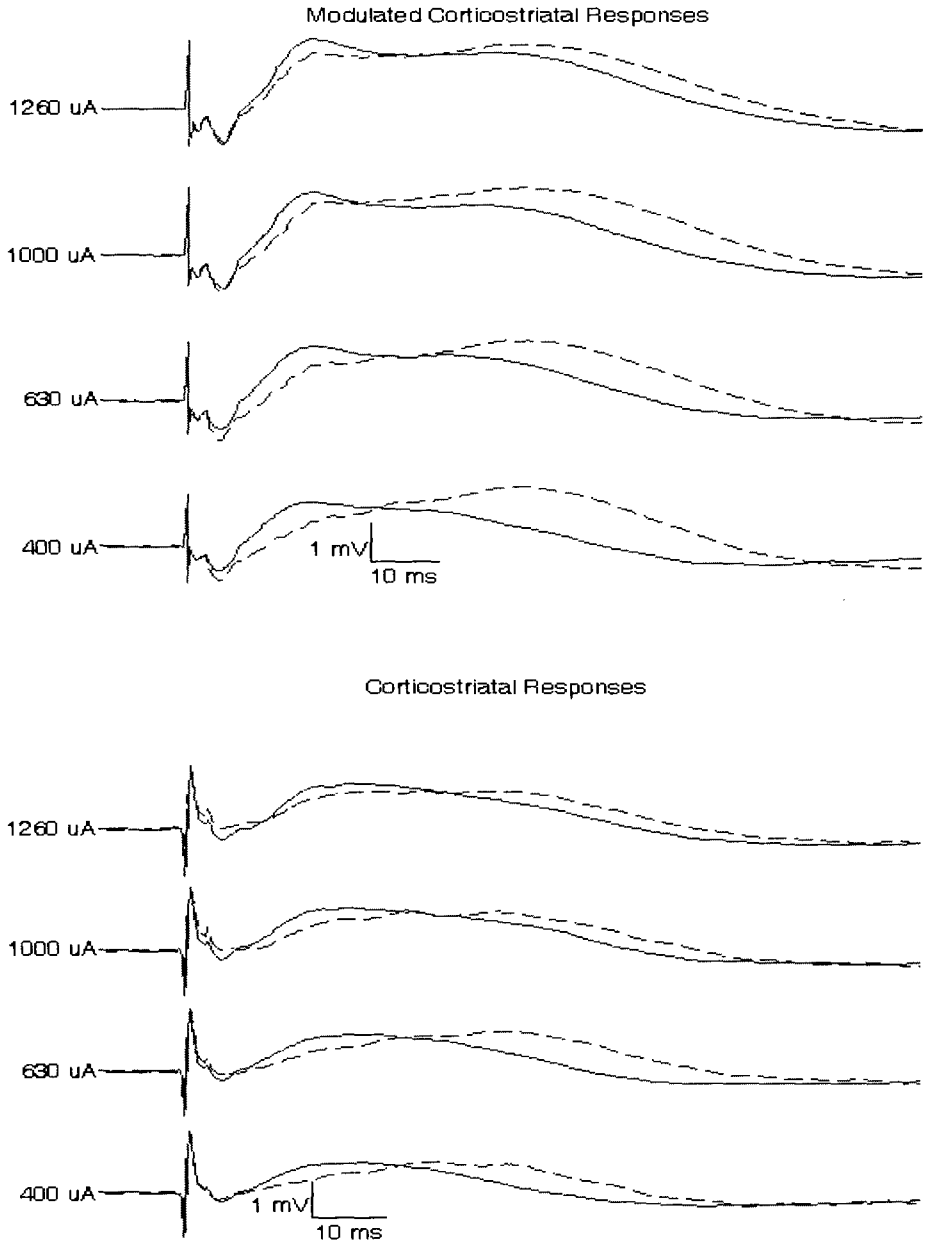


Figure 5.9: Field Potentials evoked by paired cortical and SNc stimulation (upper panel) and cortical stimulation (lower panel). Solid lines represent baseline responses and dashed lines are 24 hours after the last stimulation train session.

5.4 Discussion

The purpose of this experiment was to investigate the contribution of the nigral dopamine on LTP induction in the corticostriatal pathway. Recordings in the striatum following the paired stimulation period clearly demonstrate the modulatory effect of dopamine on the induction of LTP/LTD in the striatum of the freely behaving animal.

The magnitude and duration of the changes observed in early and late components of the evoked potential were greater following paired stimulation of the corticostriatal and nigrostriatal pathways compared to stimulation of the corticostriatal pathway alone, although the interaction effect was not quite significant for the early component (Figs 5.5 and 5.6). Thus, stimulation protocols pairing corticostriatal/nigrostriatal pathway stimulation appear to be more effective, at least for the early component, than stimulation protocols that only stimulate the corticostriatal pathway. The lack of effect in the late component when comparing stimulation conditions might reflect a ceiling effect as the late component effects ere relatively large, even in the unpaired group.

Although the early component effect was not quite significant in the group by sessions interaction effect, the stimulation-induced amplitude change was significantly different between the paired and unpaired groups over intensities for the input/output measurements (group by intensities interaction effect). The stimulation-induced change in the early component has consistently been a decrease in response amplitude.

As pointed out earlier, this could reflect either a synaptic depression or a synaptic enhancement. The lack of high frequency components in the early component strengthened the argument for depression. However, in this experiment, we did see high-frequency components riding on the population EPSP (the early component) (Figure 5.3). These components may reflect enhanced cell discharge (as they do for corticocortical responses). So, there is still a possibility that the early component effect represents an enhancement (LTP) effect, but physiological tests at the cellular level are required to settle this issue.

It should also be pointed out that even 3 weeks after the stimulation period ended, the modulated LTP response did not return to baseline, whereas the unmodulated response did eventually return to baseline. The modulated response showed a small reduction in the peak amplitude one week following the end of stimulation, but then remained stable for the duration of the decay testing phase.

The increase and stability in average peak amplitude for the modulated group suggests excitatory actions of dopamine on striatal neurons, as has been demonstrated at cortical sites (Gonzalez & Hablitz, 2003). In order to be certain that these effects are due to stimulation-triggered DA release in the striatum, it will be necessary to run additional experiments in which the DA levels are monitored during the release period. However, the electrode tips were found to be within the SNc, the stimulation intensity was relatively low, and the effects on LTP were in part consistent with what could be expected from previous findings.

Gonzalez, Hablitz (2003) used slice preparation to demonstrate effects of dopamine on evoked activity in the prefrontal cortex. Whole cell recordings from pyramidal cells exposed to bath-applied dopamine showed an increase in postsynaptic currents, although they noted that the effects they reported may be less pronounced under normal conditions.

Researchers have suggested that dopamine receptors modulate multiple voltage-dependent currents, including high-voltage gated Ca^{2+} currents in medium spiny neurons that project from the striatum. It seems likely that dopamine acts postsynaptically on D1-like receptors (Wang & O'Donnell, 2001). Paired-pulse test provides further evidence for this point. Paired-pulse stimulation has been used to evaluate possible presynaptic changes in synaptic transmission. Gonzalez and Hablitz (2003) reported that neither pair-pulse ratios nor excitatory post-synaptic current rise times were affected by dopamine. If dopamine had an affect on presynaptic mechanisms, excitatory post-synaptic current rise times and or pair pulse ratios have been altered.

The precise mechanism by which dopamine regulates striatal plasticity is not completely understood, but research findings indicate that induction of plasticity in the striatum requires an interaction between the actions of dopamine and other neurotransmitters, with glutamate being the primary excitatory neurotransmitter. Of course, many questions about dopamine-mediated control of corticostriatal plasticity remain. One question that will be addressed in the next

chapter is the role of timing in the interactions between dopaminergic and glutamatergic input.

Chapter 6

Paired Corticostriatal and Substantia Nigra Stimulation: Order and Delay Effects

6.1 Introduction

As demonstrated in the previous experiment (chapter 5), and in vitro electrophysiological recording experiments, corticostriatal excitatory synaptic transmission can be significantly modulated by dopamine (Brown et al., 1999; Centonze et al., 2001; Centonze et al., 1999). These and many other researchers have proposed that dopamine acts as a neurobiological substrate of reward, facilitating trial-and-error-based improvements in performance. A basic rule of positive reinforcement is that motor responses will increase in magnitude and vigor if followed by a rewarding event.

The interaction of dopamine and glutamate in the striatum is considered vital for the formation of motor memories in movement control. Animal research suggests a close temporal relationship between dopamine receptor and glutamate receptor activation in the striatum, as it relates to the formation of motor memory (Brown et al., 1999; Montague et al., 1996; Schultz, 1998). Depending on the type of interaction that occurs between glutamate and dopamine, striatal synapses can experience either a long-last increase (LTP) or an enduring decrease (LTD) in

synaptic strength. Thus, the exact mechanism by which dopamine reinforces behaviour remains unclear.

The ability for humans to learn and improve new motor skills can be attributed to reinforcement learning. Reinforcement learning considers the animal's responsiveness or motivational drive state associated with engaging in self-generated naturally rewarding behaviours (van de Laar et al., 2004). In reinforcement learning, systems learn through interaction with the environment in an attempt to optimize performance.

Reward signals through the dopaminergic nigrostriatal pathway affect cortical states and subsequent behaviour by increasing or decreasing reward signals.

Biologically, systems may experience a delay between motor activity and reward signals (Aosaki et al., 2004; Brown et al., 1999). This delay affects striatal plasticity (in the form of LTP/LTD), which adjusts the response of the basal ganglia to cortical input. When the reward signal falls within a critical temporal window with respect to the motor signals, the motor signals are reinforced and nonrewarding actions are suppressed (Schultz, 1998).

Previous models of reinforcement learning in the striatum, specifically temporal models (Houk, 1995) have been criticized because they fail to demonstrate dopamine signaling in the intact system where typical interactions of various pathways can occur without pharmacological manipulation (Brown et al., 1999).

The current experiment attempts to address the effect of different temporal activation patterns in the corticostriatal and nigrostriatal pathways on striatal plasticity in the awake animal. Theta burst stimulation will be used to activate the two pathways either simultaneously or with a short interval delay for one of the pathways. The temporal design of the experiment will compare the results of activating the two pathways under conditions where the "reward" stimulus" (SNc stimulation) is delivered before or after cortical stimulation.

6.2 Materials and Methods

6.2.1 Animals

A total of 23 male Long-Evans rats were used in this experiment. They weighed between 350g and 500g. Animals were prepared using the same procedures as those in the previous corticostriatal/SNc LTP experiment.

6.2.2 Stimulation and Recording

Testing was also done in the same manner as in the previous paired stimulation experiment, except for the stimulation train intensity, the number of trains applied, and the order and intervals between the stimulation trains delivered to the two pathways. The I-O tests included ten responses evoked at each of seven intensities (30, 80, 160, 400, 630, 1000, 1260 μA). An example input/output function is shown in Fig. 6.1.

Input/Output Function

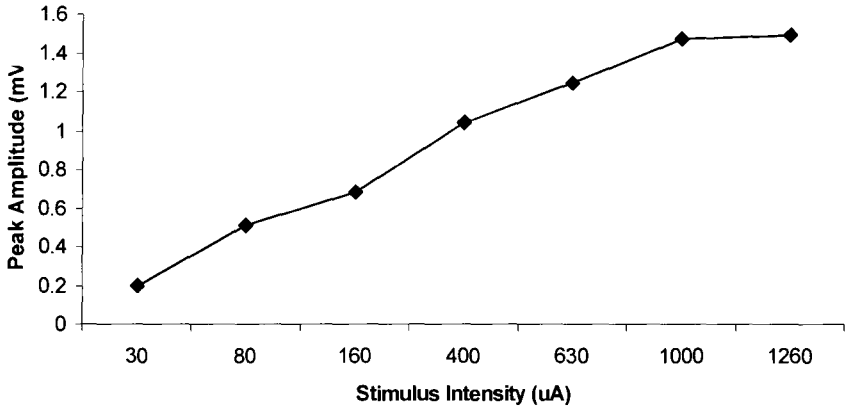


Figure 6.1: Example input/output function. The amplitude of the field potential is plotted as a function of the intensity of the test pulse.

Three baseline I-O tests, separated by 48 hr, were taken to confirm the stability of the evoked responses. The three baseline I-O's were then averaged together in order to produce a single baseline measure.

The animals were randomly assigned to one of four groups: 1) a group receiving paired stimulation of the cortex and the SNc, with the cortex stimulation preceding the stimulation of the SNc. 2) a group receiving paired Cortex/SNc stimulation with the SNc stimulation preceding the cortical stimulation. 3) a group receiving simultaneous stimulation of the cortex and SNc stimulation, and a control group receiving only test pulses. These stimulation patterns enabled us to take a first step toward observing the affects of altering order and temporal delay in the combined stimulation of the cortex and SNc (Fig 6.2).

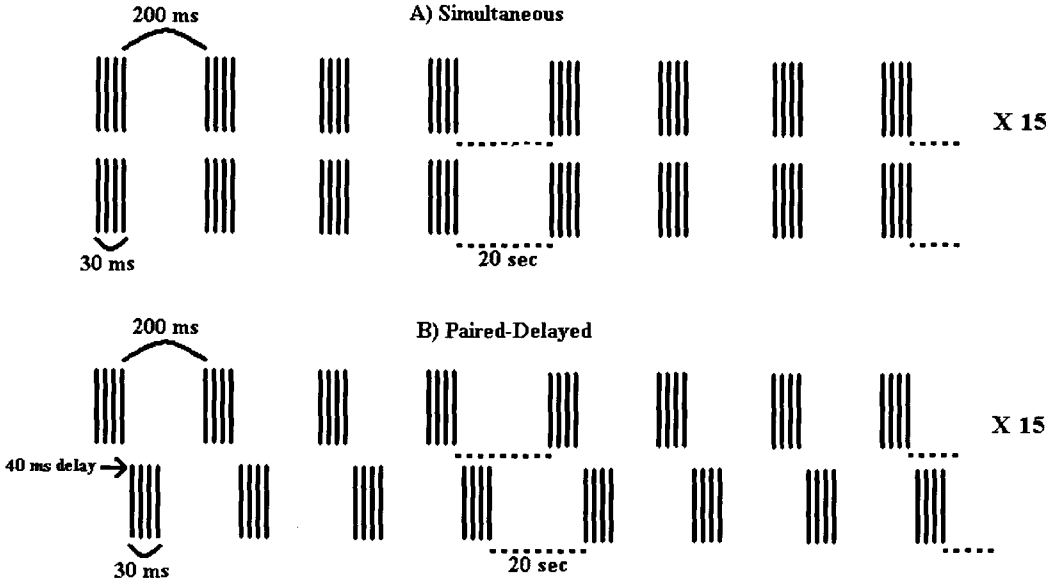


Figure 6.2: Illustrates stimulation timing patterns of a) simultaneous TBS train and b) paired-delayed (40 ms) TBS train.

6.2.3 Test for Long-term Potentiation.

TBS sessions commenced one day after baseline I-O tests were completed. TBS consisted of brief trains of repeating short-duration (30 ms) 4-pulse high-frequency bursts. The pulse frequency within the bursts was 100 Hz, and a block of 4 of these bursts made up one stimulation train. The stimulation trains were separated by 200 ms. The stimulation pulse intensity was set at 500 μA. A stimulation session involved 30 trains delivered in this 5 Hz pattern. One 30-train set was delivered each day for 12 consecutive days. Input-output tests were taken every 3 days during train sessions.

For the group receiving cortical stimulation preceding SNc stimulation, the TBS to the corticostriatal pathway was delivered 40 ms before TBS was delivered to the nigrostriatal pathway, creating a 10 ms separation between trains (or a 40 ms delay from the start of one train to the start of the next train) (Fig. 6.2). For the group receiving SNc stimulation preceding cortical stimulation, the TBS to the nigrostriatal pathway was delivered 40 ms before TBS was delivered to the corticostriatal pathway. TBS was delivered to both pathways simultaneously in the 3rd paired cortical/SNc stimulation group. The control group did not receive any high-frequency stimulation.

6.2.4 Data Analysis

Data was analyzed in the same manner as outlined in the previous corticostriatal LTP experiments (chapters 4 and 5). A sample sweep is shown in Fig. 6.3.

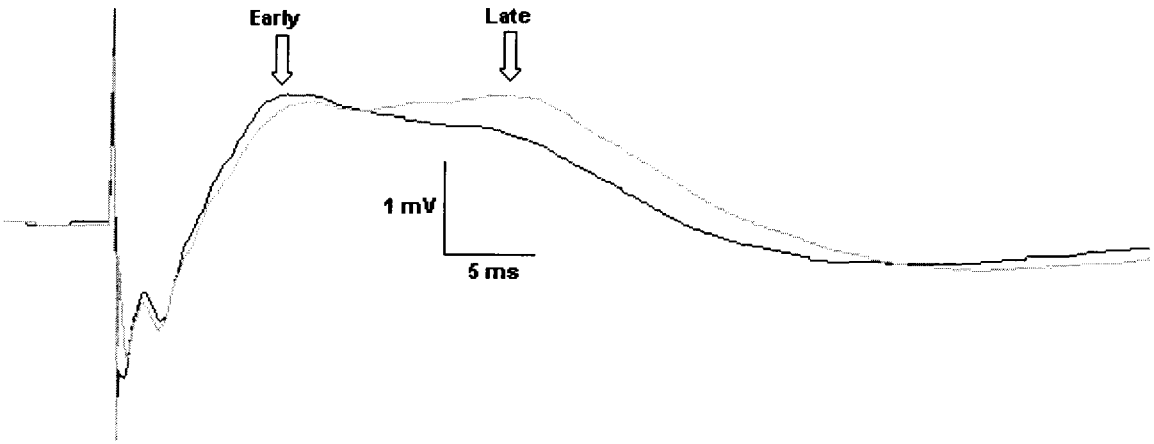


Figure 6.3: Latencies of early and late components of the corticostriatal pathway.

6.2.5 Histology

As before, the animals were perfused upon the completion of the experiment. The brains were stained with 2% Ferrocyanide and Neutral Red to confirm location of the electrode tips.

6.3 Results

6.3.1 Session Effects

The theta burst stimulation triggered the characteristic downward shift in early component field potential amplitude in all experimental conditions (Fig 6.4), and most clearly in the paired stimulation conditions. An ANOVA revealed a significant group by session interaction for the early component, (group by session: $F_{18, 138} = 1.846$, $p = .026$). When the cortical TBS was simultaneously paired with nigral TBS, the LTP expressed in the early component was changed

by a magnitude of 0.5 mV by the ninth day of stimulation. When the cortical stimulation preceded SNc stimulation, or the SNC stimulation preceded cortical stimulation, the amplitudes changed by 0.4 mV and 0.2 mV, respectively.

The late component effects were also significant (group by session: $F_{18, 138} = 7.637$, $p < .0001$). The largest effect was again seen in the simultaneous group, with the cortical_substantia nigra group (the substantia nigra stimulation following the cortical stimulation) achieving similar levels. When the substantia nigra stimulation preceded the cortical stimulation, the effect was considerably diminished.

6.3.2 Corticostriatal input/output functions

TBS produced a small depression in the early component under each experimental condition (Fig 6.6) with larger effects when the paired stimulations were simultaneously applied. Paired stimulation led to a larger change in the average peak amplitude of the early component compared to the other experimental groups. When the TBS was delivered simultaneously to the cortical and nigral pathways, the amount of change observed at the end of the stimulation session (at 630 μA) was approximately 40% (Pre/Post Ctx+SNc Session Effect: $p = .016$), compared to an amplitude reduction of 31% (Pre/Post Ctx_SNc Session Effect: $p = .026$) for the group receiving cortical stimulation first. Changes in the group receiving SNc stimulation first were even smaller with a 17% decrease (Pre/Post SNc_Ctx Session Effect: $p = .083$).

Simultaneous paired stimulation produced a larger peak amplitude change in the late component, when compared to the group receiving cortical stimulation first. This was evident at the middle intensity (630μA) where the magnitude of change for the peak amplitude of the simultaneous paired group was 58% compared to 50% for the group receiving cortical stimulation first (Ctx+SNc vs. Ctx_SNc Group by Session Interaction Effect: $p = .54$) (Fig. 6.7).

Stimulation to the substantia nigra first produced a 17% increase in the late component. A comparison of the response amplitude changes for the stimulation orders revealed a significant difference when comparing the changes observed in the group receiving nigral stimulation first to the other two groups; simultaneously stimulated cortex and substantia nigra group (SNc vs. Ctx+SNc Group by Session Interaction Effect: $p = .024$) and group receiving cortical stimulation first (SNc vs. Ctx_SNc Group by Session Interaction Effect: $p = .031$).

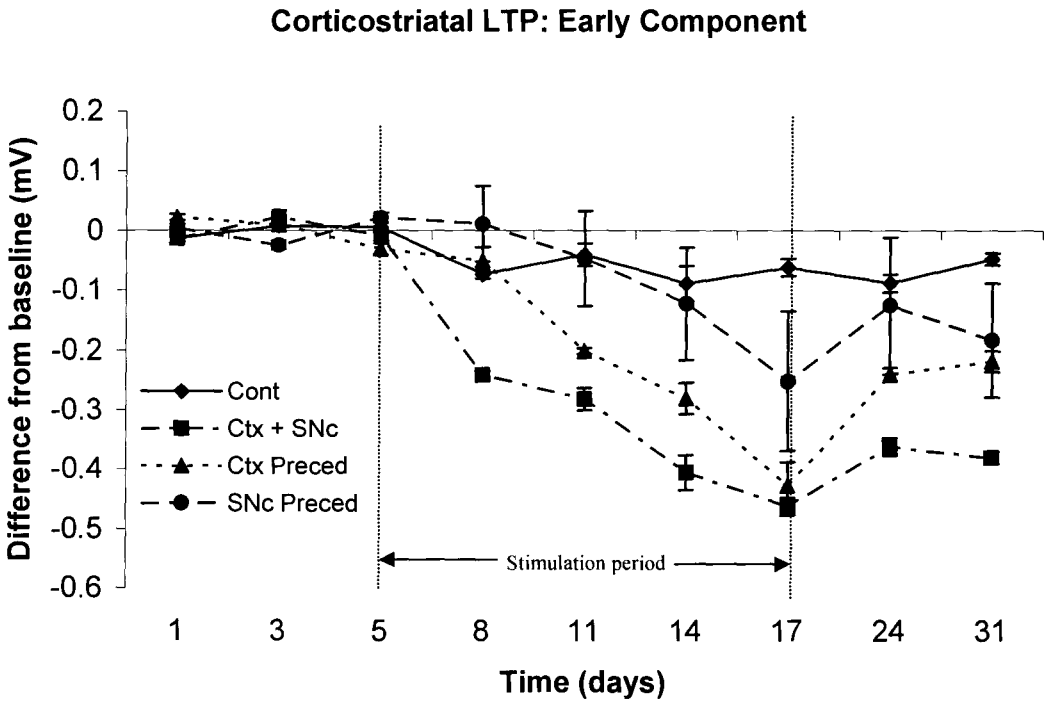


Figure 6.4: Peak amplitude change from baseline. The measures were taken from the early components of both experimental and control groups.

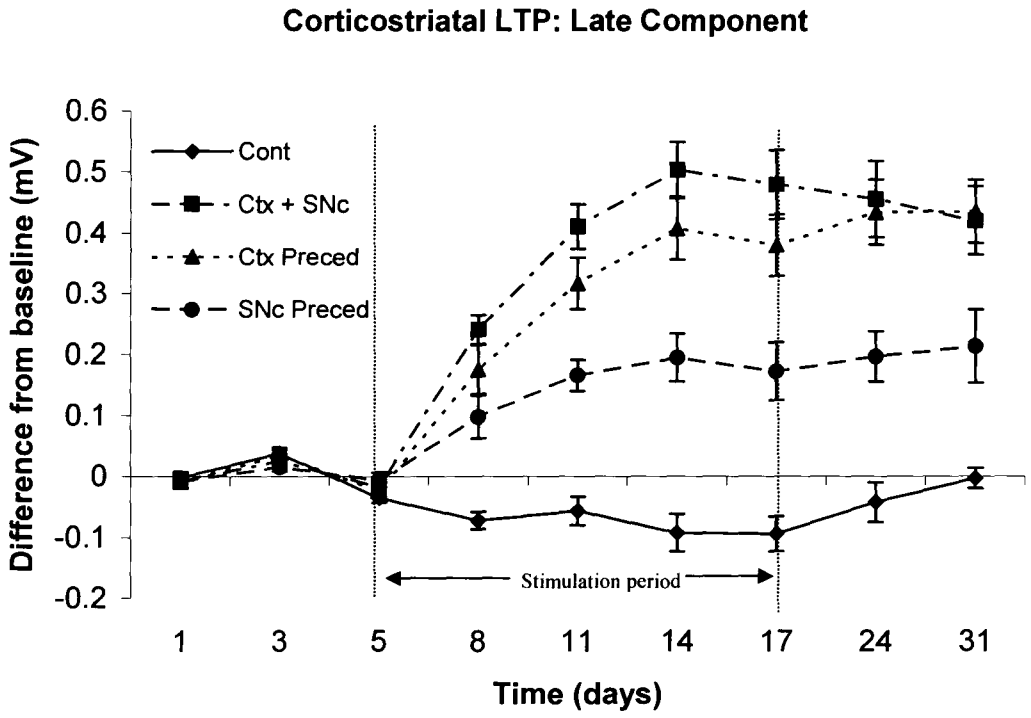


Figure 6.5: Peak amplitude changes from baseline. The late component measures are shown for both experimental and control groups.

Corticostriatal Pathway Input/Output Functions: Early Component

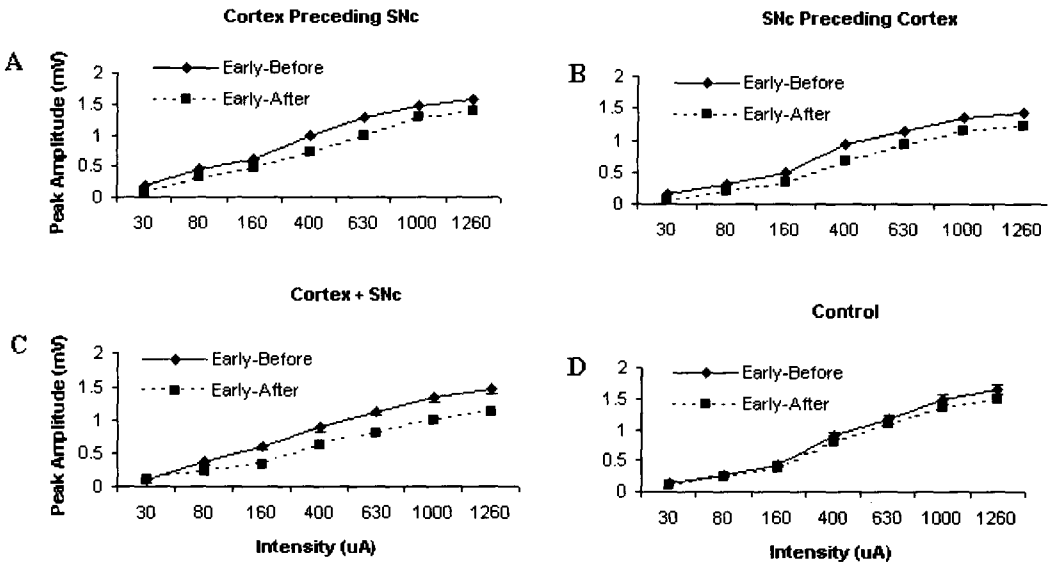


Figure 6.6: Early component input/output functions. Measures were taken from the striatal responses, evoked by cortical stimulation, before and after stimulation (figure title indicates stimulation condition). Solid lines are baseline measures, dashed lines are 24 hours after last day of high-frequency stimulation.

Corticostriatal Pathway Input/Output Functions: Late Component

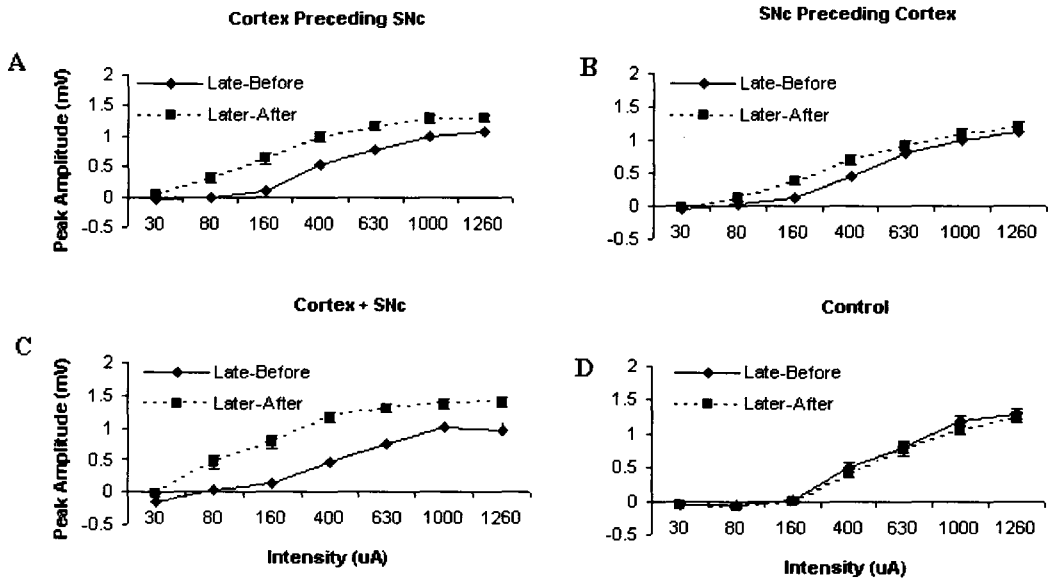
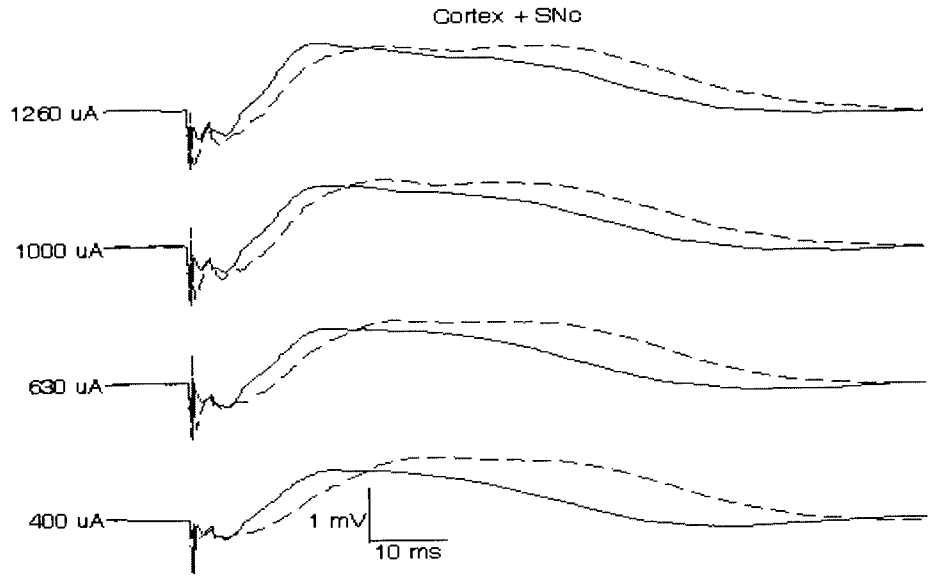
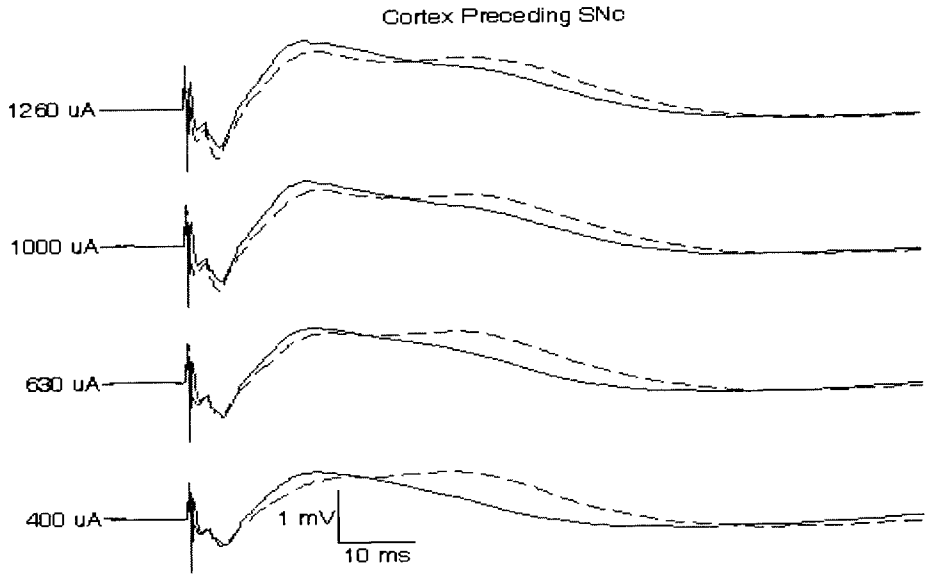


Figure 6.7: Late component input/output functions. Measures were taken from the striatal responses, evoked by cortical stimulation, before and after stimulation (figure title indicates stimulation condition). Solid lines are baseline measures, dashed lines are 24 hours after last day of high-frequency stimulation.

Representative Sweeps





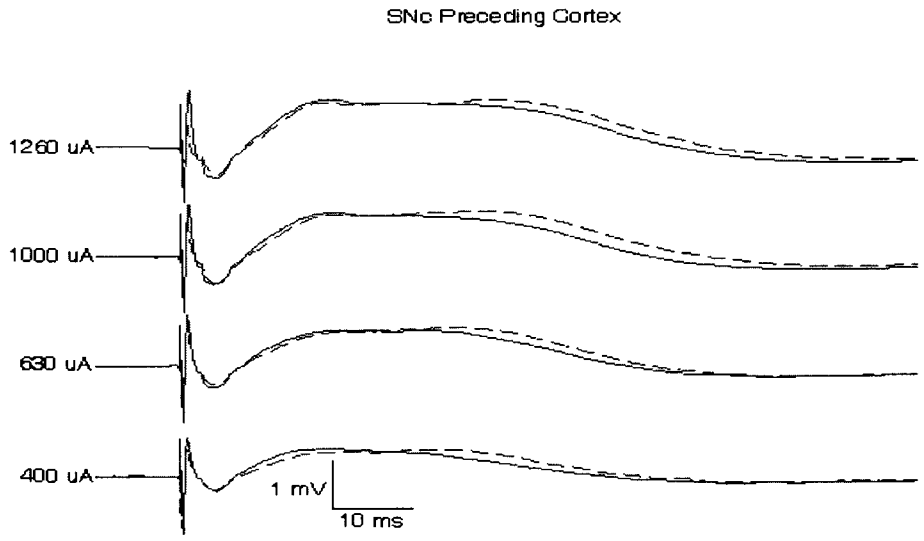


Figure 6.8: Field Potentials evoked in the group receiving paired cortical and SNc stimulation (upper trace), group receiving cortical stimulation before SNc stimulation (middle trace), and group receiving SNc stimulation before cortical stimulation (lower trace). Solid lines represent baseline responses and dashed lines are 24 hrs after the last day of stimulation train session.

6.4 Discussion

The experiment reported in this chapter dealt with the temporal requirements for dopaminergic neuromodulation of plasticity in the striatum. The results support the argument that different patterns of activation of cooperative afferents differentially effect the quality of LTP induction in the common striatal target site.

Although the substantia nigra has been shown to facilitate sensorimotor learning and striatal plasticity (Centonze et al., 2001; Centonze et al., 1999), and the coordinated activation of corticostriatal and nigrostriatal pathways is considered vital for the execution and control of motor behaviour, the time period during which dopamine release can affect plasticity in activated glutamatergic afferents has not been determined. Determining the neuromodulatory window during which dopamine release can alter LTP or LTD effects in the striatum should further our understanding of the cellular mechanisms underlying reward-related learning.

Simultaneous stimulation and stimulation in which the onset of the substantia nigra trains either preceded or followed the onset of the cortical trains by 40 ms had different effects on LTP induction. The results showed that the magnitude of change in both components was greatest when the corticostriatal and nigrostriatal pathways were stimulated simultaneously. Imposing a 40 ms delay between the cortex and substantia nigra stimulation produced effects either equivalent to simultaneous stimulation of slightly weaker. Applying substantia

nigra stimulation prior to cortex stimulation on the other hand, produced consistently weaker effects. This is consistent with what is known about reward mechanisms, which generally act on *prior* events. In other words, the substantia nigra stimulation should be simultaneous with or follow cortical stimulation in order to facilitate cortical plasticity. From the available literature, it is possible to assume that these patterns of stimulation lead to an optimal enhancement of postsynaptic signaling cascade involving Ca2+, PKA, and CaMKII (Gonzalez & Hablitz, 2003).

Although the ordering effects here are roughly consistent with expectations, accepting that dopamine acts as a reward with respect to reinforcement learning, we might have predicted that LTD would replace LTP when the "reward" signal preceded the "action." Brown et al., (1999) used model simulations to show that if the reward is delivered before the conditioned stimulus, in our case dopamine before glutamate, a depression occurs. One possible reason why we did not observe LTD in the case where reward preceded the conditioned stimulus could be the result of a relatively short delay (10 ms) between the stimulation of the two pathways, nigrostriatal and corticostriatal respectively, and the fact that there was still overlap in the stimulation periods. Another possible reason that there was still a potentiation effect in the reverse-order condition is that reinforcement is built in to the stimulation protocol, even without substantia nigra stimulation. There is known to be a cooperativity effect among activated afferents. The reason for this is that increases in the number of

afferents activated increases the postsynaptic depolarization achieved, which leads to larger influxes of the calcium trigger. A more optimal test for substantia nigra modulation might be to use even weaker cortical stimulation that is hovering near the threshold level for inducing LTP (or LTD).

Further research is required to extend the temporal manipulations begun here. For example, rather than staggering the stimulation trains between the two pathways, we could stagger the theta bursts (e.g. allow one complete 4 train set before activating the dopaminergic pathway). Perhaps a better approach would be to go back to the less effective spaced single trains, which would simplify the temporal manipulations. This would have the additional advantage of producing a lower level of LTP, against which the modulatory effect can more effectively show its facilitating effects.

Chapter 7

7.1 General Discussion

The ability of neurons in the brain to make new connections and reorganize existing ones is essential to learning and memory. A persistent augmentation of synaptic strength, as a result of experience, is thought to mediate information storage in the brain (Ericsson, 2008; Thelen, 1995; Thelen et al., 1993). Research has shown that there are a number of possible mechanisms that could lead to a change in synaptic strength.

Presynaptically, the probability of vesicle release can be increased if the density of calcium channels on the membrane is increased or altered, thereby increasing conductivity (Chen et al., 2002; von Gersdorff & Matthews. 1999). Synaptic strength can also be altered by increasing the number of vesicles of transmitter released per action potential (von Gersdorff & Matthews. 1999). A third possibility is a change in the density of postsynaptic receptors, which ultimately would lead to an increase or decrease in the magnitude of the postsynaptic potential (Boeckers, 2006). The mechanisms that lead to a stable enhancement or decrease in synaptic strength are activated during the induction of long-term potentiation (LTP) and long-term depression (LTD).

LTP, a model of activity-dependent changes in synaptic strength, has been studied extensively since the 1970s. At many excitatory synapses, increases in postsynaptic calcium arising from activation of NMDA-type glutamate receptors

induce LTP. The other model that has recently received much attention, LTD, leads to a reduction in synaptic transmission.

The notion that an increase in synaptic strength would necessarily lead to an enhanced behavioural response, and a decrease in synaptic strength lead to a decreased behavioural response, is incorrect. For instance, a decrease in synaptic strength at a postsynaptic neuron that, in turn, produces an inhibitory effect at its targets can lead to an enhanced behavioural response, as seen in learning models involving the cerebellum (Ito, 1989). Classical conditioning of the eye-blink response is mediated by such a mechanism. The parallel fibre to Purkinje cell connection requires disinhibition, which leads to increased motor activity (Freeman & Rabinak, 2004).

It is generally agreed that the common biochemical pathway for the induction and expression of both LTP and LTD is an elevation of intracellular Ca^{2+}, activation of enzymatic cascades, and, in short-term forms of LTP and LTD, a modification of postsynaptic AMPA and metabotropic subtypes of glutamate receptors. Changes lasting longer than hours appear to require protein synthesis and possibly growth of new synaptic connections (Abraham and Bear, 1996). Interactions among increases and decreases in synaptic strength underlie the formation of memories and behaviour (Abraham, 1996; Abraham and Bear, 1996).

However, most of the LTP and LTD research so far has been done in hippocampal slice preparations. In order to validate LTP and LTD as a model for

memory, experiments must be conducted in a variety of systems, including the striatum, in the awake, freely behaving animal.

7.2 Corticostriatal/Thalamocortical loop

The basal ganglia are involved in the normal processing of motor, associative and limbic functions (Albin et al., 1989; Alexander & Delong, 1986). In 1989, Albin, Young, and Penney introduced a model explaining the function of the basal ganglia. Based on neurochemical and anatomical data, this model explains how the basal ganglia influences cortical activity. The influence of basal ganglia output over the cortical motor areas was viewed as an increase or a decrease in excitation of the cortex by the thalamus, as a result of excitation and inhibition at earlier stages in the basal ganglia-cortical loop (Albin et al., 1989). Thus, motor output results from the patterning of activities within the basal ganglia-cortical loop.

The basal ganglia are comprised of complicated structures with extensive and complex connectivity (Alexander & Delong, 1986). Over the last few years, speculation about the complex nature of the basal ganglia has shifted attention from simple models to models that involve the interaction of different nuclei and pathways (Calabresi et al., 1994; Centonze et al., 2001; Centonze et al., 1999; Gubellini et al., 2003). Very little is known about the potential for modification in any of the component pathways.

Many electrophysiological studies have emerged regarding the role of the basal ganglia in brain function (Calabresi et al., 1998; Centzone et al., 2003; Pisani et al., 2003; & Plenz & Kitai, 1998). These studies have confirmed the functional importance of the striatum to sensorimotor learning, although the principles underlying its operation have so far been based on incomplete anatomical and physiological data. I anticipate that the physiological data that I have provided will serve as a small but valuable step toward the eventual development of a new and influential generation of learning models of intact basal ganglia motor systems.

This thesis consisted of a series of five experiments designed to test mechanisms of LTP and LTD in two excitatory glutamatergic pathways of the cortical-basal ganglia motor loop in freely behaving animals. This thesis reported experiments on 1) the effects of high-frequency and theta burst stimulation of the thalamocortical pathway on LTP/LTD induction (Chapter 2); 2) the effects of low-frequency stimulation of the corticostriatal pathway on LTD induction (Chapter 3); 3) the effects of high-frequency and theta burst stimulation of the corticostriatal pathway on the induction of corticostriatal LTP (Chapter 4); 4) the effects of dopaminergic modulation of the corticostriatal pathway on the induction of corticostriatal LTP following theta burst stimulation (Chapter 5); and 5) the effects of dopaminergic modulation of the corticostriatal pathway LTP while varying the temporal order of activation of the substantia nigra input relative to the motor cortex input (Chapter 6).

7.3 Thalamocortical Plasticity

The experiment on thalamocortical plasticity was undertaken for two main reasons. First, it has been argued that the thalamocortical pathway is simply a relay pathway incapable of demonstrating synaptic plasticity, in either direction (Glees & Le Gros Clark, 1941; Zeki & Shipp, 1988). It is important to know if this argument holds true for thalamocortical LTP or LTD in freely behaving animals. Second, as a result of anesthetized and in vitro LTP experiments, it has been theorized that thalamocortical pathways do not support LTP or LTD induction in the adult brain (Crair & Malenka, 1995; Feldman et al., 1999). Experimental support for these arguments have been mixed. Clearly, thalamocortical plasticity has implications for learning and memory in the context of motor learning, so this issue merits further attention.

The effects of thalamocortical stimulation on both LTP and LTD were examined. The term thalamocortical indicates the general direction of the pathway, in which the thalamus (the presynaptic region) sends projections to the cortex (the postsynaptic region).

In my hands, high-frequency and theta burst stimulation to the thalamocortical pathway failed to produce either LTP or LTD in the awake freely moving animal. In fact, the average peak amplitude in both the early and late components remained at baseline throughout the experiment, regardless of the type of stimulation applied. It is possible that the inability to elicit a potentiation

or depression in the thalamocortical pathway, is because the thalamus is a simple relay station.

Models of basal ganglia function often view the primary role of the thalamus as relaying of information processed in the basal ganglia to the cortex (Haber & Calzavara, 2008). More likely, the thalamus serves an important information processing function, which simply requires no further modification in the adult system. Of course, modification of thalamic processing could be accomplished by plasticity in the reverse pathways from the cortex to the thalamus, and area of study that needs more work. Finally, it is also possible that the thalamocortical pathways are capable of modification, but that this modification requires different activation parameters than those used here.

Another argument in favor of some residual plasticity in the thalamocortical system is that some researchers have been successful in demonstrating thalamocortical facilitation, although in slice and anesthetized preparations (Crair & Malenka, 1995; Feldman et al., 1999; Iriki et al., 1989). For example, Iriki et al. (1989) found that the thalamocortical pathway in anesthetized preparations was capable of supporting associative LTP. Although the experiments of Crair and Malenka (1995) demonstrated plasticity in thalamocortical synaptic transmission, they also found that modification could only be achieved during a critical period early in life. They elicited LTP in prepared slices from postnatal rats pups less than a week in age (postnatal day 1-7), but they were unable to induce LTP in older rat pups (postnatal day 8-17).

Crair and Malenka (1995) stimulated the ventrobasal nucleus of the thalamus while recording from layer 4 of the S1 barrel cortex. In addition to the differences in preparation and age, the different cortical target site in their study may partly account for the difference in findings.

In the chronic preparation, studies by Eckert and Racine (2006) and Ivanko (1997) appear to support the view that LTP cannot be induced in the thalamocortical pathway of non-anesthetized, intact adult animals. Although Eckert and Racine (2006) were unsuccessful in inducing LTP in the thalamocortical pathway following high-frequency stimulation, and unsuccessful in inducing LTD in response to low-frequency homosynaptic stimulation, they did find that associative high-frequency stimulation of both callosal and thalamic pathways actually produced an LTD effect in the thalamic pathway. One possible reason why we failed to demonstrate a similar finding (homosynaptic stimulation) could be due to a difference in pulse intensity. Eckert and Racine (2006) used a relatively low pulse intensity (500 μA), whereas this experiment used a much higher pulse intensity (1mA). Given their observation of LTD following high-frequency stimulation, we need to be even more cautious in viewing thalamocortical pathways as non-plastic. In any case, LTP has not been seen in the adult, and given that LTD is generally more difficult to induce than LTP, I decided to concentrate on properties of the primary *input* pathway, the corticostriatal pathway.

7.4 Corticostriatal LTD

Unlike the findings of the experiment in chapter 2, in which the thalamocortical pathway showed no indication at all of being able to support either LTP or LTD in the adult freely behaving animal (in response to high frequency stimulation), the results reported in chapter 3 (Corticostriatal LTD) showed induction curves that trended in the right direction. However, there were no significant results for the early component, so no conclusions can be yet be made as to whether the corticostriatal pathway is capable of supporting LTD.

Activity-dependent changes in synaptic efficacy, in the form of LTD, is considered critical for the normal development of neural circuits and for memory formation in the adult (Bliss, 1993; Ito, 1989). Expression of LTD at various sites has been attributed to both presynaptic and/or postsynaptic events. Presynaptically, alterations in neurotransmitter release have been discussed a possible factors relating to LTD (Ito, 1989). Postsynaptically, changes in receptor density and or conductance are possible changes that have been shown to play a role in LTD induction (Nicoll & Malenka, 1995).

I started with an exploration of LTD induction in the corticostriatal pathway, because an earlier pilot study showed a slight decease in evoked potential amplitudes following the application of a 300 Hz tetanus to the motor cortex. I thought that low-frequency stimulation applied to the same pathway might elicit a more robust LTD effect. Our lab has been successful in using low-frequency stimulation to induce LTD in other cortical regions, thus I felt it

appropriate to use the same protocol to induce LTD in the striatum of the freely behaving animal (Froc et al., 2000).

Despite the fact that the magnitude of decrease in both the early and late components were not significant following low-frequency stimulation, the trend of the responses suggest that improvements made to the stimulation protocol, or repeating the experiment with larger numbers of animals, might lead to a reliable LTD induction in the corticostriatal pathway. As to improvements in protocol, a further examination of stimulation parameters and electrode placements is warranted. For example, an increase in the stimulation frequency (e.g., to 3-10 Hz) might be more effective. Increasing the intervals between stimulation trains is another potential area of exploration.

In the current experiment, two inter-train intervals were used for cortical stimulation, 30 and 60 minutes. Again, keeping in mind that the current results were not significant, the trend for the 60 min interval to be more effective than the 30 min interval suggests that further increases to the inter-train interval may be worthy of exploration. Parameter mapping is tedious, but the results can be critical for achieving a clear understanding of response properties.

7.5 Corticostriatal LTP

The results presented in chapter 4 supported the prediction that both high-frequency and theta burst stimulation would produce long-lasting changes in response amplitude in the corticostriatal pathway. I also found that theta burst stimulation was more effective than high-frequency stimulation. Following theta burst stimulation, both the early and late components showed amplitude changes, whereas high-frequency stimulation only induced LTP in the late component. The peak amplitude of the late component was approximately 18% greater following theta burst stimulus than following high-frequency stimulation.

Although the mechanism for this effect remains unclear, it has been shown, in the hippocampus, to be due to suppression of inhibition during the stimulation period. The 200 ms delay between the short pulse bursts places the bursts following the first one in the period of maximal suppression of inhibition. Thus, the following trains trigger larger responses in the target neurons. The resulting increase in depolarization leads to a greater activation of NMDA receptors and a greater influx of calcium ions (Calabresi et al., 1992b). Even when the NMDA receptor does not play a critical role (e.g. NMDA-independent LTP), depolarization and calcium influx is still a critical trigger.

Calabresi et al., (1992b) studied the effects of high frequency stimulation (100 Hz) of the corticostriatal pathway using slice preparations. Their study demonstrated a contribution of both dl-alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-d-aspartate (NMDA) receptors in

striatal LTP following the application of selective receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2-amino-5-phosphonovalerate (APV) respectively.

Kasanetz, Riquelme, and Murer (2002) also showed an involvement of NMDA receptors in LTP induction in anesthetized rats. However, the contributions of AMPA and NMDA receptors to LTP induction in the corticostriatal pathway of the unanesthetized, freely behaving animal remains to be tested.

7.6 Modulated Corticostriatal LTP

Previous studies have demonstrated a role for dopamine in corticostriatal circuit plasticity. The experiment described in chapter 5 was designed to investigate dopaminergic modulation of the post-stimulation effects of corticostriatal activation in the awake-behaving rat following theta burst stimulation. Paired activation of the corticostriatal and nigrostriatal pathways led to a potentiation of both early and late components that remained stable for an extended period (Figures 5.5 & 5.6). The increased amplitude and stability of LTP following theta burst stimulation indicates a modulatory action of dopamine on corticostriatal synaptic plasticity.

The modulatory effects of dopamine on LTP in the corticostriatal pathway has also been demonstrated in slice. Centonze et al., (2001) were able to show that dopaminergic D1 receptor activation is necessary for the induction of LTP in

striatal synapses. They were also able to present evidence that showed that LTP induction was blocked when dopaminergic D2 receptors were active. These data indicate that dopaminergic D1 and D2 receptor activation differentially affect the expression of LTP in vitro and may very well behave similarly in the chronic preparation. Therefore it appears that dopamine could have multiple roles during motor learning. The actions of dopaminergic D1 and D2 receptors on LTP induction in the awake freely behaving animal still need to be determined.

Although this section has been discussing the results of chapter 5, which examined the affects of dopamine on LTP induction in the corticostriatal pathway, recent evidence has emerged suggesting the striatum may play a role in drug addiction (Kauer & Malenka, 2007). Drug addition is a persistent, relapsing brain disorder characterized by a progressive increase in drug taking, and marked by a slow ability to respond to naturally rewarding stimuli. It is well known that the ventral striatum is involved in drug induced changes in neural function, including drug addiction. It has also been suggested that the *dorsal* striatum is involved in the maintenance of drug seeking behaviour and that is effect is mediated by glutamate and dopamine neurotransmission (Kauer & Malenka, 2007).

Dopamine appears to reduce glutamate release via the dopamine D2 receptor (Bamford et al., 2004). Bamford et al., (2004) addressed this issue by testing the effects of repeated exposure to the psychostimulant methamphetamine, which releases dopamine and alters glutamate release at corticostriatal synapses. They found a presynaptic LTD effect, with a reduction in the frequency of

spontaneous firing of postsynaptic currents in the medium-spiny neurons in the striatum that lasted up to 140 days following methamphetamine withdrawal. They propose that drug addiction effects depend partly on dopamine induced changes in glutamatergic transmission in the dorsal striatum (the role of the ventral striatum had already been demonstrated).

Despite the contribution of slice work toward our current understanding of striatal physiology and plasticity, surprisingly little is known about the functional properties of striatal synapses in chronic preparations. The severed connections in the slice preparations removes the possibility of observing normal (or quasi normal in the case of electrical stimulation) interactions among various regions of the brain in their intact state. This fact is often over-looked because slice preparations yield an abundance of information in a relatively short period of time. In vitro preparations often allow for more experimental control of variables, but they might produce results that do not reflect normal physiological properties. Therefore, the results reported here are an important contribution to the neural plasticity knowledge base.

7.7 Spike-timing Dependent Corticostriatal Plasticity

The basal ganglia is known for its role in mediating the reinforcement effects of behaviour (Beiser & Houk, 1998; Reynolds et al., 2001). It is generally believed that the dopaminergic reward signals strengthen the likelihood of

repeating the 'rewarding' behaviour, and they do this, at least in part, via modulation of corticostriatal plasticity.

The temporal relationships among co-active inputs, particularly when one of those inputs is a "reward" signal, play a major role in determining the resulting modulatory effect, and the outcome of learning strategies aimed at building accurate predictions based on past experience (e.g., Montague et al., 1996; Schultz et al., 1997; Schultz et al., 1997). In effect, the temporal model computes the estimated reward to that of the actual reward received, which mimics reinforcement learning situations.

Chronic recording experiments show that striatal neurons are sensitive to motivationally significant cues in the environment and show firing patterns which are consistent with adaptive changes during learning (Schultz, 1998). Schultz (1998) measured the dopaminergic activity of monkeys that were trained to associate a sensory cue with a juice reward. Schultz (1998) observed an increase in the activation of dopaminergic neurons as the monkey learns to expect a juice reward that reliably *follows*, within a fixed time interval, a sensory cue. Schultz noted that the dopamine firing ceased at the expected time of the reward. At the end of the learning period if the animal fails to receive a reward after the presentation of the visual cue, a phasic depression in dopamine cell firing occurs.

The results of the study suggest a temporal relationship exists in the striatum with respect to the release of nigral dopamine from the nigrostriatal system and cortical glutamate from the corticostriatal system. Later studies by

Schultz (2002) indicate that the interaction between dopamine and glutamate at dendritic spines located on striatal spiny medium neurons is central to corticostriatal synaptic plasticity. The precise mechanisms, as well as the precise temporal requirements for these interactions is still a matter of debate and further research. There is surprisingly little information pertaining to the mechanisms that underlie reinforcement learning in the basal ganglia (Bertin et al., 2007; Reynolds et al., 2001).

The experiment described in chapter 6 addressed the question of how various patterns of dopamine release from the substantia nigra pars compacta affect LTP induction in the striatum. When theta burst stimulation was applied simultaneously to the corticostriatal and substantia nigra pathways, a robust LTP was induced in both the early and late components. These effects were also seen when the theta burst stimulation was first applied to the corticostriatal pathway followed 10 ms later by a stimulation to the substantia nigra pathway. In terms of the amount of change observed following stimulation, the simultaneous stimulation pairing produced the largest change in evoked potentials, followed closely by the delayed pairing where the substantia nigra was stimulated after the cortical stimulation. The smallest change was observed when the cortex was stimulated 10 ms *after* the substantia nigra stimulation. The results strongly suggest that dopamine activation is necessary for this form of temporally-based learning.

A somewhat similar experiment was recently done in the slice preparation (Pawlak & Kerr, 2008). Using high-frequency stimulation, Pawlak and Kerr (2008) paired cortical stimulation with single action potentials backpropagated through dendrites of the medium-spiny neuron to examine corticostriatal spike-timing dependent plasticity. Pawlak and Kerr (2008) found similar temporal requirements. When the backpropagated spike was delayed by 10 ms, there was an enhancement of the excitatory post-synaptic potential amplitude.

An additional result of Pawlak and Kerr's (2008) study supports another prediction from reinforcement theory, that LTD results when the "reward" stimulation *precedes* the cortical input. Pawlak and Kerr (2008) were able to induce LTD by pairing a single backpropagated action potential (i.e. nigrostriatal) with an excitatory post-synaptic potential evoked by cortical stimulation (i.e. corticostriatal) delivered 30 ms later. Although I did not find LTD with the reversed stimulation pattern, I did find a greatly reduced LTP. As pointed out in Chapter 6, the cortical stimulation is capable of producing its own reinforcement effects, so the reversed sequence of stimulation may have led to an "overlay" of LTD, leading to a coincident depotentiation effect.

Pawlak and Kerr (2008) also tested the contribution of NMDA receptors in the strengthening and depression of corticostriatal synapses. They found that neither LTP nor LTD was induced when D-AP-5, an NMDA antagonist was administered. Although it remains to be tested, it seems likely that corticostriatal

LTP and LTD, in the awake behaving animal, also depend on both dopamine and NMDA receptors.

NMDA receptors have been associated with long-term potentiation in a number of cortical regions, as well as the striatum (Malenka & Nichol, 1999; Kasanetz et a., 2002; Pawlak & Kerr, 2008). Kelley, Smith-Roe, and Holahan (1997) tested the hypothesis that NMDA in the nucleus accumbens was important for operant learning in the rat. The nucleus accumbens is a region of the striatum that is known for its role in mediating the reinforcing and rewarding properties of drug abuse (Wise & Bozarth, 1987). Using a simple lever-pressing task, Kelley, Smith-Roe, and Holahan (1997) found that blocking NMDA receptor with AP-5 disrupted learning.

An interesting behavioural study was undertaken by Reynolds, Hyland, and Wickens (2001), who showed rats would learn to press a lever that delivered electrical stimulation to the substantia nigra pars compacta. After establishing intracranial self-stimulation behaviour, the rats were anesthetized and prepared for in vivo recordings. Reynolds, Hyland, and Wickens (2001) found that the rats that had received stimulation of the substantia nigra while pressing the lever exhibited potentiation of corticostriatal responses. Presumably, the effects of the corticostriatal pathway activation during lever press responses were modulated by the resulting dopamine release.

A key next step in investigating plasticity within the basal ganglia-motor loop of awake freely behaving animals, would be to further investigate the

modulatory interactions in both the descending direct excitatory pathway as well as the ascending direct pathway. Eckert and Racine (2006) in which they successfully induced thalamocortical depression, so it would be useful to replicated and extend this result. A part of the further exploration of this output pathway from the striatum might include looking at the effects of neuromodulation on thalamocortical synapses.

As mentioned earlier, an advantage of using in vivo preparations is that is that they provide us with the opportunity to investigate how coactivated pathways interact. In addition to the continued focus on the modulatory effects of dopamine, it would be useful to test the effects of other neuromodulators, such as acetylcholine, on LTP and LTD expression. Finally, although it would be more difficult, a more thorough understanding of the capacity of the other, intervening pathways in the direct and indirect loops to support long-term modification in synaptic transmission would help us to better understand the function of these cortical/basal ganglia loops in motor processing and motor learning.

Appendix 1: Characterization of Evoked Striatal Components

The primary object of this study is to characterize the two components of interest stated throughout this thesis, the early (putative monosynaptic) and late (polysynaptic) components of striatal responses. A common approach to confirming that a component is monosynaptic is to test its ability to follow high frequency trains. Polysynaptic components typically begin to fail at frequencies about 10 to 20 Hz, while monosynaptic components follow at frequencies up to 40 Hz and higher. In previous experiments on pathways of cortical origin, it has been found that polysynaptic responses begin to fail at high frequencies by the 2nd response in the stimulation train at frequencies above about 25-30 ms. Given that 2 pulses are potentially sufficient to test for multiple synaptic links, I opted for a simpler paired pulse test in this experiment. The first latency used in the following paired pulse test was 20 ms, which is equivalent to a stimulation frequency of 50 Hz. The remaining responses in the paired pulse sequence allowed us to test for post activation inhibition effects.

A.1 Materials and Methods

A.1.1 Animals

A total of 7 male Long-Evans rats were used in this experiment. They weighed between 350g and 500g. Animals were prepared using the same procedures as those in the previous corticostriatal/SNc LTP experiment.

A.1.2 Stimulation and Recording

Paired-pulse tests were conducted by delivering two stimulation pulses to the primary motor cortex at seven inter-pulse intervals (20, 40, 80, 160, 320, 640, and 1280ms). The first pulse is considered the conditioning pulse and the second pulse is considered the test pulse. The pulse intensity for the early component was set to evoke a response approximately 50% of the maximum amplitude observed in the input/output tests (630 μA). An example input/output function is shown in Fig 6.1. The peak amplitude of average responses expressed in the pair-pulse test were expressed as the difference from the first pulse. The responses were averaged across animals and plotted as a function of inter-pulse intervals (Fig 6.9).

A.2 Results

As can be seen from Figure A-1, there was no depression in the early component of the evoked response at the 20 ms inter-pulse interval (frequency equivalent: 50 Hz). The response amplitude is very close to that of the conditioning pulse. The late component, on the other hand, showed a nearly complete failure at the 20 ms inter-pulse interval.

The subsequent responses in the paired pulse series also show a very different response profile between the early and late components. The early component showed a relative strong paired pulse inhibition effect lasting for at least 640 ms. This is probably due to a variety of late inhibition effects, such as potassium based post-activation inhibition. These long lasting inhibition effects are characteristic of most cortically-induced synaptic responses (Mahon et al., 2003; Racine & Milgram, 1983). The late component showed no depression between 40 and 1280 ms.

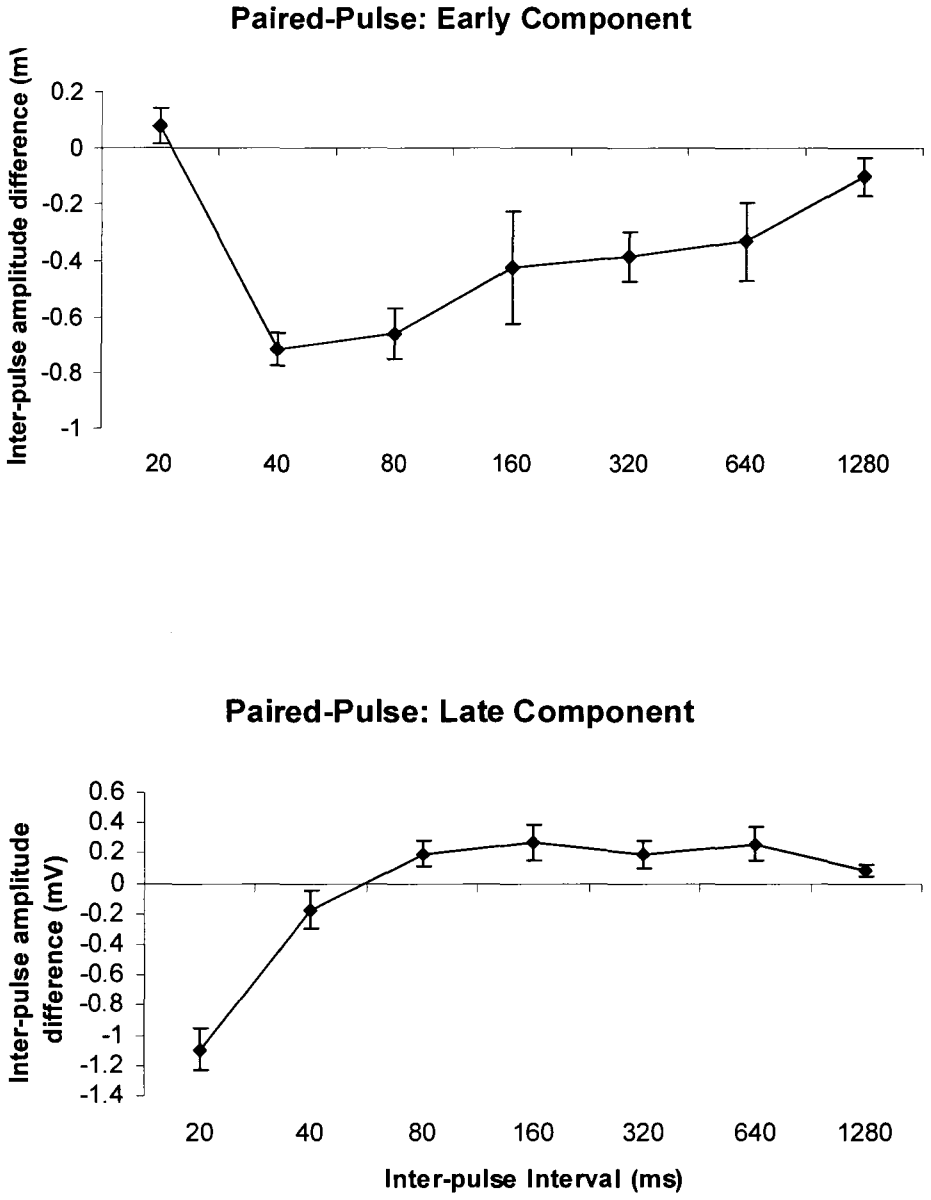


Figure A-1: Paired-pulse input/output functions. Measures were taken from the striatal responses, evoked by cortical stimulation at various paired-pulse intervals. The upper figure shows the response of the early component when the corticostriatal pathway receives paired-pulse stimulation. The lower figure shows the response of the late component.

A.3 Discussion

The responses of the early and late components in the paired pulse experiment were consistent with the behaviours expected of monosynaptic and polysynaptic responses. This interpretation is also consistent with the short latency response of the early component. However, since this was a very minimal test of the question, a great deal of caution needs to be exercised in the interpretation of the results. While the results are consistent with those found in other pathways of cortical origin, previous experiments have used more rigorous tests for monosynaptic connectivity. Clearly, further work is necessary to answer this question with confidence. Preferably, this question would be tackled at the cellular level using intracellular recording, but these experiments are very difficult to do in the intact preparation, due to tissue movement. At the very least, a battery of tests, including frequency of following, recovery from anesthesia, paired pulse, and extracellular unit recording should be run. Done properly, these tests require a variety of pulse intensities, pulse frequencies, anesthesia dosages, and so on.

Given the positive results for the LTP/LTD tests reported in this thesis, a full characterization of the corticostriatal response in the awake, freely moving anima (or at least the intact, anesthetized animal) is an obvious next step in the exploration of this corticostriatal system.

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