MECHANISMS OF NEOCORTICAL LONG-TERM POTENTIATION AND LONG-TERM DEPRESSION IN THE FREELY BEHAVING RAT

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Abstract

Long-term potentiation (LTP) and depression (LTD) are activity-dependent changes in synaptic strength that model how the brain might store memories. The mechanisms of LTP and LTD have been studied extensively in the hippocampus because the hippocampus is critical for memory storage. The neocortex is also believed to be critical for the long-term storage of memories, but less work on LTP and LTD has been done in the neocortex. Furthermore, the majority of neocortical LTP and LTD studies have been carried out in artifical brain-slice preparations. The experiments presented in this thesis test some mechanisms of LTP and LTD in the neocortex of the awake, freely behaving rat.

In the hippocampus and anesthetized neocortex, simultaneous stimulation of a 'strong' and a 'weak' input pathway can induce LTP in the weak pathway (even though stimulation of the weak pathway alone does not induce LTP). However, the results presented here show that in the neocortex of awake, freely behaving animals, simultaneous activation of strong and weak input pathways does not induce LTP in the weak pathway. In fact, under these conditions, an LTD is induced in the weak pathway. However, under these conditions, LTP induction was facilitated in the strong pathway.

Associative LTD has also been shown in the hippocampus following anticorrelated activity delivered to two input pathways. The associative LTD in the hippocampus is larger than either homosynaptic or heterosynaptic LTD. The results presented here

show that anticorrelated activity delivered on two input pathways does not induce an associative LTD greater in magnitude than homosynaptic or heterosynaptic LTD.

Hippocampal LTD and LTP have been shown to depend on activation of metabotropic glutamate receptors (mGluR). However, blocking mGluRs in freely, behaving rats partially blocks LTP (but not LTD) in the callosal pathway and does not block the induction of LTP or LTD in the thalamocortical pathway.

The results demonstrate the mechanistic differences, not only between the hippocampus and neocortex, but also anesthetized and awake neocortical preparations. The results also emphasize the need for further study on mechanisms of LTP and LTD in the neocortex of awake, freely behaving rats.

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Contents

List of Figures

List of Tables

Chapter 1

General Introduction

1.1 Dual Memory Systems

One of the most significant events in memory research was the discovery of patient H.M. H.M. underwent a bilateral temporal lobectomy, which included the hippocampus, to alleviate severe epilepsy. Following the operation, he exhibited a profound memory disorder: although all of his other cognitive functions seemed to be intact, he could not make new memories of events (Scoville and Milner, 1957). The memory deficit exhibited a peculiar temporal gradient. His memory of events early in his life were fine and could be recalled vividly. There was a partial amnesia for events prior to the surgery, a period that extended about 3 years. Finally, there was a complete lack of ability to make new memories for events following the operation. He could retain events for periods of several seconds as long as he was not distracted, but as soon as his attention was diverted he would forget the event. H.M.'s curious symptoms suggested that the hippocampus was somehow involved in the transfer of recent memories into permanent storage and that the final repository for memories

lay outside the hippocampus.

Years of research on animal models have confirmed the assertions made by Scoville and Milner (1957). In one of the most common experimental paradigms, memory is tested in lesioned and normal monkeys using a delayed nonmatching to sample task (Squire, 1992). In this task, the monkey is presented with a novel object that it must remember. Following a variable delay, the monkey is presented with two objects: the old object and a novel object. The monkey must demonstrate recognition of the old object by selecting the novel object (the choice of the novel object ensures that the monkey is not making a simple stimulus-reward association). Monkeys with hippocampal lesions perform normally at short delays (5 seconds), but perform poorly at delays longer than 30 seconds (Mishkin, 1978; Zola-Morgan and Squire, 1986). Experiments of this type clearly demonstrated that lesions of the hippocampus cause severe anterograde amnesia.

The same experimental paradigm has been used to demonstrate the retrograde amnesia originally observed in H.M. Zola-Morgan and Squire (1990) trained monkeys to recognize pairs of objects at varying intervals prior to lesioning the hippocampus. For example, at 16 weeks prior to surgery the monkeys would be trained to recognize a set of object pairs. At 12 weeks prior, they would learn a new set, and so on until undergoing the hippocampal lesion. After recovering from the operation, the monkeys were tested on all of the sets of object pairs. Sham operated monkeys showed a normal forgetting curve, performing better on the recently learned objects, but worse on the objects learned many weeks prior to the operation. Monkeys with hippocampal lesions had a profound deficit for the recently learned objects, but performed as well as sham monkeys for the older objects. This suggested that the role of the hippocampus in memory is time-dependent. Very recent memories depend critically upon the hippocampus, and there is a gradual decrease in the reliance on the hippocampus for

progressively older memories, until, finally, the hippocampus is no longer required.

Similar results have been observed in other species. A popular method for testing memory in rats is the social transmission of food preference paradigm (Galef and Wigmore, 1983). In this paradigm, a demonstrator rat consumes a particular kind of food and is then allowed to interact with a naive animal. The naive animal is then given a choice between two foods: the food consumed by the demonstrator and a novel food. Normal rats will most often choose the demonstrated food. To test the rats' memory, a delay is inserted between the social interaction and the food choice. Rats with hippocampal lesions will choose the demonstrated food if tested immediately, but choose randomly if tested 24 hours later (Winocur, 1990). Thus rats with hippocampal lesions exhibit anterograde amnesia similar to that observed in humans and monkeys.

The social transmission of food preference paradigm has also been used to demonstrate a temporally graded retrograde amnesia in rats (Clark et al., 2002). In this study, hippocampal lesions were made 1, 10, and 30 days following the social learning (the interaction between the demonstrator and naive rat). The lesioned animals displayed a graded retrograde amnesia, similar to that observed in monkeys and humans. The rats lesioned 1 day following learning performed at chance. The rats lesioned 10 days following learning improved, but their performance was not significantly difference than chance. The rats lesioned 30 days following learning performed as well as controls.

If the hippocampus eventually becomes unnecessary for memory, then what is the final repository for memory? Most believe that most long-term memories ultimately reside in the neocortex (Squire and Alvarez, 1995; McClelland et al., 1995). One popular hypothesis about how an event might be stored in the neocortex is as follows. Initially the sensory information about the event is processed in the different cortical

sensory areas. The information is further processed in higher association areas and eventually fed into the hippocampus. The hippocampus forms associations between the different cortical areas and continually reactivates these associations. Over time, and with enough reinstatements, the cortical trace becomes strong enough that it can be activated on its own, independently of the hippocampus (Squire and Alvarez, 1995; McClelland et al., 1995).

This theory makes two important assumptions. One is that memories are stored as changes in synaptic strength. The other is that the hippocampus and neocortex should change their connection strengths differently. At the very least, the hippocampus should be able to change connection strengths quickly, and the cortex should change strengths more slowly. While it is not yet known if the brain does store memories as changes in connection strength, it is known that synapses are capable of changing strength in response to patterns of activity. Long-term potentiation is a model of memory storage based on activity-dependent changes in synaptic strength. It is discussed in the next section.

1.2 Long-term Potentiation

The Canadian psychologist Donald Hebb (1949) was the first to suggest that the brain could store memories as changes in synaptic strength. He suggested that when there was correlated activity between a pre- and postsynaptic neuron, the strength of the synaptic connection between the two neurons would increase. It was more than two decades before evidence was presented to support this theory.

Bliss and Lomo (1973) were the first to demonstrate an increase in synaptic strength following high frequency stimulation of an afferent pathway. They were working in the hippocampus of the anesthetized rabbit. They recorded population

(field) excitatory postsynaptic potentials (fEPSP) from the dentate gyrus evoked by stimulation of the perforant pathway. When they conditioned the perforant pathway with moderate (15 Hz for 10 s) or high frequency (100 Hz for 3 s) pulse trains, they observed a potentiation of the fEPSP that lasted for several hours. The high frequency conditioning had activated both the pre- and postsynaptic neurons, laying the groundwork for support of Hebb's idea that correlated activity was necessary to increase synaptic strength. This topic is discussed in the next section (Section 1.2.1).

The results of Bliss and Lomo (1973) were confirmed by other groups, establishing long-term potentiation as a reliable phenomenon in the hippocampus. A few years later, the opposite of long-term potentiation (LTP)—long-term depression (LTD)—was observed in the hippocampus (Levy and Steward, 1979). Thus, reliable methods for increasing and decreasing synaptic strengths had been shown in a formation known to participate in memory storage. LTP and LTD became attractive models for memory storage in the mammalian nervous system.

One of the main benefits of these models was that they provided the opportunity to examine the mechanisms underlying the change in synaptic strength. Lashley (1950) had shown that memories were not stored in localized regions of the cortex and concluded that memories must be stored diffusely. Although it is now known that memory storage is more localized than Lashley (1950) originally suggested, the traces are still believed to be diffuse. Trying to isolate such a trace to study it is a near impossible task. As a model, LTP provided a highly localized change in synaptic strength in response to correlated conditioning. This made it possible to study the mechanisms of LTP.

1.2.1 Physiological Mechanisms of LTP

One of the first properties of LTP to be discovered was that the conditioning stimulation had to exceed a certain threshold before LTP induction was successful. This was first observed by Bliss and Lomo (1973), and later tested experimentally by McNaughton et al. (1978). LTP was not induced following a weak intensity tetanization, nor was it induced if too few afferent fibers were activated (McNaughton et al., 1978). Thus, input fibers can act *cooperatively* to induce LTP.

It was also shown that afferents could act *associatively* during LTP induction (Levy and Steward, 1979). A strong input (one that reliably showed LTP) could facilitate the induction of LTP in a weak input (one that did not potentiate if conditioned alone) if the two inputs were conditioned simultaneously or if the weak input preceded the strong input (Levy and Steward, 1983). The associative property of LTP was attractive because it provided a neural explanation of classical conditioning (e.g., Kim and Thompson, 1997). The weak input corresponds to the CS, which, initially, evokes little behavioural response. The strong input fills the role of the US, which evokes a strong behavioural response. After repeated pairings, the CS (weak input) is capable of evoking a behavioural response without the US. (NB: the term 'associativity' sometimes means different things in the literature; its meaning as it relates to this thesis is discussed in section 1.4 on page 15).

Another significant property of LTP is its *specificity*. In order to be relevant from an information storage standpoint, LTP needs to promote changes specific to the synapses showing correlated activity. If LTP caused generalized changes in excitability, it would serve very little use as a mechanism for storing information. Andersen et al. (1977) were the first to show that LTP is specific to the activated pathway, causing no significant changes in control input pathways.

1.2.2 Molecular Mechanisms of LTP

A significant development in the understanding of the mechanisms of synaptic plasticity was the discovery of the N-methyl-D-aspartate (NMDA) receptor, one of the ionotropic glutamate receptors. Collingridge et al. (1983) were testing the effects of various agents selective for glutamate receptors in the hippocampal slice preparation. When applying the selective NMDA receptor antagonist DL-2-amino-5-phosphonovalerate (APV), they observed that induction of LTP was blocked even though basal synaptic transmission was not affected. This showed that, even though the synapse was still active, NMDA receptor activation was necessary for successful LTP induction, at least in the CA1 region of the hippocampus.

The NMDA receptor behaves differently than the other ionotropic glutamate receptors. Whereas the other ionotropic receptors open in response to binding of glutamate alone, the NMDA receptor has the additional requirement that the cell membrane must be partially depolarized (Bliss and Collingridge, 1993). At the resting membrane potential ($\sim$-70 mV), the pore of the NMDA channel is blocked by a Mg^{2+} ion. This Mg^{2+} ion dislodges when the membrane is partially depolarized. Thus, the NMDA receptor only permits the flow ions when it is binding glutamate and the cell membrane is partially depolarized.

The discovery of this unique property of the NMDA receptor was very exciting because it meant that the receptor was essentially detecting correlations between pre- and postsynaptic activity (Bliss and Collingridge, 1993). Binding of glutamate meant that the presynaptic neuron was active and releasing transmitter. Partial depolarization of the cell membrane meant that the postsynaptic neuron was also active. The latter condition could explain the results of McNaughton et al. (1978): if the stimulus intensity is low, the NMDA receptor will bind glutamate, but the associated

ion channel will not open because the postsynaptic membrane is not depolarized sufficiently. The existence of a molecular coincidence detector provided crucial support for Hebb's theory of memory storage.

When the NMDA receptor is activated, it opens to permit the flow of cations into the cell. The most important of these ions, in terms of synaptic plasticity, is Ca^{2+}. The rise in intracellular Ca^{2+} concentration triggers a complex chain of events, which are only partly understood at this point (Sheng and Kim, 2002).

One signaling event that has received a lot of attention is the activation of calcium-calmodulin dependent protein kinase II (CaMKII). The rise in intracellular Ca^{2+} activates calmodulin, which in turn activates CaMKII. The activated CaMKII can interact with various proteins in the postsynaptic density, including NMDA receptors and the actin cytoskeleton, suggesting a structural role for CaMKII (Lisman et al., 2002). Activated CaMKII also phosphorylates various proteins, including AMPA receptors. Poncer et al. (2002) showed that constituitively active CaMKII by itself was sufficient to potentiate AMPA-mediated excitatory postsynaptic currents (EPSCs). Conversely, the removal of phosphate groups from AMPA receptors by protein phosphatases is thought to contribute to LTD. One of the most significant properties of CaMKII is that it can phosphorylate itself and thus remain switched on for prolonged periods of time. The prolonged activation of CaMKII could possibly be a significant event in memory processes (Lisman et al., 2002). Support for this hypothesis comes from a recent study showing behavioural memory deficits, as well as reduced synaptic plasticity, in CaMKII mutant mice (Miller et al., 2002).

Another important signaling event receiving a lot of attention in recent years is the regulation of AMPA receptor expression and insertion in the postsynaptic membrane (Sheng and Kim, 2002; Malinow and Malenka, 2002). Because AMPA receptors mediate rapid glutamatergic responses, a change in their distribution in the postsynaptic

membrane would significantly affect synaptic transmission. Recombinant AMPA receptors tagged with green fluorescent protein (GFP) are localized in the shafts of dendrites under normal resting conditions. Following high frequency stimulation and LTP induction, the AMPA receptors translocate to dendritic spines. Furthermore, blocking NMDA receptors blocks the translocation of AMPA receptors following LTP induction (Shi et al., 1999).

Similar results have been reported for endogenous receptor levels. Heynen et al. (2000) found increased levels of AMPA receptor following induction of LTP in the hippocampus *in vivo*. This increase was blocked (as was LTP induction) if NMDA receptors were blocked during tetanization. There was no difference in the amount of NMDA receptor levels before and after tetanization.

The results of these studies lead to the conclusion that part of the mechanism of LTP induction is translocation of AMPA receptors from an available intracellular pool to the dendritic spine. Interestingly, there is evidence that the opposite process, the removal of AMPA receptors, contributes to the development of LTD. In the same study that showed an increase of endogenous AMPA receptors in dendritic spines following LTP induction, a decrease in the level of AMPA receptors occurred following induction of LTD (Heynen et al., 2000).

Hebb (1949) originally predicted that 'some growth process or metabolic change' was responsible for the activity-dependent increase in synaptic strength. The mechanisms discussed so far deal mainly with metabolic changes but do not deal with new growth. Nearly twenty years ago, the maintenance of LTP was shown to be dependent on the synthesis of new proteins (Krug et al., 1984). However, it was not known whether the newly synthesized proteins resulted in changes at existing synapses or whether there was growth of new synapses following LTP induction. Recently, Engert and Bonhoeffer (1999) showed that LTP induction was associated with

the growth of new dendritic spines. Using fluorescence imaging, they monitored the structure of the dendritic tree of a neuron before, during, and after LTP induction (as measured by intracellular recording). Following the induction of LTP, new spines could be seen growing approximately 30 minutes later. Activation of NMDA receptors and initiation of the resultant signaling cascade are undoubtedly critical events for synaptic plasticity. However, it is unlikely that they fully explain the process of synaptic plasticity. Recently, attention has been given to the metabotropic class of glutamate receptors (mGluR). While the ionotropic receptors mediate the rapid excitatory synaptic transmission, mGluRs mediate slower responses through second messenger systems (Nakanishi, 1994).

1.3 LTP in the neocortex of awake animals

A number of mechanisms underlying LTP have been identified. The vast majority of this research has been done either in slice preparations of mammalian brains, or in invertebrate preparations. While these preparations facilitate the exploration of LTP mechanisms, in particular molecular mechanisms, they are not without serious shortcomings. The invertebrate preparation provides a relatively simple nervous system, but it is very far removed evolutionarily from the human brain. Mammalian slice preparations are much closer, in terms of evolution, to the human brain, but the major drawback is that only a small piece of the brain is being studied in a very artificial environment. One further criticism is that the majority of the research has focused on the role of the hippocampus. While the role of the hippocampus is certainly important, given its significance in memory function, the neocortex is equally important as it is believed to be the principal repository for long-term memories. Based on these criticisms, a significant requirement of LTP research becomes evident:

it is important that the properties of LTP discovered in the slice and invertebrate preps be tested in the neocortex of awake, freely behaving animals.

Learning and memory occur in intact animals. Establishing that LTP is the mechanism underlying learning and memory requires that experiments be done in intact animals. This thesis tests some mechanisms of LTP and LTD in the neocortex of awake, freely behaving rats. Specifically, the properties of associative LTP and LTD are examined by pairing activation of thalamocortical and callosal inputs to the motor cortex. Finally, the role of metabotropic glutamate receptors in neocortical LTP and LTD is examined.

The remainder of the introduction gives a brief introduction to thalamocortical and callosal plasticity.

1.3.1 Thalamocortical and Callosal Plasticity

The majority of work on thalamocortical plasticity has been done in the context of critical period plasticity. Wiesel and Hubel (1963) investigated receptive field properties of visual cortex neurons in normal kittens and kittens that had been reared with one eye sutured shut (monocular deprivation). In normal kittens, the majority of neurons in the visual cortex responded to input from both eyes. However, if the kitten had been monocularly deprived, the majority of neurons responded only to input from the open eye. The loss of patterned input in the one eye caused that eye to become functionally disconnected from the visual cortex. The effects of monocular deprivation were only evident during a brief period during development called the critical period (Hubel and Wiesel, 1970). If the animal was monocularly deprived after the critical period, then no effects of monocular deprivation were observed. However, the duration of the critical period itself depends on visual experience. If

an animal is reared in complete darkness from the time of birth, the duration of the critical period can be prolonged (Cynader and Mitchell, 1980).

Plasticity during the critical period became a hallmark feature of visual system development. Similar effects were observed in other mammalian species such as monkeys (LeVay et al., 1980) and rats (Fagiolini et al., 1994b). Researchers hypothesized that LTP-like mechanisms might contribute to the plasticity observed during the critical period. First, it had to be shown that LTP could be induced in the visual cortex and that it was mechanistically similar to LTP in the hippocampus. Artola and Singer (1987) showed that tetanization of the underlying white matter produced a lasting potentiation of the EPSP in slices of visual cortex. Furthermore, successful induction of LTP depended on activation of NMDA receptors. It was later discovered that the amount of LTP that could be induced in the visual cortex depended on the age of the animal. The amount of LTP induced following white matter stimulation in young rats was significantly more than that induced in adult rats (Kirkwood et al., 1995). The period of robust LTP corresponded well with the critical period for the effects of monocular deprivation. Furthermore, the period of robust LTP could be extended by dark-rearing the rats prior to LTP induction. These results supported the hypothesis that LTP-like mechanisms contributed to critical period plasticity. One drawback of these studies was that the thalamocortical pathway was not specifically tested for LTP induction. These experiments had tetanized white matter beneath the neocortex. The white matter contains axons arising from several different sources including the thalamus.

One of the reasons the previous experiments did not include LTP induction at thalamocortical synapses is because the lateral geniculate nucleus is located a significant distance away from the visual cortex. It would be extremely difficult, if not impossible, to prepare a slice preparation that preserved the thalamocortical pathway

in the visual system. Crair and Malenka (1995) addressed this issue by developing a thalamocortical slice preparation in the somatosensory system of the rat. The somatosensory system is subject to a critical period similar to that in the visual system. If the whiskers on the side of a rat's snout are cauterized during the first week of life, then the amount of somatosensory cortex that responds to these whiskers is reduced (Woolsey and Wann, 1976). Crair and Malenka (1995) found that the ability to induce LTP at thalamocortical synapses was correlated with the critical period. The largest LTP effects were seen in newborn rats, and if the slices were prepared from rats older than one week, there was little to no LTP induced.

There has been less work on thalamocortical plasticity in intact preparations. Iriki et al. (1989) tried to induce LTP at thalamocortical synapses in the motor cortex of anesthetized adult cats. They found that LTP induction was unsuccessful if the thalamocortical pathway was tetanized alone. However, if the thalamocortical pathway was tetanized at the same time as an intracortical projection, the thalamocortical pathway showed LTP. This demonstrated that the thalamocortical pathway potentiated following *associative* stimulation, but not following homosynaptic stimulation. Heynan and Bear (2001) tested LTP induction in the visual thalamocortical pathway of adult anesthetized rats. They found that LTP induction was successful following homosynaptic tetanization. In our lab, Ivanco (1997) tested LTP induction in 3 different thalamocortical pathways: the visual system, the auditory system, and the frontal cortex. None of these pathways showed LTP following homosynaptic tetanization. Thus, the results of the thalamocortical LTP in adult animals are somewhat mixed. In general, it appears that while thalamocortical LTP is readily induced in critical period preparations, it is much more difficult, but possible, in adult preparations.

Plasticity of the callosal pathway has received less attention compared to the

thalamocortical pathway. In slice preparations, induction of LTP in the neocortex following tetanization of the underlying white matter is difficult. Typically, either young animals must be used or some chemical agent must be added to the bath to reduce inhibition (e.g., Artola and Singer, 1987). In anesthetized rats, tetanization of the callosum does induce LTP in a number of neocortical sites (Racine et al., 1994b), but, as in slice preparations, the effects can only be monitored for short periods.

LTP induction in the neocortex of the chronically prepared, freely moving rat following tetanization of the callosum proved difficult at first. Racine et al. (1994a) failed to induce LTP following single conditioning sessions under a number of experimental conditions. They tried to vary the parameters of the conditioning stimulation, condition two pathways associatively, condition animals exposed to enriched environments, reduce levels of inhibition, use the conditioning train as a cue in a fear conditioning experiment, and modulate cholinergic activity. None of these conditions resulted in the induction of LTP following tetanization of the callosum.

It was later discovered that LTP could be induced reliably in the neocortex if the multiple stimulation sessions occurred over several days (Racine et al., 1995; Trepel and Racine, 1998). Trepel and Racine (1998) varied a number of stimulation parameters such as number of conditioning sessions, interval between conditioning sessions, number of trains applied during a single conditioning session, and intensity of pulses during conditioning. They found that successful LTP induction was strongly affected by the number of conditioning sessions as well as the interval between them. LTP induction only occurred following multiple conditioning sessions and the sessions were most effective if they occurred at $\sim$24 hour intervals. With these parameters, the neocortex was found to be very reactive, and robust LTP could be induced using low pulse intensities or few stimulation trains.

The discovery of the gradual, incremental induction of LTP in the neocortex fit

nicely with data from memory research in humans and animals. Memory researchers and theorists had hypothesized that the hippocampus and neocortex played different roles in memory storage (Squire and Alvarez, 1995; McClelland et al., 1995). They believed that the hippocampus was responsible for the initial rapid but temporary encoding of memory. Memory storage in the neocortex was believed to occur gradually, relying on repeated activations from the hippocampus to consolidate a memory trace. The fact that LTP induction in the neocortex followed a rule similar to memory storage provided strong support for LTP as a mechanism involved in memory storage.

The purpose of the present thesis is to build upon the initial characterization of neocortical LTP in freely behaving animals (Trepel and Racine, 1998; Chapman et al., 1998). This thesis will examine some of the mechanisms of LTP that have been shown mainly in hippocampal preparations. Specifically, the thesis will deal with three questions. First, does the property of associativity hold for neocortical LTP in chronic preparations? Second, does the property of associativity also hold for neocortical LTD? Finally, do metabotropic glutamate receptors play a role in neocortical LTP and LTD?

1.4 Correlation and Association

The essence of Hebb's (1949) theory of memory formation in neural circuits is captured by the following quote:

> When an axon of cell A is near enough to excite a cell B and repeatedly takes part in firing it, some growth process or metabolic changes take place so that A's efficiency of firing B is increased.

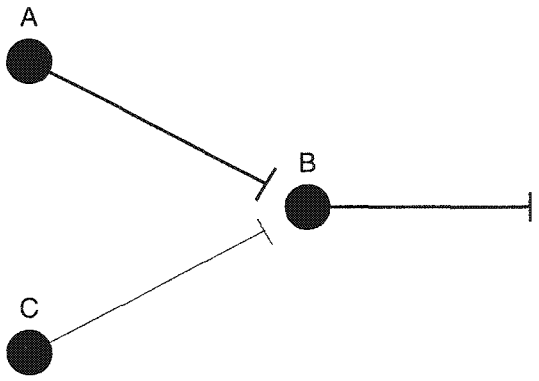

Figure 1.1: Simple circuit to illustrate Hebb's theory

This means that when cell A makes cell B fire—when their activity is correlated—the strength of the synaptic connection between the two cells will increase (Fig. 1.1). This correlation-based increase in synaptic strength has come to be known as associative learning because an association is being formed between some input and some output. However, the term 'association' has taken on different meanings in the literature.

The term associativity is sometimes used as described above, but sometimes it is used to mean some interaction between multiple inputs to a particular postsynaptic target. Following the terminology above, imagine another, independent, presynaptic cell (cell C) that is weakly connected to cell B. Because the connection between cell C and cell B is weak, cell C will not, by itself, cause B to fire very often. The lack of correlation between cell C and cell B will not result in a strengthening of the connection between them. However, if cell C is active at the same time as cell A (which is making cell B fire), then the activities of C and B will be correlated, and the connection between them will strengthen. In this instance the term associativity refers to the fact that stronger of the two inputs is, in a sense, helping the weaker

input.

These two meanings of associativity are in essence the same. In the case with the multiple inputs, the change in connection strength between the weak cell (C) and cell B is associative. The only difference is that cell C is too weak to drive cell B by itself, so another input (cell A) must drive the postsynaptic activity necessary to produce a correlation between C and B.

However, a distinction between the two meanings is being made here because they are used in the literature to focus on different underlying criteria. In some cases, associative changes in synaptic strength mean, operationally, changes based on correlated activity between a single input and a single output. In other cases, associative changes refer to changes based on correlated activation of multiple inputs. In this thesis, the term 'associative' will mean a situation where multiple inputs are active.

Chapter 2

Associative LTP

2.1 Introduction

Long-term potentiation was discovered and first characterized in the perforant pathway of the hippocampus (Bliss and Lomo, 1973). Following a high-frequency tetanus applied to the perforant path, there was a lasting enhancement of the synaptic response recorded from the dentate gyrus. Bliss and Lomo (1973) also noted that tetanizations of higher intensity resulted in larger potentiation. This was an important observation because it led to the discovery that afferents acted cooperatively during LTP induction.

A more intense current pulse delivered to neural tissue results in a greater spread of current in the tissue (Yeomans, 1990). If the electrode is situated in a bundle of axons, as in the case of the perforant path, more axons will become activated as the stimulus intensity increases. This suggests that a critical requirement of LTP induction is activation of a sufficient number of afferents.

McNaughton et al. (1978) tested this idea explicitly by exploiting an interesting

anatomical feature of the perforant pathway. The perforant pathway can be subdivided into distinct medial and lateral components based on axon origin and the locations of the synapses made onto the dentate gyrus granule cells. Axons of the lateral pathway terminate in the distal portion of the granule cell dendritic tree whereas axons of the medial pathway synapse in the proximal portion of the tree (Steward, 1976). By stimulating two separate inputs to the same population of cells, McNaughton et al. (1978) could better control the amount of afferent activation. They were now able to vary the number of input pathways activated as well as the intensity of stimulation.

One important consideration in their study was the independence of the two pathways. In order to demonstrate cooperative effects of multiple inputs, they had to demonstrate that the two pathways were independent of one another. Anatomical studies had shown the two pathways were separate from one anther, but it had to be shown that the two pathways could be stimulated independently. McNaughton and Barnes (1977) showed that two stimulating electrodes could be arranged so that activation of one pathway did not spread to the other pathway. They also showed that the two pathways did indeed converge on a common population of neurons. In addition to the physical independence it was also necessary to show that potentiation of one pathway did not spread to the other pathway. Andersen et al. (1977) demonstrated this in the CA1 region of the hippocampus. With one stimulating electrode positioned in the apical dendrites, and one in the basal dendrites, they showed that tetanization of one pathway led to a lasting potentiation that was restricted to the activated pathway. Based on these findings, McNaughton et al. (1978) could conclude that the pathways were independent, and begin to test cooperativity effects.

One of their most important findings was that at low stimulation intensities, LTP could not be induced reliably if either the lateral or medial pathways were tetanized

individually. However, LTP could be induced at low stimulation intensities if the two pathways were tetanized simultaneously. This demonstrated that the number of active afferents was critical for LTP induction and that convergent, but independent, pathways could act cooperatively in LTP induction.

The cooperative property of LTP soon extended into the idea of associativity between converging inputs. Could a weak input, one that does not potentiate when stimulated independently, be 'rescued' if it is paired with a strong input, one that does potentiate reliably following tetanization. Levy and Steward (1979) demonstrated this effect in the dentate gyrus of acutely prepared rats. During an acute experiment, the animal is anesthetized and mounted in a stereotaxic apparatus. All experiments are then performed under anesthesia. With a recording electrode in the dentate gyrus, they had one stimulating electrode in the ipsilateral entorhinal cortex and one in the contralateral entorhinal cortex. The contralateral pathway was considered the weak pathway because it failed to potentiate if tetanized alone. The ipsilateral pathway, as had been shown numerous times before, potentiated reliably following tetanization. When both pathways were tetanized simultaneously, the weak pathway potentiated.

Associative effects were later demonstrated at neocortical synapses in acutely prepared cats (Iriki et al., 1989, 1991). Iriki et al. (1989) combined tetanization of a weak pathway, which was otherwise resistant to LTP, with tetanization of a strong pathway and showed that LTP could be induced in the weak pathway. One stimulating electrode was located in the ventrolateral thalamus and the other was located in the adjacent somatosensory cortex. The recording electrode was situated in the motor cortex. In this preparation, the weak pathway was the thalamocortical pathway because it failed to induce LTP in motor cortex when tetanized independently. The strong pathway was the corticocortical pathway because it reliably induced LTP when tetanized independently. They hypothesized that the strong pathway could, in

effect, rescue the weak pathway by enabling its potentiation when both pathways were activated simultaneously. This is, in fact, what they found. When the two pathways were tetanized simultaneously, both showed a robust potentiation.

The bulk of previous research on associativity has been done in anesthetized or *in vitro* preparations, and most of that has been done in the hippocampus. Thus, it remains to be seen how the principle of associativity operates in the neocortex of the awake animal. The purpose of the present study was to examine cooperative properties of LTP at neocortical synapses in awake, freely behaving rats. The design of the experiment is similar to that of Iriki et al. (1989). A weak pathway was paired with a strong pathway to see if the weak pathway would potentiate. The recording electrode was located in the primary motor cortex and was activated via two independent input pathways: the callosal and thalamocortical pathways. The callosal pathway reliably yields LTP in awake animals, as long as the tetanizations are spaced and repeated (Trepel and Racine, 1998), but the thalamocortical pathway has proven resistant to LTP induction in awake animals (Ivanco, 1997). The results show that cooperative stimulation of the two pathways facilitates LTP induction in the callosal pathway, but, surprisingly, causes a depression of the thalamocortical pathway.

2.2 Materials and Methods

2.2.1 Animals and Surgery

Adult, male Long-Evans rats were anesthetized with sodium pentobarbital (65 mg/kg) and mounted into a Kopf small animal stereotaxic apparatus. The scalp was shaved and then disinfected prior to the first incision. The surface of the skull was exposed

and small burr holes were drilled through the skull to allow passage of the electrodes. Bregma was used as the reference point for stereotaxic coordinates (Paxinos and Watson, 1997). The recording electrode was located in the primary motor cortex and the callosal stimulating electrode was located 1 mm medial to the recording electrode in the white matter. The thalamic stimulating electrode was located in the ventrolateral nucleus. The electrode coordinates are listed in Table 2.1 and shown in Figures 2.1 and 2.2.

	A/P	M/L	D/V
Recording	+1.7	+3.0	-2.1
Callosal Stim.	+1.7	+2.0	-2.8
Thalamic Stim.	-2.3	+1.8	-6.0

Table 2.1: Coordinates for the electrode placements in mm relative to Bregma. A/P, anterior/posterior; M/L, medial/lateral; D/V, dorsal/ventral.

The electrodes were made by twisting together two Teflon-insulated stainless steel wires (inner diameter 125 μm, outer diameter 200 μm). The end of the electrode to be lowered into the brain was made by carefully cutting the pair of wires in half, thus exposing the stainless steel core of both tips. The end of the electrode to be fitted into the headcap assembly was prepared by stripping the Teflon insulation and crimping gold-plated pins to the end. The tip separations of the callosal and thalamic stimulating electrodes were 0.5 mm. The tip separation of the recording electrode was 1.8 mm, which resulted in one tip being located superficially in layer II/III and the other tip being located deep in layer V / layer VI.

Before lowering the electrodes into the brain, the dura mater was carefully punctured with a fine-gauge needle. Although stereotaxic coordinates were used to determine the initial depth of the electrode penetration, the electrodes were lowered under physiological control and the final depths of all electrodes were adjusted to

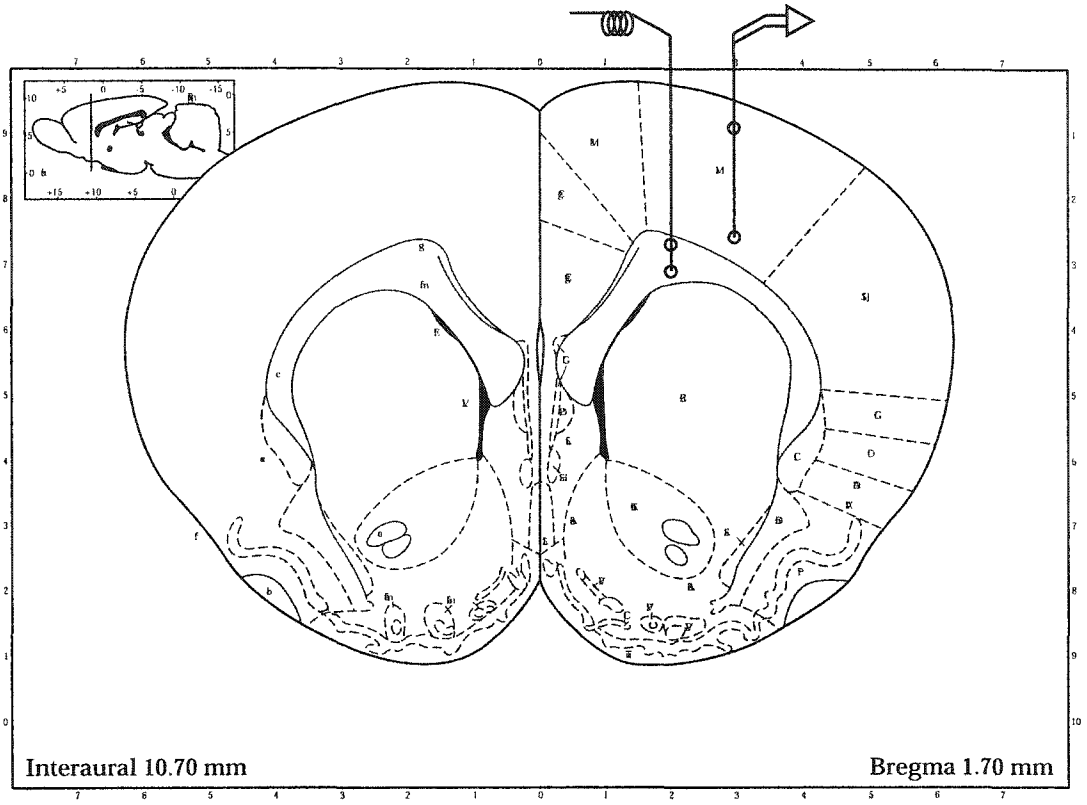

Figure 2.1: Placement of the callosal stimulating and recording electrodes

maximize the amplitude of the evoked response. The stimulating electrode was first lowered into position. Test pulses were then delivered to the stimulating electrode (0.1 Hz) as the recording electrode was gradually lowered. The response from the recording electrode was monitored for polarity reversal and adjusted to maximize both the surface-negative and deep-positive components of the field potential. Once the recording electrode was in position, the stimulating electrode was adjusted to minimize the stimulus intensity threshold. Once the optimal depths were achieved, the gold connectors of the electrodes were fitted into a miniature plastic screw connector. The whole electrode assembly was fixed to the skull surface using dental acrylic. The animals recovered for two weeks before the start of the experiment.

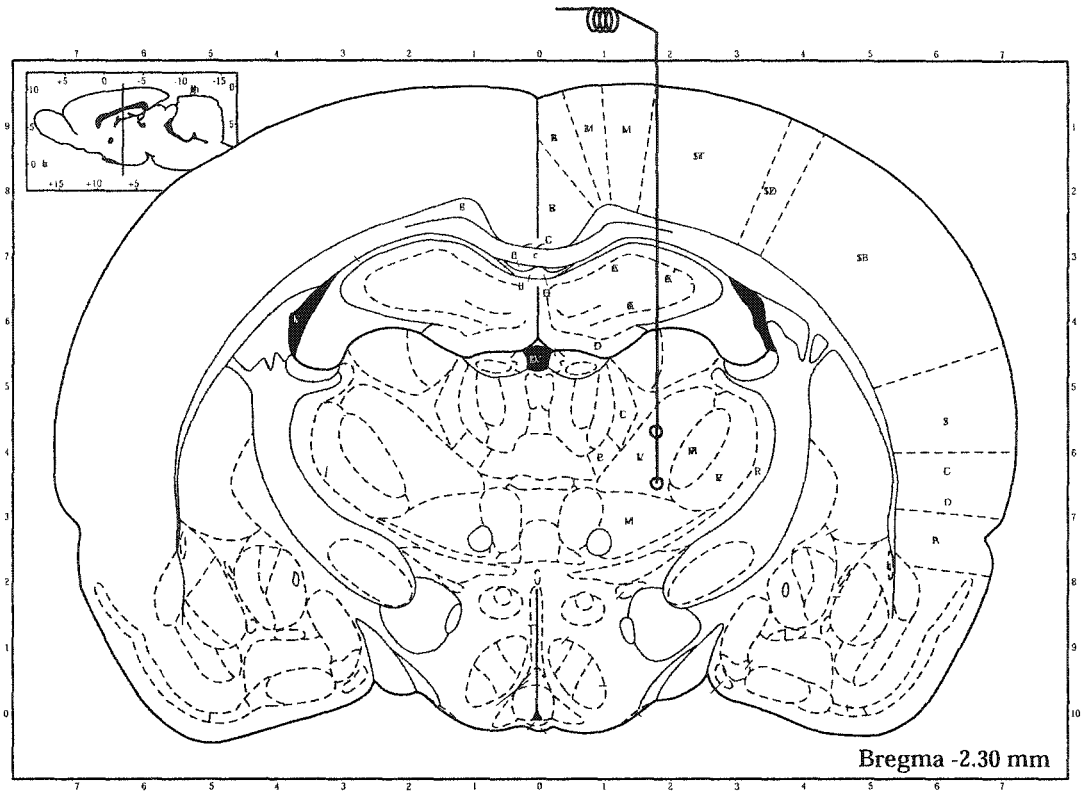

Figure 2.2: Placement of the thalamic stimulating electrode

2.2.2 Recording and Stimulation

The following general procedure was used for all data collection. Custom software written by the author in Labview (National Instruments) was used to control the data acquisition process (see Appendix A). First, a trigger pulse was sent to a Grass S88 stimulator. The grass stimulator generated a biphasic square pulse (100 μs per phase) of fixed voltage. The voltage pulse was converted to a constant current pulse by passing it through a stimulus isolation unit (Grass model PSIU6) before being delivered to the animal. The neural activity evoked by the electrical pulse was amplified and bandpass filtered (0.3 Hz–3 kHz half amplitude frequencies) using a Grass

model 12 amplifier. The amplified response was digitized (10 kHz, 12-bit) and stored on computer for off-line analysis. An example of a field potential evoked by callosal stimulation is shown in Fig. 2.3.

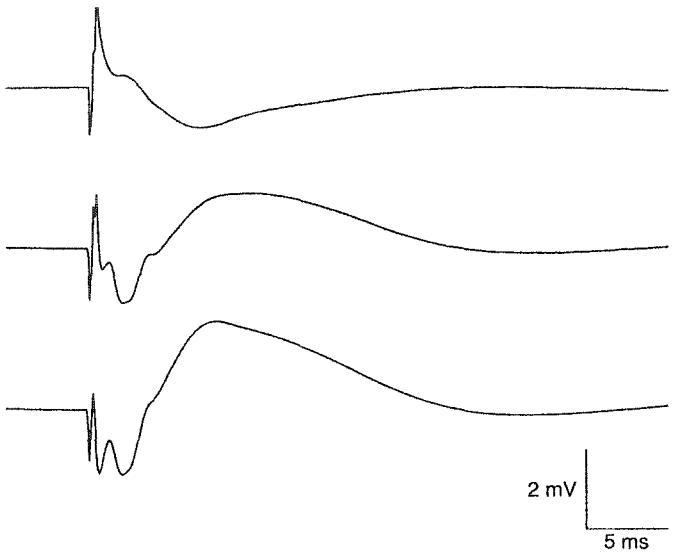

Figure 2.3: Example evoked potential. The upper trace shows the monopolar response from the superficial tip. The middle trace shows the monopolar response from the deep tip. The bottom trace shows the bipolar response.

Baseline measures of the evoked responses were taken every second day for one week to ensure stability of response amplitudes. Baselines measures consisted of full input/output functions. One input/output function consisted of measuring the amplitude of the response to varying intensities of stimulation. The intensities used to stimulate were: 15, 40, 100, 160, 250, 400, 630, and 1000 μA . An example input/output function is shown in Fig. 2.4.

Once baseline measures were complete, the animals were assigned to one of four groups. When assigning animals to groups, an attempt was made to match the response morphologies and amplitudes between groups. The first group received

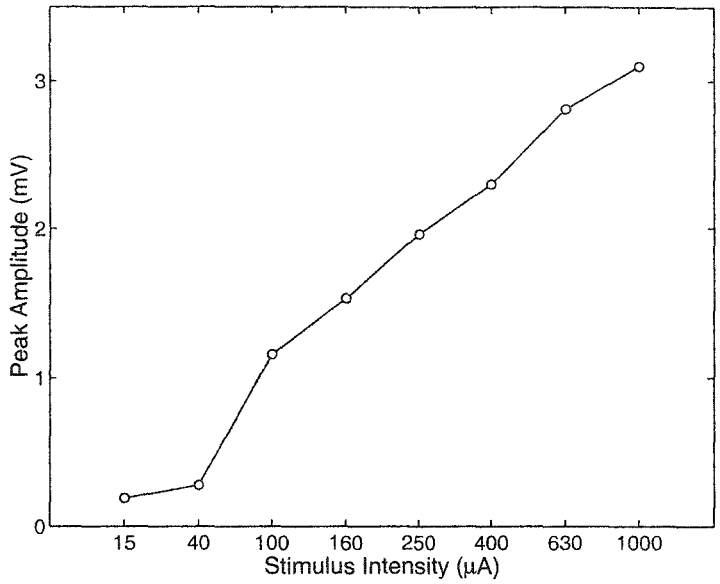

Figure 2.4: Example input/output function. The amplitude of the field potential is plotted as a function of the intensity of the test pulse.

high-frequency stimulation simultaneously to both the callosal and thalamic pathways (n = 10). The second group received high-frequency stimulation to the callosal pathway only (n = 8). The third group received high-frequency stimulation to the thalamic pathway only (n = 6). The fourth group did not receive any high-frequency stimulation (n = 7).

High frequency stimulation occurred every 24 hours for a total of 12 sessions. One session consisted of 60 trains being delivered at 0.1 Hz. A single train consisted of 8 pulses at 300 Hz. The pulse amplitude was 500 μA . This intensity was chosen because it has been shown to reliably induce LTP, but the induction rate is slower than that for higher intensities and the amplitude change is also smaller (Trepel and Racine, 1998). If pairing stimulation of the callosal and thalamic pathways does have an effect, then it can manifest as either a faster induction rate or a larger amplitude change (or both). The choice of the weaker stimulation intensity would prevent these

differences from being masked by ceiling effects.

During the LTP induction phase, input/output functions were collected every second day. Following the 12 days of LTP induction, two final input/output functions were collected at one week intervals to track the decay of any changes in amplitude.

2.2.3 Tests for Convergence

The validity of this experiment relies on the two stimulating electrodes activating different populations of fibers that converge on a common population of cells. In order to verify these assumptions, the following tests were performed (McNaughton and Barnes, 1977). First, to test that different populations of fibers were being stimulated, the two pathways were tested for heterosynaptic facilitation effects. Paired-pulse facilitation occurs when two pulses are delivered in rapid succession and the second of the two evoked responses is larger than the first. Paired-pulse facilitation is thought to occur presynaptically, through an accumulation of Ca^{2+} in the presynaptic terminal caused by the first pulse (Katz and Miledi, 1968). If the two stimulating electrodes are activating distinct populations of fibers, then there should be no heterosynaptic facilitation—a pulse delivered to one pathway should not facilitate the response to a pulse delivered to the other pathway. If the electrodes are activating overlapping populations of fibers, then heterosynaptic facilitation should occur.

Figure 2.5 shows the results of the homosynaptic and heterosynaptic facilitation tests. The thalamic pathway showed a marked paired-pulse facilitation (C), but the callosal pathway did not (A). This result alone suggests that the two pathways contain different populations of fibers because one shows facilitation and one does not. This conclusion is further strengthened by a lack of any heterosynaptic facilitation (B and D). In particular, a lack of heterosynaptic facilitation when the thalamus

was stimulated first (which shows strong homosynaptic facilitation) provides strong evidence for independence of the two pathways.

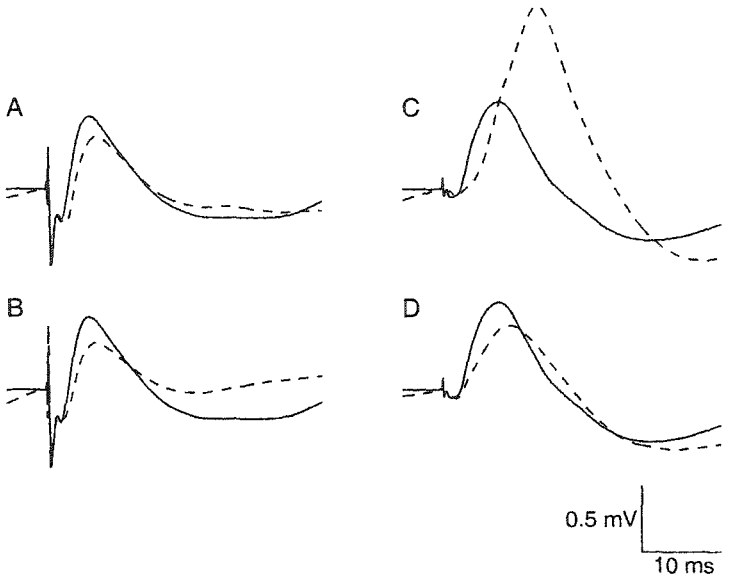

Figure 2.5: Test for the independence of the two pathways. Responses to homosynaptically and heterosynaptically delivered paired-pulses (IPI = 50 ms). The left column shows the response of the callosal pathway when the first pulse is delivered to the callosal (A) or thalamic (B) pathways. The solid lines show the response to the first pulse, the dashed lines show the response to the second pulse (in B, the callosal response to the first pulse is plotted instead of the thalamic response for ease of comparison). The right column shows the response of the thalamic pathway when the first pulse is delivered to the thalamic (C) or callosal (D) pathways.

Two tests were performed to determine if the two pathways converged onto a common population of cortical neurons. First, a high frequency train was delivered to one pathway and immediately followed by a single test pulse to the other pathway. The high frequency train should drive any post-synaptic neurons close to the reversal potential for the synaptic response. This should prevent much further current flow when the test pulse is delivered to the other pathway, resulting in a greatly reduced field potential (McNaughton and Barnes, 1977). This is indeed what occurs (Fig. 2.6).

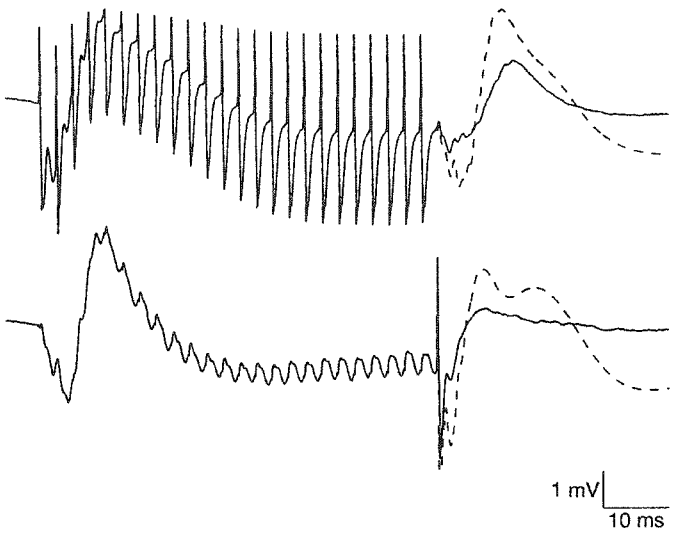

Figure 2.6: Test for convergence of the two pathways. In the upper trace, a 400 Hz, 60 ms train was delivered to the callosal pathway, followed by a single test pulse to the thalamic pathway. A normal thalamic response is plotted as a dashed line for reference. In the lower trace, the train is delivered to the thalamic pathway, followed by a test pulse to the callosal pathway. In both cases, the response evoked by the single test pulse is much smaller than normal.

The second test for convergence compares the size of the potential evoked by simultaneous activation of the two pathways to the arithmetic sum of the independently evoked responses. If the two pathways converge on the same population of neurons, the potential evoked by coactivation should be smaller than the sum of the independently evoked potentials (McNaughton and Barnes, 1977). As shown in Figure 2.7, the potential evoked by coactivation is much smaller than the sum of the two independently evoked potentials.

2.2.4 Histology

Following the recording of the final input/output function, an anodal DC current was passed through the tip of each electrode (10 μA for 20 s). The passage of anodal

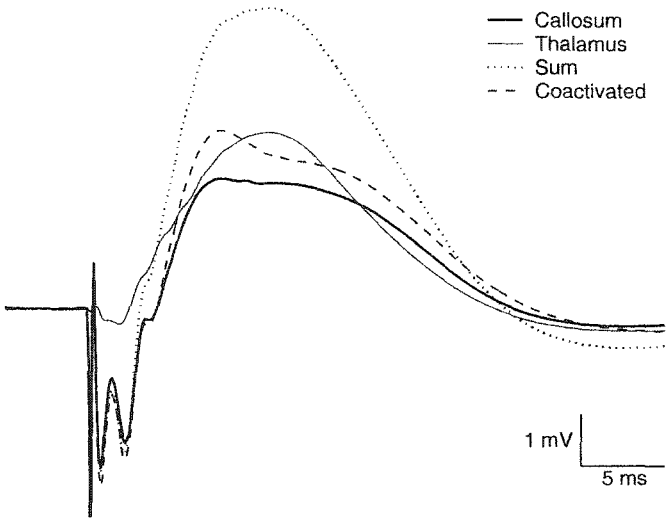

Figure 2.7: Test for convergence of the two pathways. Shown are field potentials evoked by independent and coactive stimulation, as well as the linear sum of the two independently evoked potentials. The smaller coactive potential (compared to the linear sum) confirms convergence of the two pathways.

current through the stainless steel electrode causes a small amount of Fe^{2+} atoms to be deposited in the tissue at the tip location. This deposit can be visualized with the Prussian Blue stain (described below).

Following the marking of the tip locations, the animals were perfused through the heart with phosphate-buffered saline (PBS, pH 7.4) followed by cold 10% formalin in PBS. Following extraction, the brains were post-fixed for 24 hours and cryoprotected (30% sucrose in PBS) for another 24–48 hours. 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 Prussian Blue followed by a Neutral Red counter-stain. The Prussian Blue stain, which stains Fe^{2+} deposits blue, consists of:

- 2% potassium ferrocyanide

- 1% hydrochloric acid

Neutral Red is similar to Cresyl Violet in that they both stain acidic proteins and thus primarily stain cell bodies. The difference is that the Neutral Red provides a red stain colour which was necessary to visualize the Prussian Blue stain. Following staining, the tissue was dehydrated, cleared with Xylenes, and coverslipped. Representative photomicrographs of electrode placements are shown in Figure 2.8.

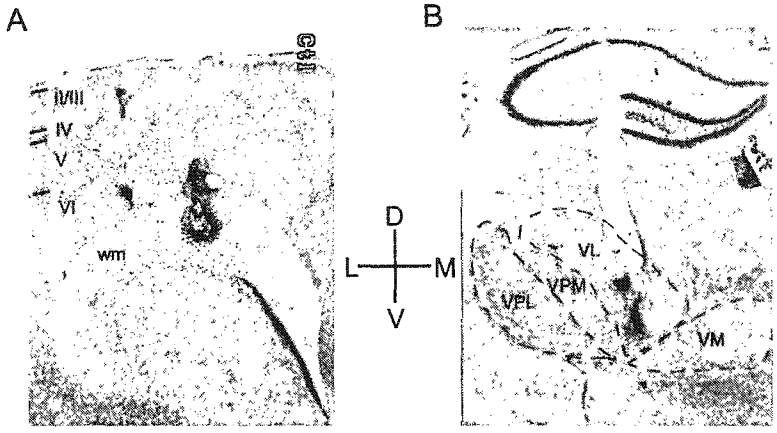

Figure 2.8: Representative photomicrographs showing placement of electrodes. A, Callosal stimulating (located medially) and recording (located laterally) electrodes. The dark regions indicate the prussian blue stain marking the tip locations. B, Thalamic stimulating electrode.

2.2.5 Data Analysis

The amplitude of the evoked potential served as the primary measure throughout most of these experiments. The bipolar response was used for most of the data analysis because it provides the largest amplitude response with a very high common mode rejection of any noise present. For the callosal pathway the amplitude of the evoked potential was tracked at two latencies. The first latency (mean 7.1 ms) is

termed the early component, and the second latency (mean 19.6 ms) is termed the late component (Trepel and Racine, 1998). An example is shown in Fig. 2.9. For the thalamic pathway the amplitude was tracked at a single intensity as there was no evidence of a late component (mean 10.3 ms).

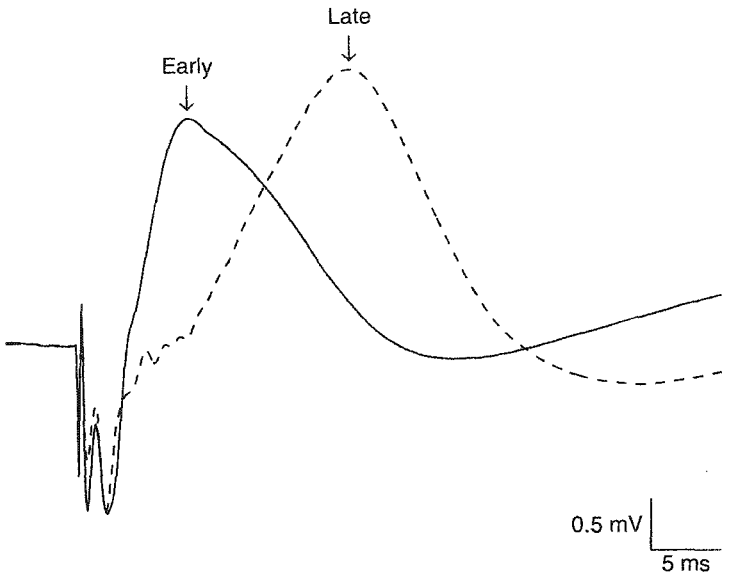

Figure 2.9: Latencies of early and late components of the callosal pathway

Analysis of the raw data was performed with custom software written by the author in Matlab (see Appendix A). The basic procedure was to plot a baseline and post-LTP sweep, as in Fig. 2.9, and then manually select the latencies at which the amplitudes are to be measured. Once the latencies were selected, the amplitudes of the evoked-potential components were automatically extracted for each day. For each animal, the amplitudes were standardized as changes relative to the average of the four baseline measures. Thus, any changes in amplitude are expressed as changes in millivolts relative to the average baseline value. A repeated measures ANOVA was performed on the amplitude measures to establish significance (using STATISTICA

software). Measurements of amplitude were always done at an intensity near the middle of the input/output function, either 160 or 250 μA , where the difference between the baseline and potentiated response is reliably the greatest.

The amplitudes of control animals change very little over the course of the experiment. This makes it difficult to select the latencies visually because late component peaks are not often clear in control responses. Consequently, a slightly different procedure was used for their analysis. For these animals, the average latencies of the potentiated animals were used to extract the amplitudes.

2.3 Results

Combined tetanization of the callosal and thalamic pathways had different effects on the two pathways. The callosal pathway exhibited increased potentiation, compared with the potentiation that occurs following tetanization of the callosal pathway alone. The thalamic pathway, however, exhibited a depression following the paired tetanization as well as following stimulation of the thalamic pathway alone.

2.3.1 Callosal Pathway Raw Data

Figure 2.10 shows representative raw data from the callosal pathway following the different stimulation procedures. Potentiation of the callosal pathway is characterized by an apparent decrease in the amplitude of the early component and an increase in the amplitude of the late component (Trepel and Racine, 1998; Chapman et al., 1998). An example is shown in Fig. 2.10(B). The apparent decrease in the early component is due to a potentiation of population spikes, which is visible as the small ripples in the potentiated sweep (Chapman et al., 1998). To the recording electrode, population spikes look like negative going wave forms. As they potentiate, they become more

negative. When this is superimposed on the positive going field potential, it looks like a decrease, or reversal, in amplitude, but it is a measure of potentiation.

Combined tetanization of both pathways (Fig. 2.10 A) produced a potentiation similar in morphology as that produced by callosal tetanization alone (Fig. 2.10 B). The main difference between the two conditions is that the potentiation of the late component was larger for the paired condition, although this is not immediately apparent from the raw data (statistical analyses will be discussed below).

Tetanization of the thalamic pathway had no effect on the callosal pathway (Fig. 2.10 C). Thus the synergistic effect of thalamic stimulation exists only when paired with callosal stimulation.

Figure 2.10(D) shows the callosally evoked field potentials from a control animal, which remained stable throughout the experiment.

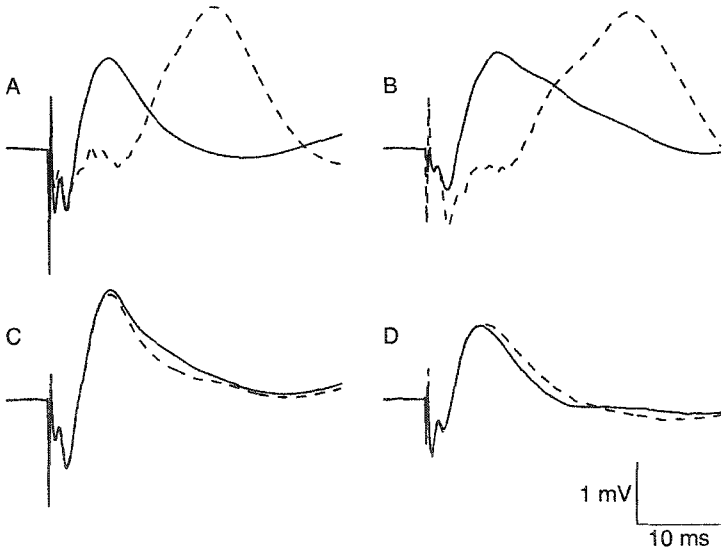

Figure 2.10: Field potentials evoked by callosal stimulation before and after tetanization of both pathways (A), the callosal pathway (B), and the thalamic pathway (C). Control animals are shown in (D). Solid lines are baseline responses and dashed lines are from the last day of tetanization.

2.3.2 Thalamic Pathway Raw Data

Figure 2.11 shows the raw data for the thalamic pathway. Surprisingly, tetanization of the thalamic pathway produced a depression of the field potential (Fig. 2.11 C). There was no evidence that this was due to potentiation of population spikes, as was the case with the callosal pathway, because there was no sign of population spike morphology and there was no development of the late component.

The depression was more pronounced when stimulation of the thalamus was combined with stimulation of the callosum (Fig. 2.11 A), the same condition that yielded a marked potentiation of the callosal pathway. The morphology of the depression in the paired tetanization group was similar to the thalamic-only tetanization group, except for the fact that it was more pronounced.

Tetanization of the callosum, by itself, did not have an effect on the thalamic pathway (Fig. 2.11 B). Thus, as was the case with the callosal pathway, there were no purely heterosynaptic effects. The increased depression of the paired tetanization group must be due to the effects of combined stimulation of the pathways.

Figure 2.11(D) shows the thalamic field potentials of a control animal, which did not change throughout the course of the experiment.

2.3.3 Callosal Input/Output Functions

Figures 2.12–2.13 show the data from the callosal pathway for all intensities of stimulation. The plotted input/output curves are from the last baseline measure and from the day following the last tetanization. The curves for each group have been normalized to the amplitude of the baseline response to the strongest test pulse (1000 μA).

The potentiation observed following callosal tetanization was similar to that previously reported by our lab (Chapman et al., 1998). There was a reduction in the

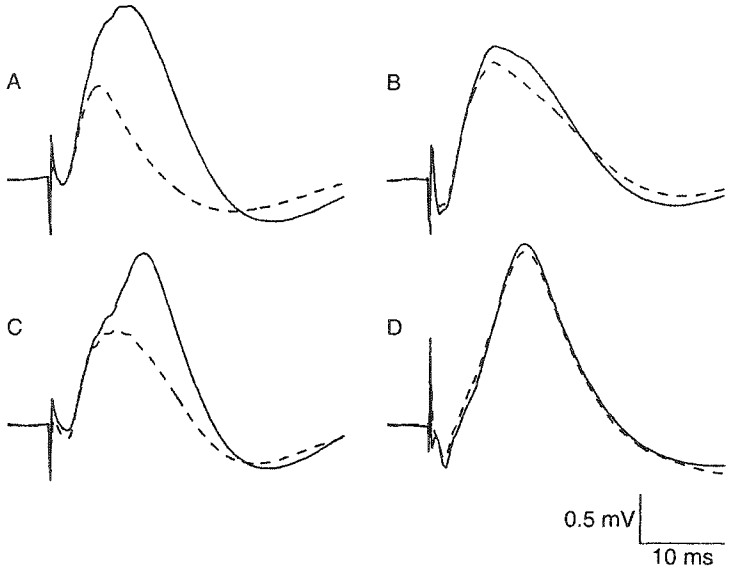

Figure 2.11: Field Potentials evoked by thalamic stimulation before and after tetanization of (A) both pathways, (B) the callosal pathway, and (C) the thalamic pathway. Control animals are shown in (D). Solid lines represent baseline responses and dashed lines are 24 hours after the last tetanization.

amplitude of the early component (due to population spike potentiation) following tetanization, an effect that was virtually uniform across intensities (Fig. 2.12). Both the main effect of group and the interaction were significant ($F_{(1,14)} = 22.24$, $p < 0.001$ and $F_{(7,98)} = 11.3$, $p < 0.001$ respectively). The early component of the paired group behaved similarly—there was a consistent reduction of amplitude across intensities. The main effect of group and the interaction were significant ($F_{(1,18)} = 27.66$, $p < 0.001$ and $F_{(7,126)} = 16.42$, $p < 0.001$ respectively). Paired tetanization of the callosal and thalamic pathways did not appear to facilitate potentiation of the callosally-evoked early component as the magnitude of potentiation was similar for the paired and callosal groups.

Tetanization of the thalamic pathway showed no heterosynaptic effects as the amplitudes of the callosal field potentials remained unchanged. The same was true

for the control group. There were no significant effects for either group.

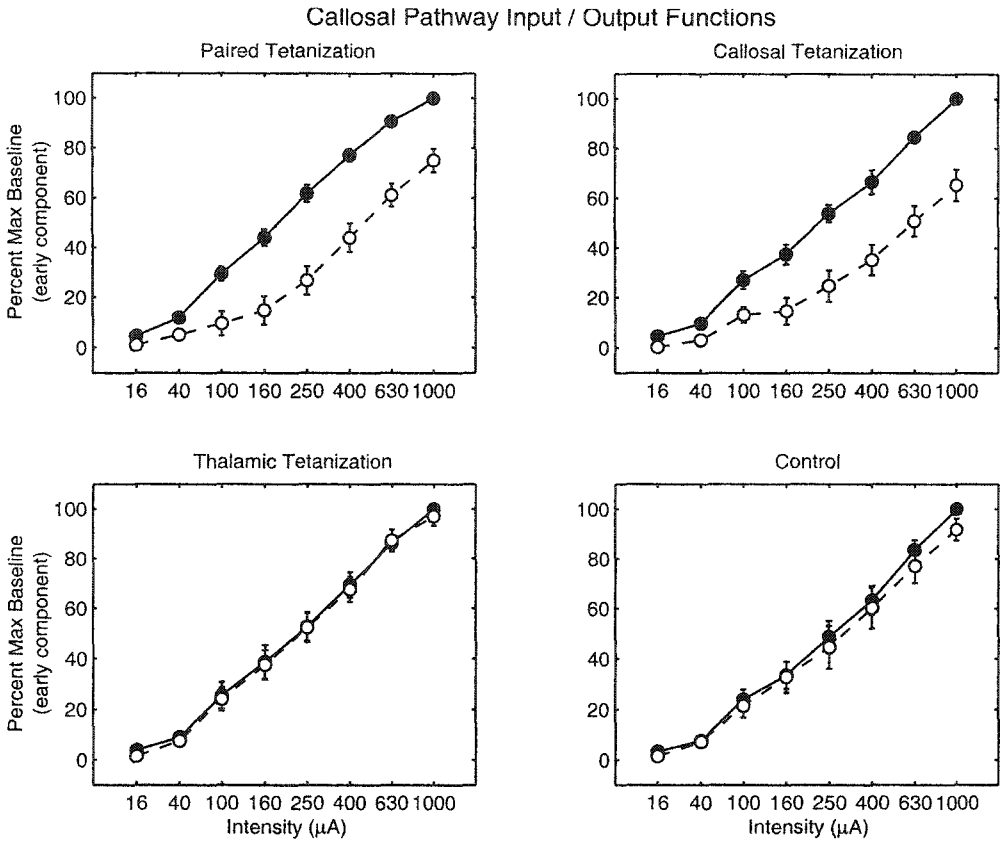

Figure 2.12: Early component input/output functions from the callosal pathway before and after tetanization (figure title indicates tetanization condition). Solid lines and filled symbols are baseline measures, dashed lines and open symbols are from the last day of tetanization.

Combined activation of the two input pathways did have an effect on the late component. For the callosal group (Fig. 2.13), the potentiation reduced the threshold to activation—the baseline amplitude at 100 μA is roughly 0%, whereas it is 20% following the daily tetanizations. At mid-range intensities, the potentiation is strongest, and it becomes less apparent at the highest intensities. Both the main effect of group and the interaction were significant ($F_{(1,14)} = 25.68$, $p < 0.001$ and $F_{(7,98)} = 9.86$,

$p < 0.001$ respectively).

When comparing the paired tetanization group to the callosal tetanization group, it is clear that paired tetanization of callosal and thalamic pathways produced a larger potentiation (Fig. 2.13). From the curves, it is difficult to tell whether the threshold to activation was lowered further. At 40 μA the potentiated amplitude is greater, but it is not a big difference. However, the difference at 100 μA is approximately 60% greater than for the callosal-only tetanization group. It is possible that a test intensity, between 40 and 100 μA would have revealed more clearly a reduced threshold. At middle intensitites the potentiation is more pronounced for the paired tetanization condition. Furthermore, the potentiation is still apparent at the highest intensities, which was not observed for the callosal-only tetanization condition. The main effect of group and the interaction were both significant ($F_{(1,18)} = 50.21$, $p < 0.001$ and $F_{(7,126)} = 29.46$, $p < 0.001$ respectively).

The input/output functions for the thalamic-only tetanization condition are virtually identical before and after tetanization. Thus, there was no purely heterosynaptic effect of tetanizing the thalamic pathway; the increased potentiation observed in the paired condition must be a result of the combined activation of the two pathways. The input/output functions for the control group show that the amplitudes remained stable over the course of the experiment. There were no significant effects for the thalamic-only and control groups.

2.3.4 Thalamic Input/Output Functions

Figure 2.14 shows the data for all intensities of stimulation. Tetanization of the thalamic pathway caused a depression of field potential amplitudes at all intensities (Fig. 2.14 C). The threshold to activation was raised and the middle and high inten-

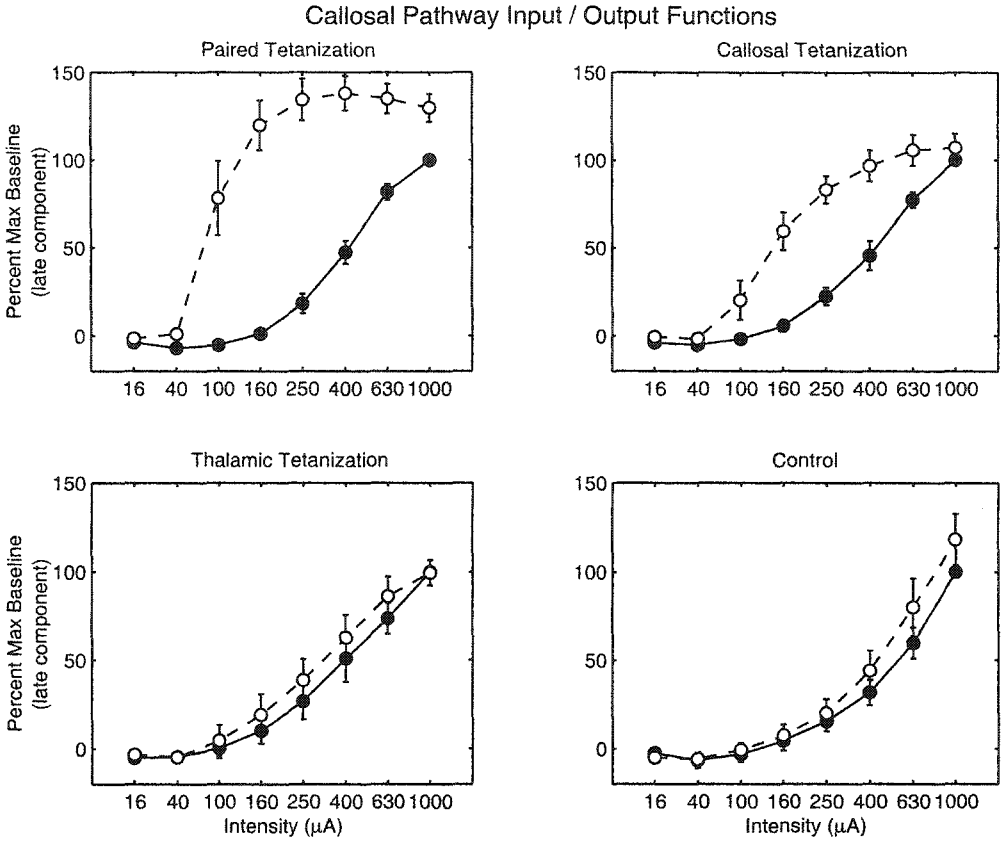

Figure 2.13: Late component input/output functions from the callosal pathway before and after tetanization (figure titles indicate different tetanization conditions). Solid lines and filled symbols are baseline measures, dashed lines and open symbols are from the last day of tetanization.

sities show a consistent reduction of amplitude, although neither the main effect or the interaction were significant ($F_{(1,10)} = 4.54$, $p = 0.06$ and $F_{(7,70)} = 1.64$, $p = 0.14$ respectively).

When tetanization of the thalamic pathway was combined with tetanization of the callosal pathway the depression was pronounced (Fig. 2.14 A). The threshold was raised and the amplitudes at the higher intensities were depressed by approximately 20%. Both the main effect of group and the interaction were significant ($F_{(1,18)} = 32.6$,

$p < 0.001$ and $F_{(7,126)} = 7.64$, $p < 0.001$ respectively).

Tetanization applied to the callosal pathway alone had no effect on the thalamic input/output function (Fig. 2.14 B). Thus the depression in the paired group was not simply due to heterosynaptic effects, but was a result of the combined activation of the two pathways.

The input/output functions of the control animals did not change over the course of the experiment (Fig. 2.14 D).

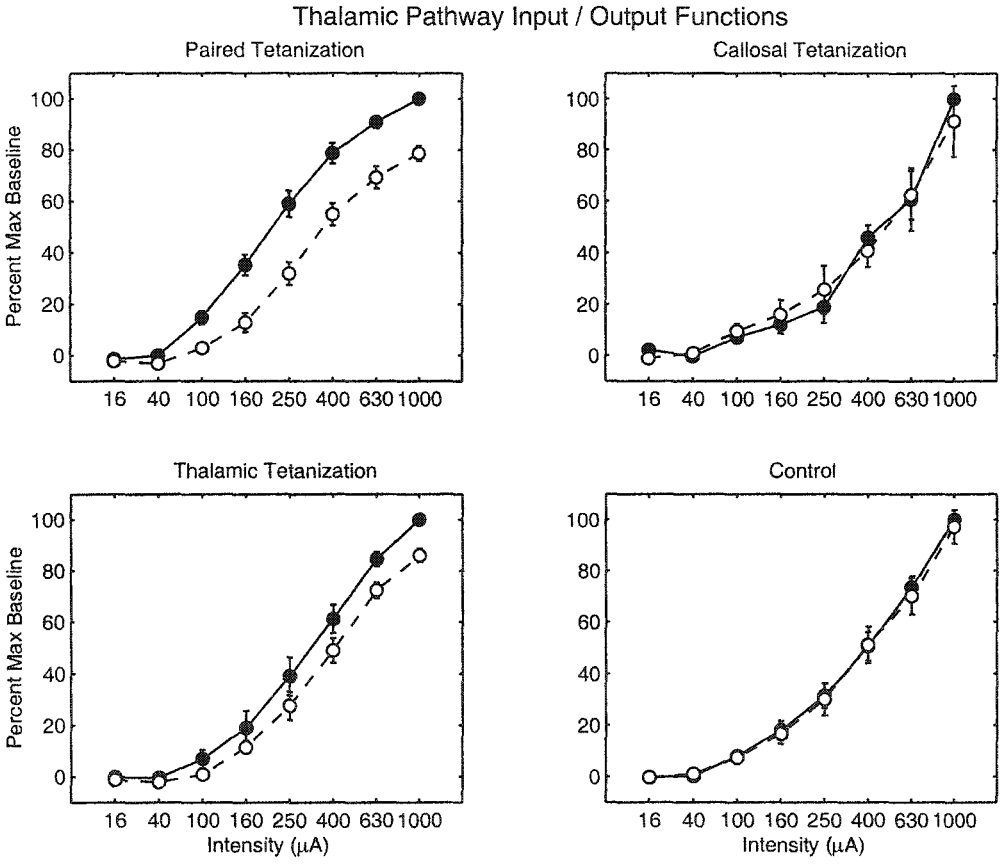

Figure 2.14: Thalamic input/output functions before and after tetanization (figure titles indicate tetanization condition). Solid lines and filled symbols are baseline responses, dashed lines and open symbols are from the last day of tetanization.

2.3.5 Callosal Group Data Across Days

Figure 2.15 shows the early component of the callosal pathway for the different tetanization conditions. The data in this figure was generated by analyzing a single intensity of test pulse (160 μA) across days. Both the paired and callosal-only tetanization groups behaved similarly with respect to the early component. The thalamic tetanization and control groups showed no lasting changes.

Potentiation of the early component developed gradually for both the paired and callosal only tetanization groups. Signs of potentiation were evident within the first couple of days following the start of tetanization. The potentiation increased throughout the 12 days of tetanization, without showing signs of saturation. Following tetanization, the potentiation decayed gradually over two weeks to roughly half of its peak value. There was no difference between the paired and callosal tetanization although there was a slight trend toward stronger potentiation in the paired group.

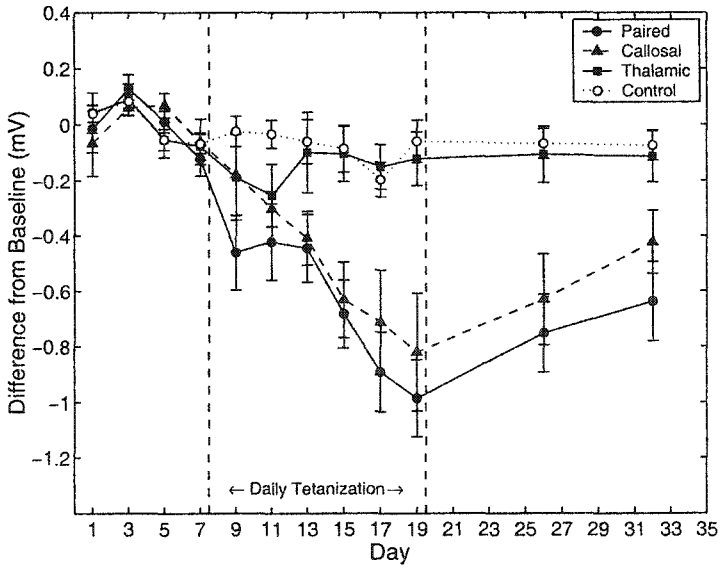

Figure 2.15: Change in the early component of the callosal pathway across days

The paired and callosal tetanization groups behaved differently with respect to potentiation of the late component (Fig. 2.16). In general the potentiation for both groups was slightly slower to develop compared to the early component. Once the potentiation began, the paired group was faster to develop than the callosal group, although both groups continued to potentiate through the 12 days of tetanization. The magnitude of the potentiation in the paired group was significantly greater than the callosal group at the end of the 12 days of tetanization ($p < 0.001$, Tukey HSD).

Following the tetanization, the paired group decayed faster than the callosal group. After one week, the difference between the groups was smaller, but still significant ($p < 0.05$, Tukey HSD). After two weeks the difference between the groups was smaller still and was no longer significant. It is interesting to note that although the paired group produced a larger, faster potentiation effect, it was not longer lasting.

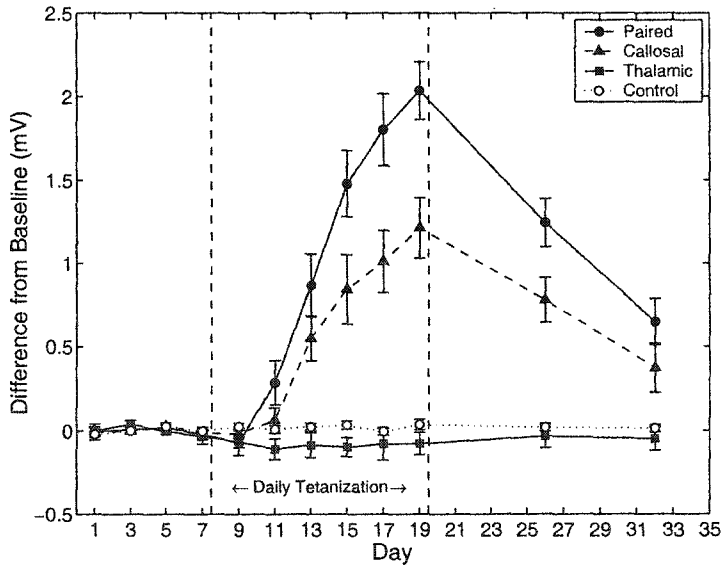

Figure 2.16: Change in the late component of the callosal pathway across days

2.3.6 Thalamic Group Data Across Days

The thalamocortical pathway depressed in the paired and thalamic only tetanization groups, but it did not change in the callosal only tetanization group or the control group (Fig. 2.17).

Both the thalamic only and paired tetanizations produced a depression ($F_{11,121} = 6.73$, $p < 0.001$ and $F_{11,165} = 8.81$, $p < 0.001$). For the thalamic group, the depression was evident within the first few days of stimulation. After 4 days of tetanization, the depression asymptoted and did not develop further. Like the thalamic only group, the paired group depression developed within the first few days of tetanization. The paired group, however, continued to depress after the thalamic group had asymptoted and thus the decrease in field potential amplitude was larger. After 8 days of tetanization, the paired group also asymptoted and did not depress further for the remaining 4 days. Although the depression produced following paired tetanization appeared larger, it did not prove to be significantly different from the thalamic tetanization group ($F_{1,14} = 0.75$, $p = 0.2$).

The depression of the thalamic pathway differed from the potentiation of the callosal pathway with respect to decay rate. Recall that the callosal pathway, though it was still potentiated two weeks after tetanization, decayed significantly over the two weeks. The depression of the thalamic pathway did not show any sign of decay, and the field potential amplitudes remained steady at their depressed levels.

Tetanization of the callosal pathway itself did not exert any heterosynaptic effects on the thalamic pathway. Field potential amplitudes of the callosal group remained constant, much like the control group.

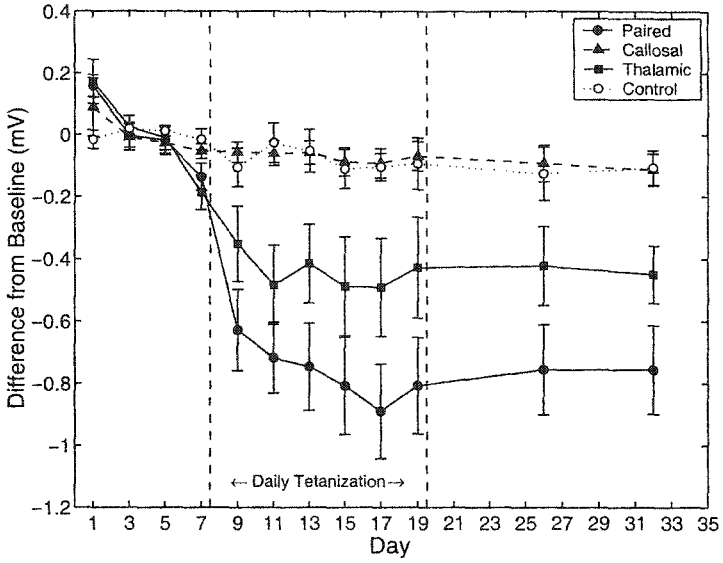

Figure 2.17: Change in the thalamic pathway across days

2.3.7 Laminar Analysis

In an attempt to understand the laminar contributions to the observed effects, the monopolar responses were analyzed individually. Although having only two tips in the cortex will not provide much laminar resolution, it should be informative about whether the effects are occurring superficially or deep in the cortex. For these analyses, the amplitudes of the monopolar responses were normalized to the amplitude of the bipolar response from the day after tetanization.

Previous current source density (CSD) analyses have shown that tetanization of the callosum potentiates a sink located in upper layer V (Chapman et al., 1998). The present analysis of the callosal pathway agrees with this (only the data from the paired and callosal tetanization groups are presented as they were the only ones to show an effect). Both the early (Fig. 2.18) and late (Fig. 2.19) components of the callosal pathway appear to be driven by changes in the deep layers. Most of the potentiation

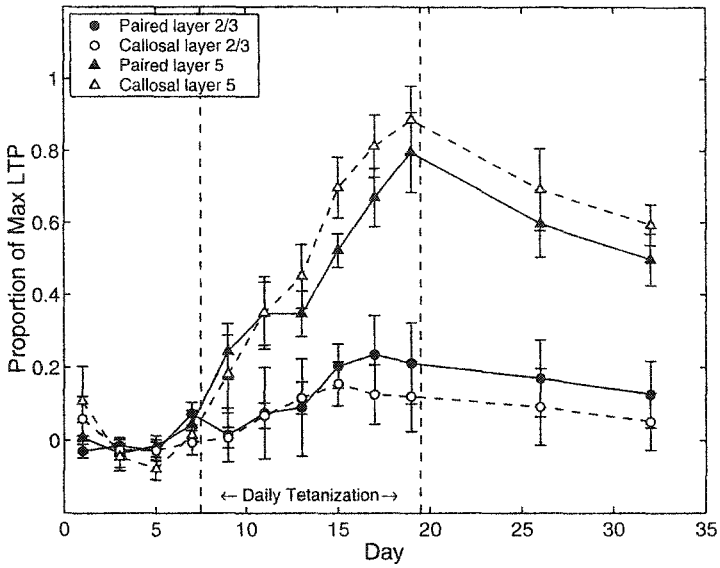

Figure 2.18: Laminar analysis of the callosal early component. Data from the superficial and deep tips of the recording electrode are plotted individually for both the paired and callosal tetanization groups. The data from each animal were normalized to the amplitude of the bipolar response on the last day of tetanization.

appeared to occur in the deep layers, with little occurring in the superficial layers. The same trend was observed for the late component, but it was not as pronounced; voltage changes in the superficial layers contributed slightly more to the potentiation. There was no obvious difference between the paired and callosal tetanization groups suggesting that the laminar profiles for the enhanced LTP of the paired group was similar to that of the callosal group, except in magnitude.

The same trend did not appear to hold true for the thalamic pathway (only the data from the paired and thalamic tetanization groups is presented). Although the data is noisier, both the superficial and deep layers appeared to contribute equally to the depression. Again, there was no apparent difference between the paired and thalamic groups.

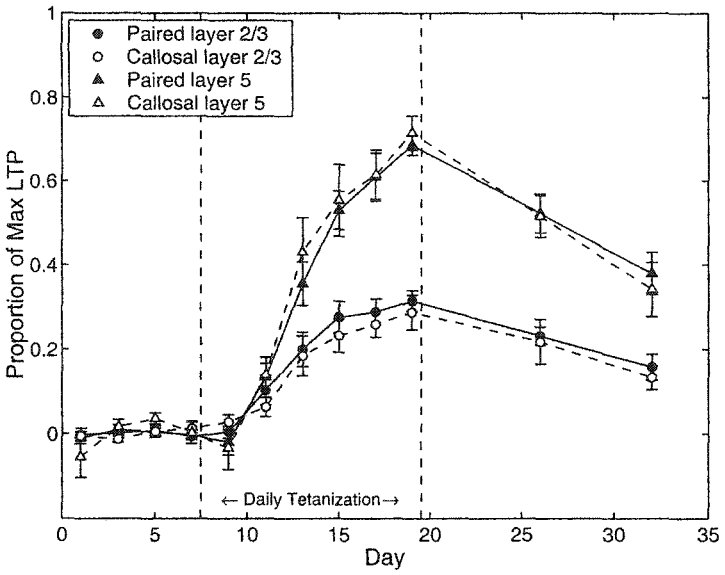

Figure 2.19: Laminar analysis of the callosal late component. Data from the superficial and deep tips of the recording electrode are plotted individually for both the paired and callosal tetanization groups. The data from each animal were normalized to the amplitude of the bipolar response on the last day of tetanization.

2.3.8 Correlations

Possible correlations were examined for two situations. In the first situation, the relationship between early and late component potentiation was examined for both the paired and callosal-only tetanization groups (Fig. 2.21). These groups are plotted with different symbols, but the data were pooled for the analysis. Somewhat surprisingly there wasn't a strong correlation between the two components. It might be expected that the magnitude of the change in the monosynaptic (early) component would contribute significantly to the magnitude of change in the polysynaptic (late) component. However, the early component data did not account for much of the variability in the late component data ($r^2 = 0.19$, $p = 0.07$).

In the second situation, the magnitude of the potentiation observed in the callosal

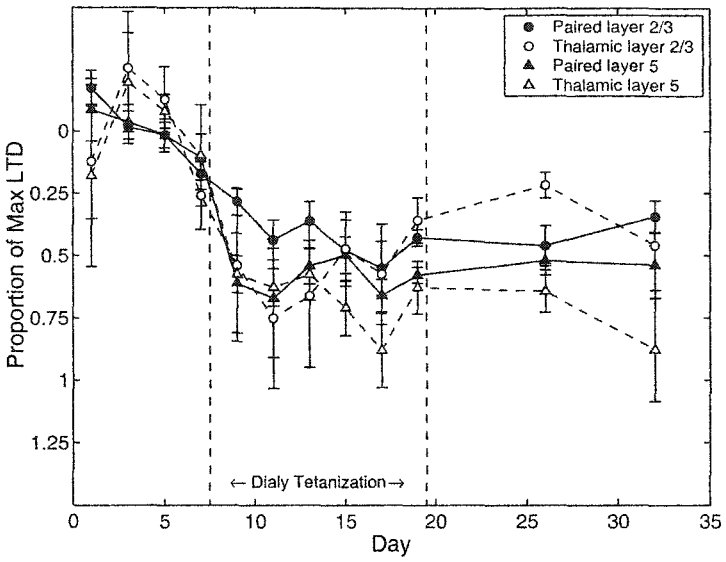

Figure 2.20: Laminar analysis of the thalamic pathway. Data from the superficial and deep tips of the recording electrode are plotted individually for both the paired and thalamic tetanization groups. The data from each animal were normalized to the amplitude of the response on the last day of tetanization.

pathway was compared to the magnitude of depression observed in the thalamic pathway (for the paired tetanization group). The analysis was performed for both the early and late components of the callosal pathway. There was a weak correlation between the thalamic depression and the callosal early component, but it was not significant (Fig. 2.22, $r^2 = 0.27$, $p = 0.12$). There was no correlation between the thalamic depression and the callosal late component ($r^2 = 0.02$, $p = 0.72$). The lack of relationship between the two pathways is perhaps not surprising as there were no purely heterosynaptic effects observed (e.g., tetanization of the callosal pathway alone did not affect the thalamic pathway).

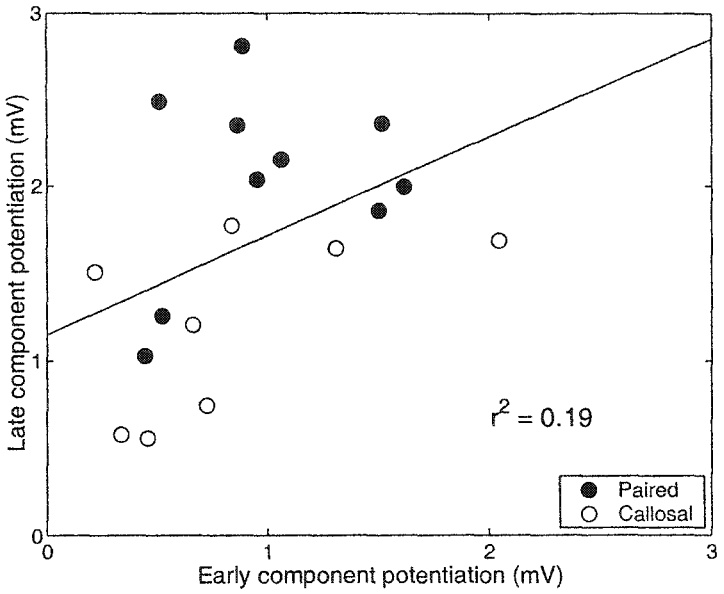

Figure 2.21: Correlation between potentiation of callosal early and late components

2.4 Discussion

There were two main questions about associative LTP addressed in this experiment. First, would cooperative stimulation of afferents facilitate LTP induction in a pathway known to support LTP, if the stimulus intensity was reduced? Second, would cooperative stimulation of afferents induce LTP in a pathway that does not support LTP if stimulated independently? The answer to the first question is a qualified 'yes' as facilitation of LTP induction was observed in the callosal pathway late component, but not the early component. The answer to the second question is no. Not only did the thalamic pathway fail to show LTP, a depression was induced following cooperative stimulation.

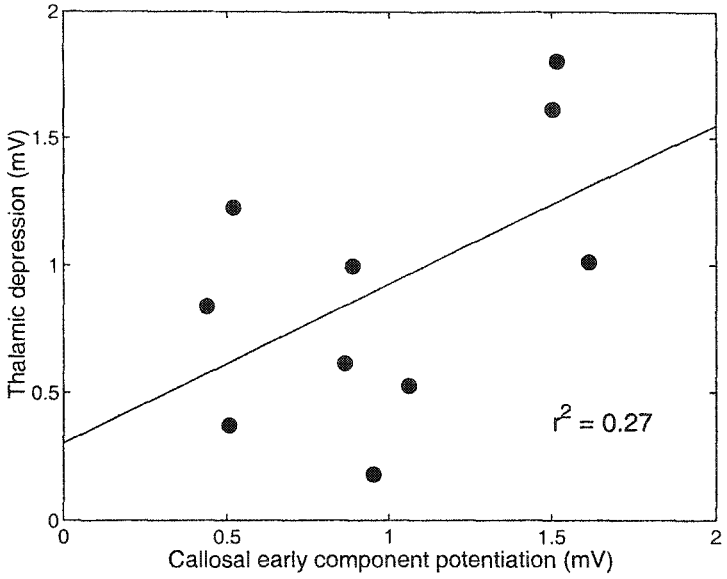

Figure 2.22: Correlation between callosal early component potentiation and thalamic depression

2.4.1 Callosal LTP

The callosal pathway exhibited a facilitation of LTP for the late component, but not the early component. The facilitation of the late component supports the hypothesis that coactivation of multiple input pathways facilitates LTP induction. However, this affect was curiously absent from the early component. Either there was a facilitation that was somehow masked, or there was no facilitation.

One possible mechanism for masking could be competition between opposing field currents. Potentiation of the early component involves both potentiation of the field EPSP and potentiation of population spikes. These changes are opposite in polarity to one another (with our electrode configuration) thus both can increase in magnitude and the final waveform will appear approximately the same. A facilitation could have occurred, but with both the spike and field potential increasing they could

effectively cancel one another. The end result could appear similar to the callosal-only tetanization condition. Single-unit recording could resolve this issue. Chapman et al. (1998) showed that there was increased unit discharge following LTP induction. If it could be shown that there was even greater unit discharge in paired animals, this would support the hypothesis that there is a facilitation of LTP in these animals.

It is also possible that a facilitation effect was masked by a ceiling effect. Although a reduced stimulus intensity was chosen for the tetanizations, it is possible that it was still too intense. The group data (Fig. 2.15) shows that there was slight trend toward a facilitation of the early component, but it was not significant. Perhaps an even lower intensity of tetanization would reveal a facilitation.

The other possibility is that there was no facilitation of the early component. If this is the case then there are two likely reasons why late component LTP would facilitate in the absence of any early component facilitation. One possibility is an anatomical restriction—it is possible that the first physical interaction between the two pathways occurs polysynaptically, at least with respect to the callosally evoked late component. From the available literature, it is difficult to confirm or reject this hypothesis. Both the thalamic and callosal projections terminate in many layers of the motor cortex (Donoghue and Parham, 1983; Akers and Killackey, 1978; Herkenham, 1980; Yamamoto et al., 1990). Anatomically speaking, then, the two pathways appear to have the opportunity to interact monosynaptically. Whether or not they do remains to be tested. Another possibility is a mechanistic difference between the two components. Some evidence suggests that the two components share at least some similarity mechanistically. Both components potentiate following high frequency tetanization and this potentiation is equally blocked in both components by the NMDA receptor antagonist CPP (Trepel and Racine, 1998). However, some evidence suggests that the two components are different mechanistically. The lack of

correlation between the magnitude of LTP in the two components (Fig. 2.21) suggests that the two are largely independent. Furthermore, the two components respond differently to paired pulse stimulation: the late component facilitates much more than the early component (Chapman et al., 1998). Thus, it is likely that there is some mechanistic difference between the monosynaptic early and polysynaptic late components, even though they both depend on NMDA receptor activation.

Chapman et al. (1998) suggested that potentiation of local horizontal connections could contribute to the late component. It should be possible to test the horizontal connections for a facilitation of LTP in the *early* component following paired tetanization with thalamic fibers. If the early component of the horizontal fibers does show a marked facilitation, then this would be evidence, although not conclusive, that horizontal fiber potentiation contributes to the callosally evoked late component.

2.4.2 Thalamocortical Depression

The depression of the thalamocortical pathway following both thalamic stimulation and paired stimulation is surprising. Previous studies in motor cortex have shown that, although the thalamocortical pathway fails to potentiate when stimulated alone, it will potentiate when paired with corticocortical stimulation (Iriki et al., 1989, 1991). Furthermore, in the visual system, thalamocortical LTP is possible following tetanization of the thalamic pathway alone (Heynan and Bear, 2001).

The difference between this study and the previous work is that this study was done in awake animals whereas the previous work used anesthetized preparations. This is the most likely explanation for the different outcomes of this study and the previous ones. Prior to the discovery of the spaced and repeated tetanization pattern that is necessary to induce LTP in awake animals (Trepel and Racine, 1998), previous

attempts in our lab to induce LTP in awake animals, via a number of induction protocols, proved unsuccessful (Racine et al., 1994a). However, attempts to induce LTP in anesthetized animals proved successful for a number of cortical sites (Racine et al., 1994b). This suggests that it is easier, for some reason, to induce LTP in anesthetized animals than in awake animals.

The anesthetic used in the previous studies was sodium pentobarbital, which significantly raises the overall level of inhibition by altering properties of the GABA receptor (Steinbach and Akk, 2001). The increased inhibition effectively removes the polysynaptic components from the evoked response (Chapman et al., 1998), reducing the circuit to a monosynaptic system. For the thalamocortical projection, this would *eliminate* a significant inhibitory component. In addition to its primary projection to the motor cortex, the ventrolateral thalamus sends collaterals to the reticular nucleus of the thalamus (Ahlsen and Lindstrom, 1982). The reticular nucleus contains primarily inhibitory neurons that project back to the ventrolateral nucleus (Cicirata et al., 1990). The reticular projection is the only significant inhibitory component of the ventrolateral nucleus, which does not contain inhibitory interneurons (Sawyer et al., 1991). Thus the reticular projection forms a negative feedback loop that would be functionally removed under anesthesia. In the awake animal, it is possible that the thalamocortical collaterals that project to the reticular nucleus would potentiate following tetanization, thereby increasing the strength of the negative feedback. This could lead to the observed decrease in the amplitude of the cortical field potential.

Potentiation of the negative feedback loop, in the awake animal, could explain a large part of the observed depression. The larger depression in the paired tetanization group suggests a cortical component, but the increased depression could be due to potentiation of a corticofugal projection from the motor cortex to the reticular nucleus (Steriade and Wyzinski, 1972). The additional cortical activation of the paired group

could further potentiate the inhibitory projection from the reticular nucleus to the ventrolateral and further depress the cortical evoked potential.

This hypothesis could be tested by administering an NMDA blocker locally in the reticular nucleus during tetanization to prevent any LTP occurring at the thalamocortical collateral and corticofugal synapses (assuming LTP occurs at this site and it is NMDA-dependent). This could unmask any potentiation occurring at the thalamocortical synapses.

2.4.3 Callosal and Thalamocortical Plasticity

The two pathways tested in this experiment responded very differently to associative high-frequency tetanization. The callosal pathway showed a robust potentiation following tetanization. Furthermore, this LTP was facilitated by paired tetanization with the thalamic pathway. Conversely, the thalamic pathway depressed following tetanization, and this depression was exacerbated following paired tetanization with the callosal pathway. The callosal LTP decayed at a faster rate than the thalamocortical depression.

These differences in plasticity are perhaps representative of the different functional roles subserved by the different pathways. Thalamocortical pathways are typically very plastic during a relatively brief period early in development but are stable thereafter. This phenomenon has been studied extensively in the visual system of monkeys (LeVay et al., 1980), cats (Hubel and Wiesel, 1970), and rats (Fagiolini et al., 1994a). Similar effects have been observed in the somatosensory cortex of the rat (Woolsey and Wann, 1976). By contrast, the superficial layers of the cortex, subserved *intra*hemispherically by local horizontal connections and *inter*hemispherically by the callosal projection, are plastic throughout adult life. Horizontal connections

are thought to mediate rapid receptive field reorganization in visual cortical neurons following retinal lesions in adult animals (Das and Gilbert, 1995). Potentiation of horizontal connections is thought to mediate acquisition of a novel motor skill in rats (Rioult-Pedotti et al., 1998, 2000). Thus it could be that the properties of the callosal pathway (reliable LTP, facilitation of LTP following paired tetanization, faster decay rate) are due to the fact that this pathway is constantly undergoing plastic change, even in adult animals. On the other hand, the properties of the thalamocortical pathway (depression following homosynaptic and paired tetanization, slow decay rate) could be due to the fact that plasticity in this pathway is limited to the critical period.

If, however, thalamocortical plasticity is reduced in adult animals, why did this pathway depress following the tetanization protocols? Induction of a true LTD following high-frequency tetanization is rare, although possible (e.g., see Trepel and Racine, 1998). Most often a low frequency stimulation pattern is used (Bear and Abraham, 1996). It could be that the thalamocortical pathway is more susceptible to depression than potentiation, even following high-frequency tetanization. This possibility could be tested by examining the relative ease of depressing the thalamocortical pathway following induction protocols known to reliably induce LTD. This possibility is explored in the next chapter.

Chapter 3

Associative LTD

3.1 Introduction

Hebb (1949) originally postulated that information could be stored in the nervous system as increases in synaptic strength following correlated pre- and postsynaptic activity. The essence of Hebb's theory was a critical landmark in memory research, but in practice a system cannot support information storage for very long if synapses can only increase in strength. For this reason, Hebb's postulate is commonly extended to include the idea that synapses can also undergo activity-dependent decreases in efficacy. Theoretically, activity dependent decreases in synaptic strength should occur under conditions opposite to those that strengthen connections—if the pre- and postsynaptic activity is *anti*correlated, then the strength of the synapse should decrease. The discovery of long-term depression provided evidence for this theory and it was an important piece of evidence in the argument for synaptic plasticity as the mechanism used to store information in the brain.

The conditions necessary to decrease synaptic efficacy can be met a number of

different ways. In the first condition, there can be postsynaptic activity but not presynaptic activity. In the second condition, there can be presynaptic activity but no postsynaptic activity. Finally, if there are two active inputs, the weaker of the two inputs can be out of phase with stronger input. Following the terminology outlined in the general introduction, this final condition is called associative LTD, and it is the subject of this experiment.

LTD was initially demonstrated as a heterosynaptic effect in the dentate gyrus of acutely prepared rats. In their study of associative long-term potentiation, Levy and Steward (1979) noted that homosynaptic tetanization of the strong, ipsilateral input to the dentate gyrus caused a depression in the weaker, contralateral input, even though the weak input had been inactive during tetanization of the strong input. This result was important because it demonstrated that synapses could support long-term decreases in efficacy as well as long-term increases. The stimulation conditions of this experiment satisfied one of the conditions for anticorrelated activity: postsynaptic activity without presynaptic activity. Experimental support for the other two conditions had yet to be demonstrated.

The third condition, or associative LTD, was the next piece of evidence to be demonstrated. Soon after their initial report of associative LTP (and heterosynaptic LTD) in the dentate gyrus, Levy and Steward (1983) showed that the correlation between the tetanization applied to the strong and weak input pathways was critical for inducing LTP or LTD. Using acutely prepared rats, they varied the timing of the tetanization applied to the contralateral (weak) and ipsilateral (strong) entorhinal cortex inputs to the hippocampus. As they had shown previously (Levy and Steward, 1979), simultaneous tetanization of both pathways induced a reliable potentiation of the weak pathway. If tetanization of the weak pathway preceded tetanization of the strong pathway, by intervals up to 20 ms, LTP could still be induced in the

weak pathway, but it was smaller that if the pathways were tetanized simultaneously. However, if tetanization of the weak pathway followed tetanization of the strong pathway, the weak pathway exhibited a *depression*. The depression occurred even for very small temporal intervals ($\sim$ 1 ms). Although no direct comparison was made, examining the figures shows that the associative depression was larger in magnitude than the heterosynaptic depression. This experiment demonstrated that activity on a weak input, when anticorrelated with activity on a strong input, can cause a long lasting depression of synaptic transmission on the weak input.

There was a long search for proof of the final condition for anticorrelated activity, where there is presynaptic activity without postsynaptic activity. Dudek and Bear (1992) discovered a stimulation paradigm that met these requirements and reliably induced LTD. They showed that several hundred pulses of stimulation, delivered at a low frequency (1–3 Hz) could reliably depress the Schaffer collateral–CA1 synapses. The low frequency activity provided presynaptic input, but without the temporal summation provided by high frequency activity, there was little postsynaptic activity. This stimulation protocol has since been used by many other groups to induce LTD. In our lab for example, this protocol reliably induces LTD in the neocortex of freely behaving rats (Froc et al., 2000).

More recently, the use of more sophisticated techniques has made it possible to more precisely control the timing of pre- and postsynaptic activity. Markram et al. (1997) performed dual whole-cell recordings of reciprocally connected neocortical pyramidal neurons. The dual whole-cell recording allowed them to precisely control the pre- and postsynaptic activity through current injections. They varied the relative timing of action potentials in the two cells. What they found was that the phase of a synaptically-evoked EPSP and the firing of an action potential in the same cell were critical. When the EPSP preceded the action potential by a short

interval ($\sim$ 10 ms), the efficacy of the synapse increased. When the EPSP followed the action potential by the same interval, the efficacy of the synapse decreased. This result further strengthens the evidence for anticorrelated activity, in a homosynaptic circuit, inducing LTD.

The evidence for associative LTD has been controversial. The associative LTD experiment by Levy and Steward (1983) used high-frequency activity on a weak input during their stimulation protocol. This left open the question whether a strong input could be depressed when its activity was anticorrelated with an equally strong converging input. Stanton and Sejnowski (1989) explored this question in the CA1 region of the hippocampus. They used two stimulating electrodes, one situated in the Schaffer collaterals and one in the subicular afferents. To one input, they applied theta-burst stimulation (brief bursts of high-frequency activity delivered at 5 Hz). This pattern of activity reliably induced LTP, but no purely heterosynaptic LTD. To the other input, they delivered single pulses, also at 5 Hz. The critical variable in the experiment was the relative phase of the two patterns of stimulation. When the two patterns were correlated (the single pulse of one pathway occurring during a burst of high-frequency activity to the other pathway), the single-pulse pathway potentiated. When the two patterns were anticorrelated (the single pulse occurring in the interval between bursts on the other pathway), the single pulse pathway depressed.

The findings of Stanton and Sejnowski (1989) were important because they demonstrated the critical importance of correlated activity between converging inputs. Unfortunately, the validity of their results was questioned because they could not be replicated by other groups (e.g., Kerr and Abraham, 1993). Since then, slight variations of the protocol used by Stanton and Sejnowski (1989) have been shown to induce associative LTD. For example, Debanne et al. (1994) stimulated two separate Schaffer collateral inputs to CA1 pyramidal neurons. Both pathways received

low frequency stimulation (0.3 Hz). On one pathway the pulses were correlated with postsynaptic depolarization (intracellular current injection). On the other pathway the pulses were anticorrelated with the postsynaptic depolarization. The pathway with correlated activity showed an increase in synaptic strength, the pathway with anticorrelated activity showed a decrease in synaptic strength. These findings restore some support for the existence of associative LTD, but the mixed findings of the past leave associative LTD on shaky ground.

The current experiment is designed to resolve some of the controversy surrounding associative LTD by testing this phenomenon in awake, freely behaving animals. In addition, the unexpected result of thalamocortical LTD following high-frequency stimulation raised the possibility that this pathway is more prone to LTD than LTP. Based on this possibility, the present experiment sought to test depression effects in the thalamocortical pathway using stimulation protocols known to induce LTD. The same electrode configuration as the previous chapter is used here. The stimulation protocol is an explicit anticorrelation procedure, similar to the one used by Stanton and Sejnowski (1989).

3.2 Materials and Methods

3.2.1 Animals and Surgery

Animals were prepared using the same procedures as those in the associative LTP experiment. The same electrode configuration and coordinates were used to implant the thalamic stimulating, callosal stimulating, and recording electrodes.

3.2.2 Recording and Stimulation

The same general procedure used in the associative LTP experiment was used here. In this experiment, one additional intensity was added to the set of intensities used in the input/output . In the associative LTP experiment, the input/output functions did not asymptote at the highest intensity of stimulation, suggesting that some potentially informative data was being missed. The set of intensities used in this experiment was: 15, 40, 100, 160, 250, 400, 600, 1000, and 1300 μA.

Baseline input/output functions were taken every second day for one week to ensure stability of responses. Following baselines, the animals were randomly assigned to one of seven groups. The first two groups examined the effects of associative LTD stimulation. Group 1 received HFS to the callosal pathway and LFS to the thalamic pathway (n = 9). Group 2 received the reverse: HFS to the thalamic pathway and LFS to the callosal pathway (n = 8). The next four groups received stimulation to a single pathway to control for heterosynaptic effects. Group 3 received LFS to the callosal pathway (n = 8), group 4 received LFS to the thalamic pathway (n = 8), group 5 received HFS to the callosal pathway (n = 7), and group 6 received HFS to the thalamic pathway (n = 6). The final group served as a control for the stability of the responses over the course of the experiment and did not receive HFS or LFS (n = 6).

The HFS and LFS protocols were configured such that the total number of pulses delivered was the same for each protocol. HFS consisted of 60 trains delivered at 0.1 Hz, where one train consisted of 10 pulses delivered at 300 Hz. LFS consisted of 600 pulses delivered at 1 Hz. For the associative stimulation conditions, where HFS was delivered to one pathway and LFS was delivered to the other pathway, the delivery of the pulses was timed such that the HFS and LFS were anticorrelated.

Delivery of a HFS train occurred between delivery of the LFS pulses. The intensity of pulses in the HFS and LFS protocols was 500 μA, which is the same intensity used in the associative LTP experiment.

Input/output measures were taken every second day throughout the 12 day stimulation period. Following the 12 days, two further input/output measures were collected at one week intervals.

3.2.3 Data Analysis

The procedure for data analysis was the same as that described in the associative LTP experiment. Briefly, custom matlab software was used to measure the amplitude of field potentials for the different days. The amplitude measurements were standardized for each rat by subtracting the raw value from the average of the 4 baseline measurements. Using these measures, a repeated measures ANOVA was used to establish significant effects.

3.2.4 Histology

As before, the animals were perfused upon completion of the experiment. The brains were processed for Prussian Blue and Neutral Red to confirm location of the electrode tips.

3.3 Results

3.3.1 Callosal Pathway

Raw Data. As previously reported by our lab (Froc et al., 2000), LFS delivered to the callosal pathway caused a depression of the field potential amplitude (Fig. 3.1).

This depression was most evident in the early component. The depression was also evident in the late component, but to a lesser extent. The LFS did not produce a change in the waveform morphology, as in the case of HFS stimulation. Surprisingly, the associative LTD condition did not produce a larger LTD. It was expected that when HFS was delivered to the thalamic pathway, in addition to the LFS on the callosal pathway, the magnitude of the LTD on the callosal pathway would increase. However, this did not happen. The magnitude of LTD was approximately the same for the two conditions.

The other associative stimulation condition, where HFS was delivered to the callosal pathway and LFS to the thalamic pathway, also yielded an unexpected result. HFS delivered to the callosal pathway produced a normal potentiation effect (as in the previous chapter), characterized as a reduction in the amplitude of the early component and an increase in the amplitude of the late component. When the callosal HFS was paired with thalamic LFS, the magnitude of the potentiation was reduced for the late component, but not the early component. The reduced LTP could be due to a small heterosynaptic depression: when LFS was delivered to the thalamic pathway alone, there was a slight depression of the callosally-evoked field potential. Surprisingly, the LFS delivered to the thalamic pathway produced more of a heterosynaptic effect than HFS delivered to the thalamic pathway, which did not impact the callosal pathway very much.

Input/Output Functions. These effects are more clearly seen in the input/output functions (Fig. 3.2). Homosynaptic LFS to the callosal pathway produced a small but reliable depression across all intensities, yielding a significant main effect ($F_{1,14} = 12.56$, $p < 0.001$). When the callosal LFS was paired with HFS to the thalamic pathway, the magnitude of depression was approximately the same, except perhaps the middle intensities, where the associative depression appears to be slightly larger.

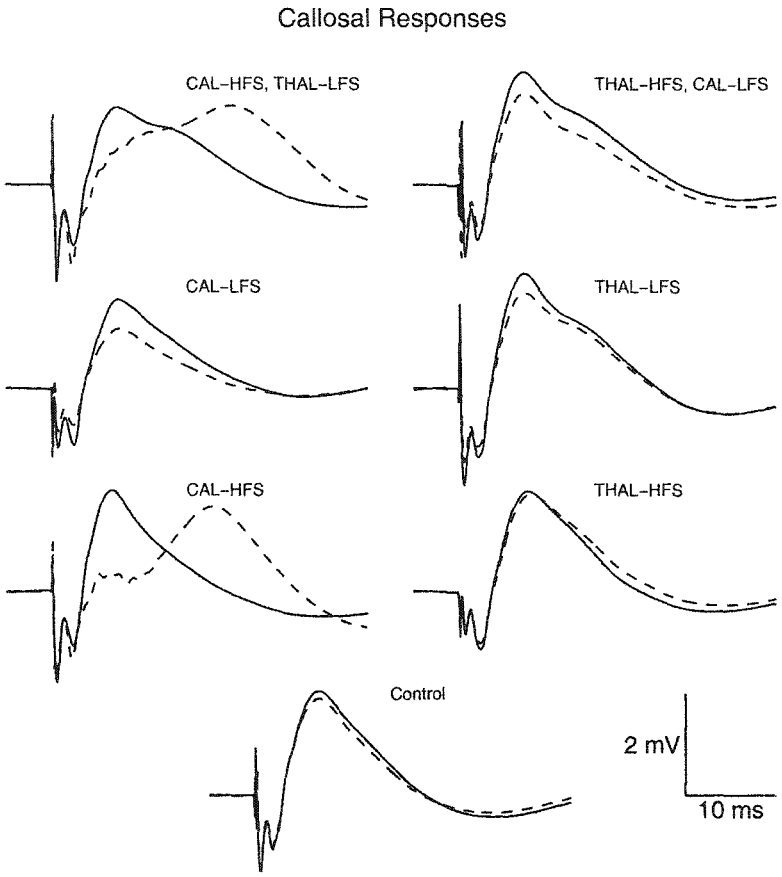

Figure 3.1: Callosal pathway raw data. Representative sweeps from the different stimulation conditions, indicated by the title above the sweeps, are shown. Solid lines are baseline responses and dashed lines are taken from the day after the last day of stimulation.

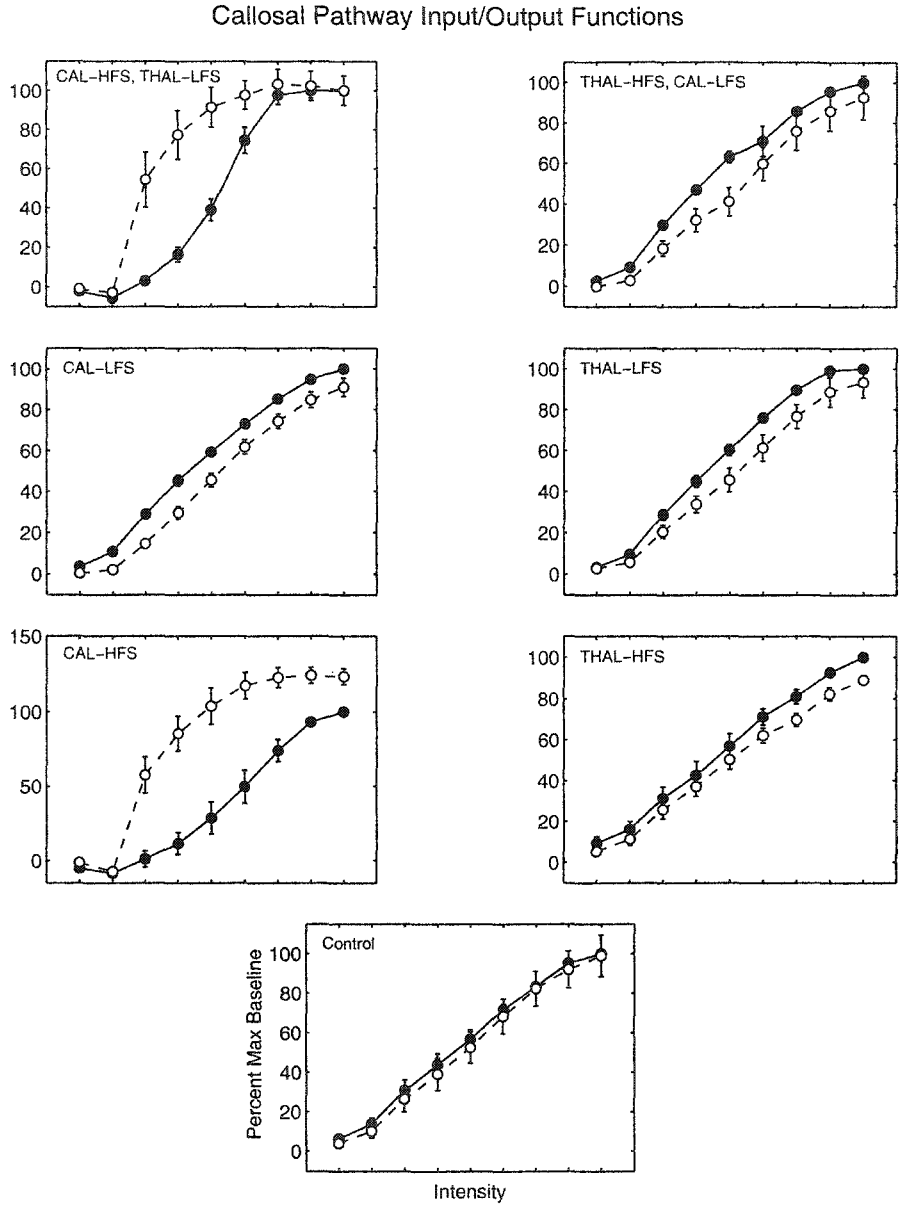

Figure 3.2: Callosal pathway input/output functions. Solid lines and filled symbols are baseline measures, dashed lines and open symbols are 24 hours following the last stimulation. The stimulation condition is indicated in the top left of each panel. Each animal's data has been normalized to the amplitude of the field potential evoked by the strongest intensity in the baseline input/output function. The intensities of stimulation are: 15, 40, 100, 160, 250, 400, 630, 1000, and 1300 μA.

In the associative condition, however, there was more variability in the size of the depression. As a result, neither the main effect nor the interaction were significant ($F_{1,14} = 3.11$, $p = 0.1$ and $F_{8,112} = 1.10$, $p = 0.37$ respectively).

HFS to the callosal pathway produced a typical LTP effect (only the input/output functions for the late component are shown for the sake of clarity). The potentiation was most evident in the middle intensities of the curve, but was still apparent even at the highest intensities. Both the main effect and interaction were significant ($F_{1,8} = 30.01$, $p < 0.001$ and $F_{8,64} = 14.42$, $p < 0.001$ respectively).

When the homosynaptic HFS on the callosal pathway was paired with LFS to the thalamic pathway, the potentiation was diminished relative to the homosynaptic condition. The magnitude of the potentiation was reduced at the middle intensities and virtually absent at the higher intensities. However, the main effect and interaction were still significant ($F_{1,16} = 12.56$, $p < 0.01$ and $F_{8,128} = 11.07$, $p < 0.001$ respectively). As noted earlier, this might have been due to a small heterosynaptic depression produced by homosynaptic LFS on the thalamic pathway. This heterosynaptic effect was small and was mainly evident near the middle intensities. The main effect was significant ($F_{1,14} = 4.82$, $p < 0.05$). This purely heterosynaptic effect could have contributed to the reduced LTP of the WM-HFS + THAL-LFS group, although the heterosynaptic depression does not seem large enough to account for the reduction of the associative group.

Surprisingly, homosynaptic HFS to the thalamic pathway produced a smaller heterosynaptic depression on the callosal pathway than the thalamic LFS condition. The depression induced by HFS was only evident at the higher intensities of the input/output function, and the main effect was not significant ($F_{1,10} = 2.6$, $p = 0.14$). The fact that LFS on the thalamic pathway produced a larger heterosynaptic depression than HFS is surprising because most heterosynaptic depressions are induced

following HFS.

Induction and Decay. The group data for the callosal pathway, plotted across days, is shown in Figure 3.3. HFS delivered to the callosum induced LTP, as expected

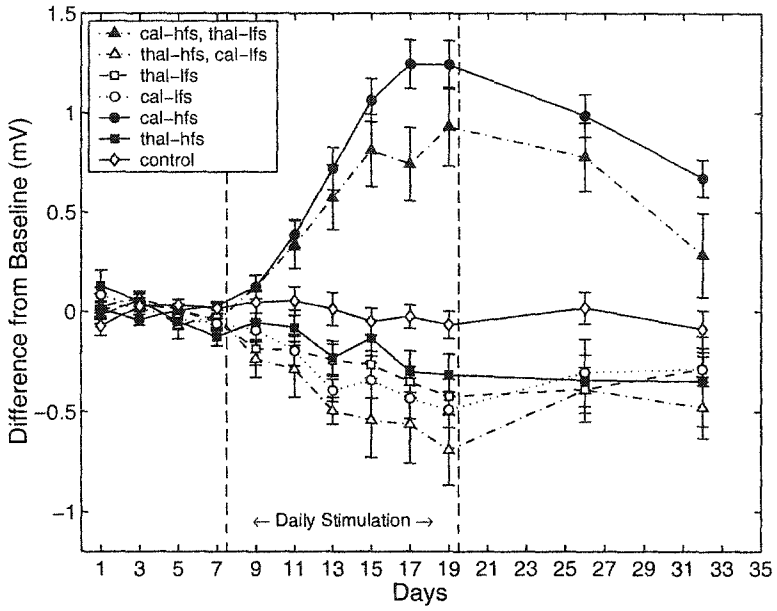

Figure 3.3: Group data for the callosal pathway plotted across days.

(only the late component is shown here for clarity, the early component is discussed later). When the callosal HFS was paired with thalamic LFS, the amount of LTP expressed in the late component of the callosal pathway was reduced significantly ($F_{11,154} = 1.89$, $p < 0.05$). The potentiation began to develop at the same time as the homosynaptic condition, but it failed to increase as much as the homosynaptic condition.

Homosynaptic LFS delivered to the callosal pathway produced a small but reliable depression that developed gradually over the course of stimulation. The depression was significantly different from control animals ($F_{11,132} = 5.52$, $p < 0.001$), but decayed gradually during the two weeks following stimulation. The associative LTD group

(WM-LFS + THAL-HFS) also produced a depression. This depression appeared to be larger, on average, than the homosynaptic group, but the effect was not significant ($F_{11,154} = 0.51$, $p = 0.89$).

Interestingly, there appeared to be heterosynaptic effects of stimulating the thalamic pathway. Both the thalamic HFS and LFS groups caused a slight depression of the callosal pathway over the course of the experiment. Although the magnitude of the depression was not large, it was significant for both stimulation conditions (thalamic HFS: $F_{11,110} = 2.09$, $p < 0.05$; thalamic LFS: $F_{11,132} = 3.37$, $p < 0.01$). In the previous chapter on associative LTP, HFS to the thalamic pathway did not have a significant effect on the callosal pathway, although there was a trend towards a depression (Figs. 2.15 and 2.16). From these mixed results it seems that heterosynaptic effects of thalamic stimulation on the callosal pathway are small and somewhat variable.

The results in the previous chapter on associative LTP showed that combined HFS to both the thalamic and callosal pathways facilitated LTP of the callosal late component, but not the early component. Figure 3.4 shows the changes in the callosal early and late components following HFS to the callosal pathway and LFS to the thalamic pathway. Similar to the associative LTP of the previous chapter, the associative condition here had no effect on the callosal early component ($F_{11,154} = 0.23$, $p = 0.99$). However, the associative condition significantly reduced the amount of potentiation induced in the callosal pathway ($F_{11,154} = 1.89$, $p < 0.05$). Thus, plasticity of the callosal late component appears to be influenced more by associative stimulation conditions.

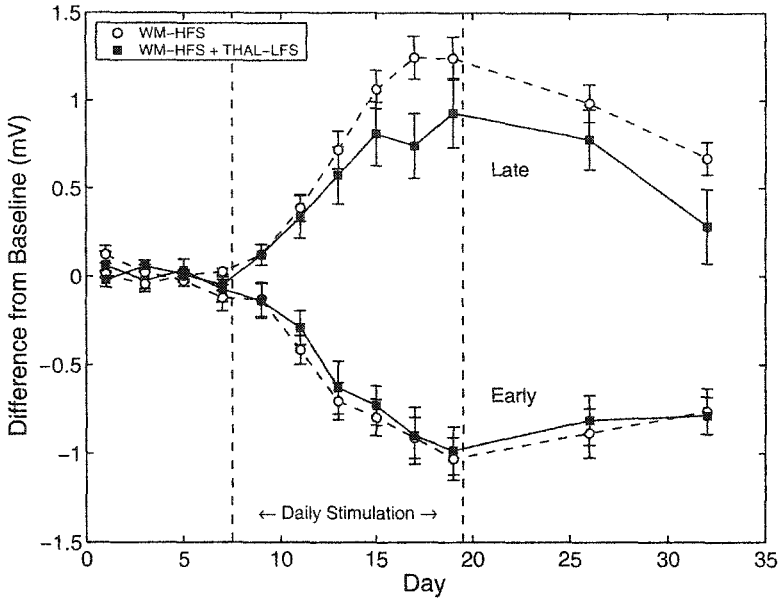

Figure 3.4: Changes in the callosal early and late components following either HFS to the callosal pathway or combined callosal HFS and thalamic LFS. The early components are the negative changes and the late components are the positive changes.

3.3.2 Thalamic Pathway

Raw Data. Examining the raw data (Fig. 3.5), it is apparent that all of the stimulation conditions, whether associative, homosynaptic, or heterosynaptic, caused varying degrees of depression in the thalamic pathway. All the stimulation conditions reduced the amplitude of the field potential of the thalamic pathway. Examining the sweeps, there is no evidence that this depression might be due to potentiation of population spikes, as is the case of callosal LTP. All of the stimulation conditions produced a morphologically similar depression. Even the magnitude of depression was relatively similar across stimulation conditions. The associative conditions (WM-HFS + THAL-LFS and WM-LFS + THAL-HFS) appeared to cause more of a reduction than the various homosynaptic stimulation conditions, but this difference was small

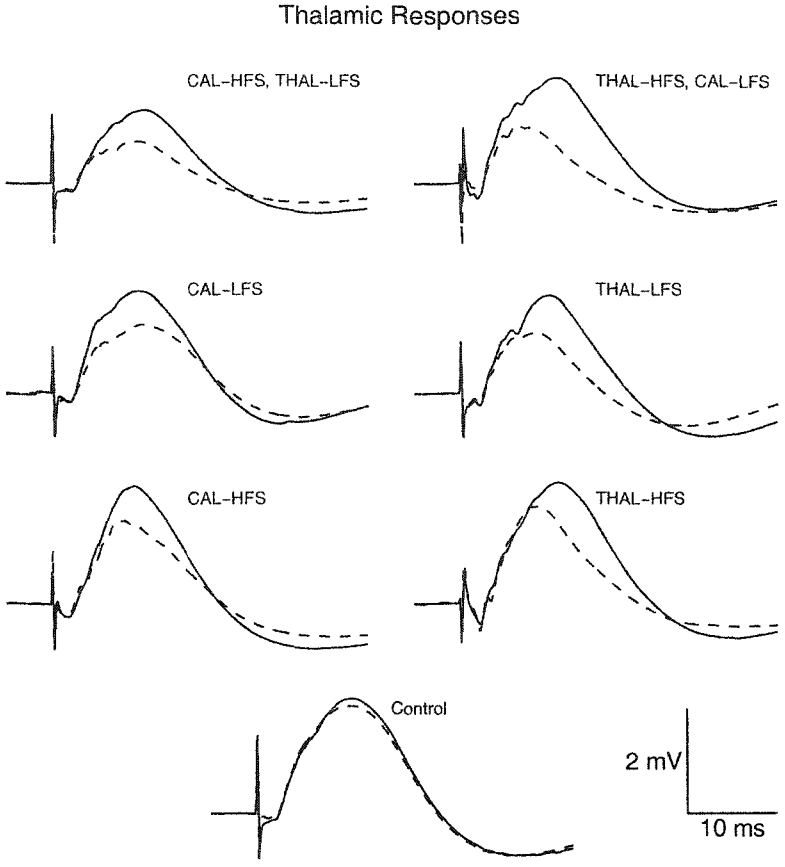

Figure 3.5: Thalamic pathway raw data. Representative sweeps from each of the stimulation conditions (indicated by figure titles). Solid lines are baseline responses and dashed lines are taken 24 hours following the last stimulation session.

and not significant (see Figure 3.7).

Input/ Output Functions. The input/output functions further characterize the depressions of the various stimulation conditions (Fig. 3.6). The trend was for the associative stimulation conditions to produce the largest depressions but the size of depressions between groups was not significant. Callosal HFS combined with thalamic LFS depressed the thalamically-evoked field potential the most. The depression was most evident at the higher stimulation intensities, where the amplitudes were ap-

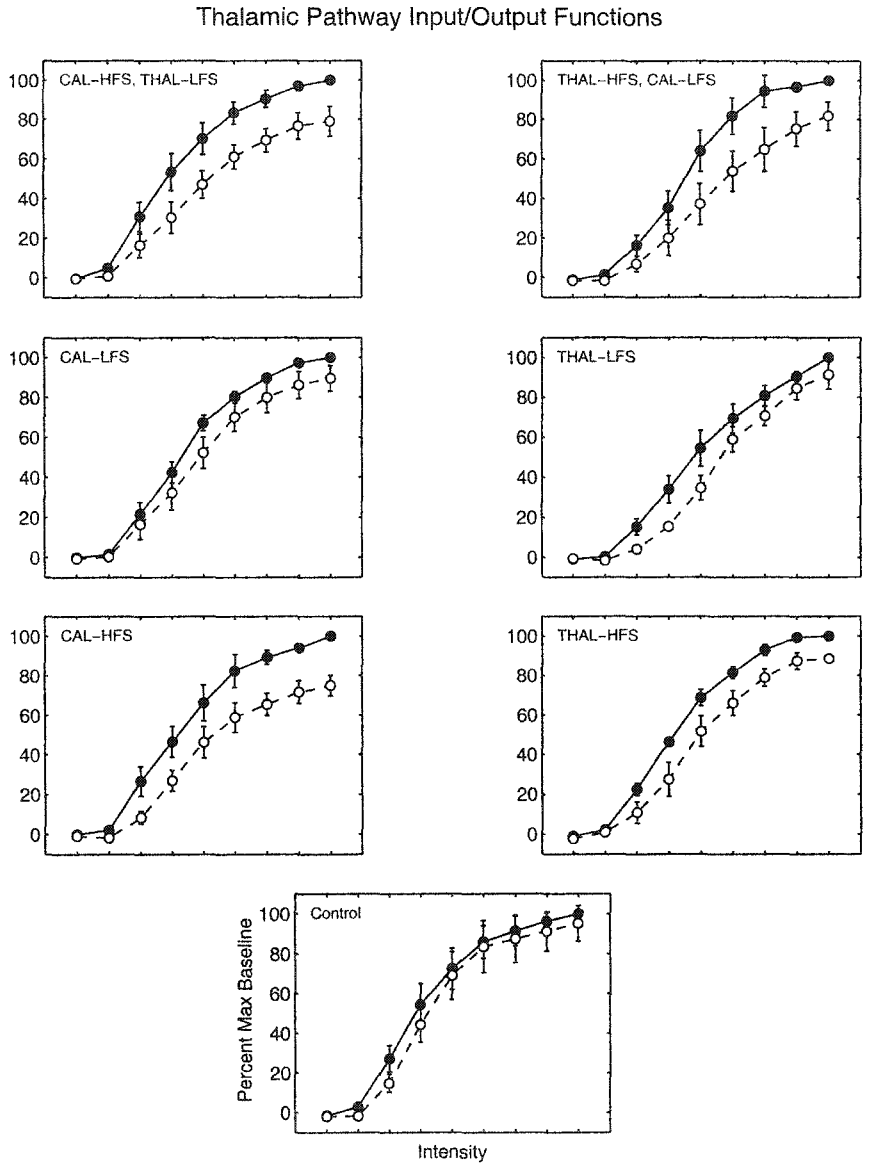

Figure 3.6: Thalamic pathway input/output functions. Solid lines and filled symbols are baseline measures, dashed lines and open symbols are 24 hours following the last stimulation. The stimulation condition is indicated in the top left of each panel. Each animal's data has been normalized to the amplitude of the field potential evoked by the strongest intensity in the baseline input/output function. The intensities of stimulation are: 15, 40, 100, 160, 250, 400, 630, 1000, and 1300 μA.

proximately 20% smaller following the associative stimulation protocol ($F_{8,128} = 2.11$, $p < 0.05$). The other associative condition (thalamic HFS + callosal LFS) also depressed the thalamic pathway by about 20%, evident mainly at the higher intensities ($F_{8,112} = 2.10$, $p < 0.05$).

LFS to the thalamic pathway alone did not cause a very large depression relative to the associative conditions. The depression was most evident at the middle intensities, but the amplitudes were consistently reduced across the other intensities as well ($F_{1,14} = 5.41$, $p < 0.05$). HFS to the thalamic pathway caused a larger depression than the LFS and it was also evident across all intensities ($F_{8,80} = 2.06$, $p < 0.05$).

Activation of the callosal pathway produced heterosynaptic depression of the thalamic pathway. In particular callosal HFS produced a marked depression of the thalamic field potential ($F_{8,80} = 2.25$, $p < 0.05$). This heterosynaptic depression could explain a large portion of the associative condition where callosal HFS was paired with thalamic LFS as thalamic LFS alone did not produce much of a depression. When LFS was delivered to the callosal pathway, there was a trend toward depression of the thalamic field potential, but this did not reach significance ($F_{8,112} = 1.17$, $p = 0.32$).

Induction and Decay. From the group data, the depression of the thalamocortical pathway caused by the various stimulation conditions appears to have developed at the same rate (Fig. 3.7). For all the stimulation conditions, the depression developed progressively over the course of the 12 day stimulation protocol. There was a tendency for the associative conditions to produce the largest depression (WM-HFS + THAL-LFS: $F_{11,143} = 3.19$, $p < 0.001$; WM-LFS + THAL-HFS: $F_{11,132} = 3.87$, $p < 0.001$). The homosynaptic thalamic stimulation conditions also interacted significantly with controls (THAL-HFS: $F_{11,110} = 2.96$, $p < 0.01$; THAL-LFS: $F_{11,132} = 2.09$, $p < 0.05$). Heterosynaptic depression of the thalamic pathway following activation of the callosal

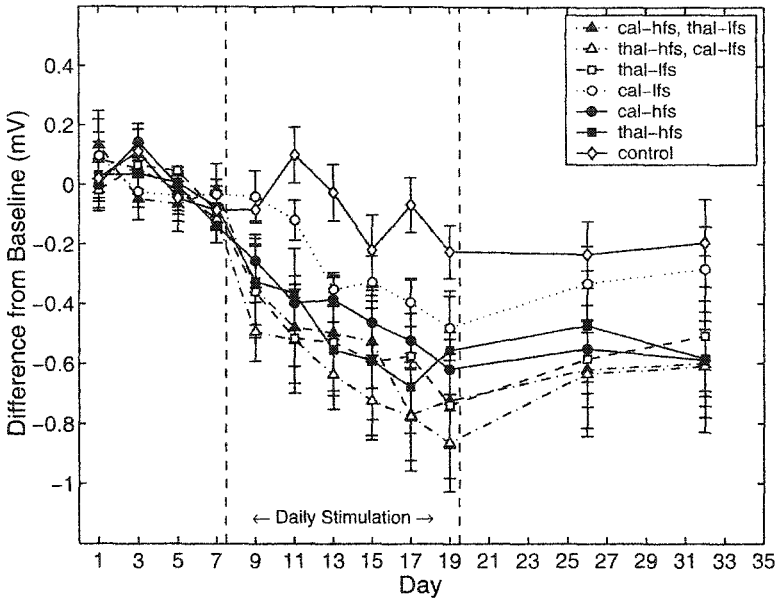

Figure 3.7: Changes in the thalamic pathway across days for the various stimulation conditions.

pathway was only significant for the HFS condition ($F_{11,110} = 3.32$, $p < 0.001$) and not for the LFS condition ($F_{11,132} = 1.66$, $p = 0.09$). None of the stimulation conditions differed significantly from one another with respect to the amount of depression they produced.

Similar to the results described in the previous chapter, the thalamic pathway depression exhibited a slower decay than the callosal pathway effects. In particular, the homosynaptic HFS conditions (WM-HFS and THAL-HFS) remained relatively stable during the two weeks following the stimulation protocol. There was a trend for the associative conditions to decay during the first week following stimulation, but remain stable during the second week. By contrast, the homosynaptic LFS conditions tended to exhibit a steady, gradual decay over the two weeks, similar to the decay observed in the callosal pathway effects.

3.4 Discussion

This experiment was designed to answer two main questions. One examined the possibility of inducing associative LTD in the neocortex. In general, there were no clear effects of the associative stimulation pattern compared to homosynaptic LFS. The other question tested the relative ease of inducing LTD on the thalamocortical pathway. In general, the thalamocortical pathway was depressed by approximately the same amount no matter what type of stimulation was applied. Stimulation protocols known to induce LTD in other pathways (i.e., LFS) were no more effective than stimulation protocols known to induce LTP (i.e., HFS).

3.4.1 Associative vs. Homosynaptic LTD

On the callosal pathway, the associative anticorrelation stimulation did not appear to increase the amount of LTD, compared to the homosynaptic LFS. Homosynaptic LFS to the callosum induced a small, but reliable LTD. This LTD was smaller in magnitude than that previously reported (Froc et al., 2000), but that could be due to a difference in pulse intensity. Froc et al. (2000) used a relatively high pulse intensity (1200 μA), whereas this experiment used a much lower pulse intensity (500 μA). One of the early stages of LTD expression is a rise in intracellular calcium (Mulkey and Malenka, 1992). It could be that the lower pulse intensity used in this experiment was too small to trigger the necessary rise in calcium postsynaptically.

Whatever the cause, the smaller LTD observed here was partly intentional. The lower pulse intensity was chosen so that possible associative increases in LTD would not be masked by ceiling effects. Thus the LTD expressed here following homosynaptic LFS is not asymptotic, maintaining the potential for further LTD induction following associative stimulation. Since the amount of LTD following the associa-

tive stimulation protocol was approximately the same as the homosynaptic LFS, it appears that the two stimulation protocols are inducing the same LTD.

The history of associative LTD has been controversial. The notion of associative LTD is an attractive idea from a theoretical standpoint because it satisfies one of the conditions necessary to decrease synaptic strength following anticorrelated activity. The initial report of associative LTD by Stanton and Sejnowski (1989) was therefore quite promising. However, subsequent failures to replicate their findings called the validity of the original result into question. Because the evidence for associative LTD remains divided some believe that it is not a real phenomenon. For example, Bear and Abraham (1996) expressed the viewpoint that the associative stimulation protocol 'simply provides another way of inducing homosynaptic LTD'. The lack of evidence for associative LTD in the present experiment supports this viewpoint.

On the thalamic pathway, all of the patterns of stimulation caused depressions of roughly equal magnitude. These findings, taken together with the results of the previous chapter, indicate that the thalamic pathway behaves somewhat differently than the callosal pathway in the freely behaving animal. In the previous chapter it was shown that the thalamocortical pathway depressed following homosynaptic HFS. In this experiment, homosynaptic LFS induced a depression roughly equal in size to the one following homosynaptic HFS. Furthermore, the associative conditioning protocol (WM-HFS + THAL-LFS) did not increase the amount of depression observed.

It is difficult to explain why both HFS and LFS would induce LTD in the thalamocortical pathway other than it might be more prone to LTD than LTP. Previous work in our lab also has shown a similar trend in thalamocortical pathways outside of the motor system. Ivanco (1997) tested the possibility of LTP induction on three thalamocortical pathways: medial geniculate to auditory cortex, lateral geniculate to visual cortex, and mediodorsal nucleus to frontal cortex. None of these pathways

showed signs of potentiation following standard homosynaptic HFS conditioning. Furthermore, the thalamocortical pathway of the auditory system showed a significant depression following HFS. In the visual system, there was a trend toward depression, but it was not significant.

However, the thalamocortical pathway is capable of supporting LTP, as demonstrations of LTP in anesthetized preparations have been reported for the motor system (Iriki et al., 1989) and the visual system (Heynan and Bear, 2001). One of the most likely possibilities for the difference between awake and anesthetized animals is a difference in inhibition between the two preparations. As mentioned in the previous chapter, the thalamic reticular nucleus is a major source of inhibition to the ventrolateral thalamus (Sawyer et al., 1991). The reticular nucleus also sends projections to the lateral geniculate nucleus (Wang et al., 2001). Anesthetic could possibly functionally remove these inhibitory components of the thalamocortical pathways (because anesthetic eliminates polysynaptic activity). The absence of functional inhibitory inputs might be the condition that permits LTP induction in anesthetized animals. Thus a useful experiment would be to silence the inhibitory projection from the reticular nucleus to the thalamus, although this might be difficult because of the small and irregular shape of the reticular nucleus.

As with the callosal pathway, the associative anticorrelation conditioning did not increase the amount of depression observed in the thalamocortical pathway. Again, this argues in favour of the similarity between homosynaptic and associative LTD.

3.4.2 Callosal Early and Late Components

There was one significant effect of the associative stimulation protocol on the callosal pathway. When callosal HFS was paired with thalamic LFS, the amount of LTP

expressed in the callosal late component was reduced relative to callosal HFS alone. Remarkably, the callosal early component expressed the same amount of LTP in the two conditions. Although it is difficult to explain why LFS on the thalamic pathway would interact with the HFS to cause this effect in the late component but not the early component, it does fit with data from the first chapter. In the first chapter, two pieces of evidence were given demonstrating the independence of the two callosal components. First, there was no correlation between the amount of LTP expressed by the two components following either homosynaptic or associative HFS conditioning. Second, the late component showed a facilitation of LTP during the associative conditioning, but the early component did not. Here again, the late component is showing an effect of associative conditioning, but the early component is not.

Chapter 4

Metabotropic Glutamate Receptors in LTP and LTD

4.1 Introduction

Glutamate is one of the major excitatory neurotransmitters in the brain. It acts both pre- and postsynaptically at a number of different ionotropic and metabotropic receptors. Ionotropic glutamate receptors mediate rapid synaptic reponses by opening to allow the passage of cations into the cell. Metabotropic receptors mediate slower synaptic responses because they are coupled to a G-protein signalling mechanism. The ionotropic class of glutamate receptors are divided into three categories: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), kainate, and N-methyl-D-aspartate (NMDA) receptors. The ionotropic receptors, in general, have been the subject of numerous experiments, but the NMDA receptor, in particular, has received a lot of attention. The NMDA receptor is unique because it detects coincident pre- and postsynaptic activity (Bliss and Collingridge, 1993). This property qualifies it as

a key participant in the process of memory formation, as suggested by Hebb (1949).

Metabotropic glutamate receptors have received less study, compared with the ionotropic receptors. This is partly due to the lack of selective agonists and antagonists. However, the recent development of a broad range of selective agents has prompted a surge of new studies on mGluRs. The results of these investigations demonstrate the important role mGluRs play in activity-dependent plasticity.

4.1.1 mGluR Structure and Function

So far, eight different subtypes of mGluR have been discovered (mGluR1–mGluR8). All the subtypes are coupled to G-protein signaling cascades (Nakanishi, 1994). Structurally, they resemble other G-protein receptors because they have 7 putative transmembrane domains. However, their extracellular ligand-binding domain is significantly larger than other G-protein receptors, making them somewhat unique.

Based on differences in intracellular signaling pathways and sequence homology, the 8 subtypes have been divided into 3 groups (Figure 4.1). Group 1 mGluRs acti-

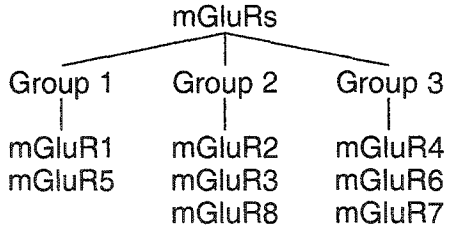

Figure 4.1: Grouping of mGluR subtypes

vate phospholipase-C, which hydrolyzes phosphatidylinositol 4,5-biphosphate (PIP_2) into inositol 1,4,5-triphosphate (IP_3). IP_3 in turn causes the release of Ca^{2+} from intracellular stores. mGluRs belonging to groups 2 and 3 are coupled to an inhibitory

G-protein that inhibits the production of cAMP by adenylyl cyclase (Nakanishi, 1994; Fagni et al., 2000).

4.1.2 mGluRs in LTP and LTD

mGluRs are thought to play a role in both LTP and LTD induction, although the evidence supporting a role in LTD is more substantial (Anwyl, 1999). In terms of LTP induction, clear effects have been observed in the hippocampus. In hippocampal slices, activation of mGluRs alone (in the absence of high-frequency stimulation) induces LTP. Bartolotto et al. (1994) applied the broad spectrum agonist 1S,3R - 1-amino-1,3-cyclopentanedicarboxylate (ACPD), and found a lasting potentiation in the CA1 region. Furthermore, tetanically-induced LTP can be blocked by antagonizing mGluRs. Breakwell et al. (1996) used a broad spectrum antagonist, (+)-alpha-methyl-4-carboxyphenylglycine (MCPG), and found that high-frequency stimulation failed to induce LTP in CA1.

Evidence of mGluR involvement in LTD has been demonstrated in the hippocampus and neocortex. Blocking mGluRs, either with the broad spectrum MCPG or the selective group 1 blocker (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA), prevents the induction of one form of LTD in the CA1 region of the hippocampus (Oliet et al., 1997). Furthermore, bath application of the group 1 mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) induced LTD in area CA1 without low-frequency stimulation (Huber et al., 2001). Similar effects have been observed in the dentate gyrus. Bath application of DHPG induces reliable LTD (Camodeca et al., 1999).

Experiments examining the role of mGluRs in neocortical LTD suggest that activation of group 1 mGluRs is sufficient, but not necessary for LTD induction. Bath application of the group 1 mGluR agonist quisqualate induced LTD without low-

frequency stimulation (Kato, 1993). Also, blocking mGluRs with MCPG has been shown to prevent the induction of LTD following LFS. More recently it was shown that MCPG is not as effective an antagonist of mGluRs as originally believed. Although it does block the actions of artificial agonists, it does not prevent the hydrolysis of PIP_2 into IP_3, at least in the neocortex (Huber et al., 1998). If a selective group 1 mGluR antagonist (LY341495) is used, the induction of LTD with LFS is *not* blocked. Furthermore, mutant mice lacking mGluR5 (group 1) show normal LTD following LFS (Sawtell et al., 1997).

4.1.3 mGluRs in Learning and Memory

The results from the LTP and LTD experiments discussed above demonstrate that mGluRs play a role in synaptic plasticity. Their role in learning and memory has also been investigated.

Balschun et al. (1999) examined the role of mGluRs in both synaptic plasticity and learning and memory. They used an *in vitro* preparation and an *in vivo* preparation to test the role of mGluRs in LTP induction in CA1. They also blocked mGluRs during a variety of learning and memory tasks. In all experiments, the same antagonist of group 1 mGluRs was used ((S)4-carboxyphenylglycine, 4-CPG). In the slice preparation, they found that administration of 4-CPG blocked induction of LTP following weak high-frequency stimulation (100 Hz for 400 ms), but failed to block LTP induction following strong high-frequency stimulation (10 bursts of 4 pulses at 100 Hz). In the intact animal, they found that intraventricular administration of the mGluR blocker prevented LTP induction, even following the strong conditioning paradigm. The memory tests produced mixed results. Intraventricular administration of the mGluR blocker failed to significantly impair memory performance in an 8-arm radial

maze. However, memory performance was significantly impaired on a Y-maze spatial alternation task.

Nielsen et al. (1997) examined the role of group 1 mGluRs in memory using a fear conditioning paradigm. They used the selective group 1 antagonist (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA), administered systemically at two different dosages (0.18 and 1.8 mg/kg). Rats were injected with either AIDA or saline 20 minutes prior to undergoing fear conditioning. The fear conditioning contained both a contextual and cued component. Contextual fear conditioning depends on the hippocampus, whereas cued fear conditioning does not depend on the hippocampus (Philips and LeDoux, 1992). At the start of each trial, the rat was placed in the apparatus. Following a 2-minute adaptation period, the amount of freezing was scored during a 20 s period in which no stimuli were presented. This measure was taken as an indicator of contextual fear conditioning. Next, a 20 s tone was presented, followed by a brief foot shock. The amount of freezing during the tone was taken as an indicator of cued fear conditioning. Control rats showed memory for both contextual and cued fear conditioning by freezing for longer periods of time on successive trials. Rats injected with both dosages of AIDA showed memory for cued but not contextual fear conditioning. The duration of freezing increased on successive trials in the cued condition, but remained the same in the contextual condition.

The results of the behavioural experiments by Balschun et al. (1999) and Nielsen et al. (1997) show that blocking group 1 mGluRs can impair memory performance on some tasks, particularly those that depend on an intact, functioning hippocampus.

4.1.4 Current Experiment

The previously cited experiments provide convincing evidence that mGluRs (particularly group 1) play a role in hippocampal synaptic plasticity. The evidence for the role of mGluRs in neocortical LTP and LTD is more controversial, but there is some evidence showing that group 1 mGluRs are involved in neocortical LTD.

Most of the studies on mGluRs have been performed in hippocampal slice preparations, with fewer studies done in neocortical slice preparations. There have been few studies in freely behaving hippocampal preparations, and no experiments in freely behaving neocortical preparations. The experiment presented in this chapter tests the role of group 1 mGluRs in neocortical LTP and LTD in freely behaving animals. Although several selective antagonists of mGluRs are now available, few of these cross the blood-brain barrier and must therefore be administered via cannula in intact preparations. To eliminate the need to implant cannula, this study uses one of the antagonists that is active systemically and crosses the blood-brain barrier (AIDA). AIDA is a highly selective group 1 mGluR antagonist and it has been shown to block the induction of LTD as well as the acquisition of contextual fear conditioning.

4.2 Materials and Methods

4.2.1 Animals and Surgery

The same surgical procedures used in the previous experiments were used for these experiments. The one difference was the electrode configuration. In the previous experiments, the animals were implanted with two stimulating (callosal and thalamic) electrodes and one recording electrode. For these experiments, the animals were implanted with only a single stimulating electrode and a recording electrode. Thus, each

animal had either a callosal or a thalamic stimulating electrode, but not both. The reason for the difference is that, while the previous experiments dealt with associative effects between pathways, the present experiment examines the role of mGluRs in LTP and LTD, which does not require activation of multiple pathways. The coordinates for the placements were the same as in the previous experiments.

4.2.2 Recording and Stimulation

The same general procedure used in the previous experiments was used here, with the exception of the following differences. In the previous experiments, baseline input/output measures were taken every two days for 7 days. In this experiment, baseline measures were taken every two days for 5 days. The baselines were stable over the course of the 5 days so the fourth measure was not necessary.

In the previous experiments, conditioning stimulation (HFS or LFS) was given once every 24 hours for 12 days. The pulse intensity used for the conditioning was 500 μA in all cases. In this experiment, conditioning stimulation was given once every 24 hours for 10 days. The pulse intensity of the conditioning stimulation was doubled to 1000 μA. Again, the reason for the difference has to do with the questions being asked in each experiment. The experiments on associativity used a lower stimulation intensity over more conditioning sessions to allow the expression of possible interactions between the homosynaptic and associative conditions. The present experiment sought to test the role of mGluRs in LTP and LTD. For this reason, it was desirable to induce large, reliable LTP and LTD, which occurs readily using higher intensities of stimulation (Trepel and Racine, 1998; Froc et al., 2000).

The HFS used in this experiment consisted of 60 trains (0.1 Hz) of 8 pulses at 300 Hz. LFS consisted of 900 pulses at 1 Hz. These patterns are slightly different

than those used in the previous experiments. HFS in the previous experiments used 10 pulses per train instead of 8. LFS consisted of 600 pulses instead of 900. The conditioning used in the previous experiments was altered to match the absolute number of pulses delivered during the different conditioning protocols.

Following the 10 days of conditioning, two additional input/output measures were taken at one week intervals.

4.2.3 Drug Administration

The group 1 metabotropic glutamate receptor antagonist AIDA ((RS)-1-aminoindan-1,5-dicarboxylic acid; Tocris) was used in this experiment. It was administered into the intra-peritoneal cavity at a dosage of 0.25 mg/kg. The drug was initially dissolved in 1.1 equimolar sodium hydroxide, and further diluted in 0.9% saline to a final concentration of 0.25 mg/ml.

Drug administration began after the 5 days of baseline measures and preceded each of the 10 daily conditioning stimulations. Following an input/output measure, the animal was injected with AIDA and returned to his home cage. After 90 minutes, conditioning stimulation (either HFS or LFS) was administered.

Control animals received injections of saline 90 minutes prior to conditioning.

4.2.4 Design

The experiment consisted of 12 groups of animals. The design of the experiment is shown in Table 4.1.

	AIDA	Saline
Callosal Pathway	HFS (n = 6)	HFS (n = 4)
	LFS (n = 7)	LFS (n = 7)
	No Stim (n = 5)	No Stim (n = 6)
Thalamic Pathway	HFS (n = 6)	HFS (n = 6)
	LFS (n = 7)	LFS (n = 5)
	No Stim (n = 5)	No Stim (n = 6)

Table 4.1: Experimental design

4.2.5 Data Analysis

The procedure for data analysis was the same as that described in the associative LTP experiment. Briefly, custom matlab software was used to measure the amplitude of field potentials for the different days. The amplitude measurements were standardized for each rat by subtracting the raw value from the average of the 4 baseline measurements. Using these measures, a repeated measures ANOVA was used to establish significant effects.

4.2.6 Histology

As before, the animals were perfused upon completion of the experiment. The brains were processed for Prussian Blue and Neutral Red to confirm location of the electrode tips.

4.3 Results

4.3.1 Behaviour following drug administration

Rats injected with AIDA behaved the same as the saline controls. There was no change in locomotor behaviour, grooming, or eating and drinking. When the rats

were handled again 90 minutes following the injection, they vocalized normally and struggled like control animals.

There did not appear to be any behavioural consequence of chronic administration of AIDA. Animals remained healthy throughout the experiment, continuing to eat and drink normally. The AIDA rats gained weight at the same rate as control animals.

The brains of AIDA rats appeared the same as saline rats. There were no observable structural differences between the two conditions.

4.3.2 Callosal Pathway

Raw Data. Surprisingly, blocking mGluRs partially blocked induction of LTP, but did not affect the induction of LTD. The main effect of blocking mGluRs on callosal plasticity was a partial block of LTP in the early, but not late, component (Fig 4.2). The morphology of the early component in the AIDA rats was similar to the saline animals, but the amplitude change was reduced. Late component potentiation was approximately the same in AIDA and control rats, providing further evidence that the two components are independent.

Antagonizing mGluRs did not affect the induction of LTD on the callosal pathway. LFS induced a marked depression that was similar in morphology and amplitude to the saline control animals. The depression produced by the LFS protocol in this experiment (900 pulses at 1 Hz, 1000 μA pulse intensity) produced a larger depression than the LFS protocol used in the previous experiment (600 pulses at 1 Hz, 500 μA pulse intensity).

Administration of the mGluR blocker alone, in the absence of any conditioning stimulation, did not effect the morphology of the callosal responses. The responses of both the AIDA and saline rats remained stable over the course of the experiment.

Input/Ouput Functions. The input/output functions for the callosal pathway are shown in Figure 4.3. The most notable effect of mGluR antagonism on callosal plasticity was a partial block of LTP in the early, but not late component. Comparing the early component curves for the saline and AIDA animals, it is clear that the magnitude of the shift in the saline animals is larger ($F_{8,64} = 2.85$, $p < 0.01$). However, early component LTP was not completely blocked in the AIDA animals as the baseline and post-conditioning curves were significantly different ($F_{8,80} = 4.81$, $p < 0.001$). For the saline animals, the effect was also significant ($F_{8,48} = 2.91$, $p < 0.001$).

LTP in the late component of the callosal pathway did not appear to be affected by mGluR antagonism. The post conditioning input/output function resembled that from a typical LTP group. The largest potentiation occurred in the middle intensities and was only slightly evident at higher intensities. The interaction was significant ($F_{8,80} = 19.97$, $p < 0.001$). The post-conditioning input/output function for the saline rats was similar except for an atypical effect at the highest intensity. Potentiation, as usual, was evident in the middle intensities, but the highest intensity showed a small depression. One possible explanation for this is a small number of animals in this group (4). Two rats in this group developed infections prior to completion of testing and were removed from the experiment.

Blocking mGluRs did not affect the induction of LTD in the callosal pathway. Rats treated with AIDA exhibited a consistent depression across intensities, similar to the saline controls. The main effect was significant for both the AIDA and saline conditions ($F_{1,12} = 26.15$, $p < 0.001$ and $F_{1,12} = 7.78$, $p < 0.05$ respectively).

In the control animals, administration of AIDA in the absence of any conditioning stimulation had no effect on the input/output function. The pre- and post-conditioning curves were approximately the same for both the AIDA and saline control animals. Although administration of AIDA produced no lasting effect, there

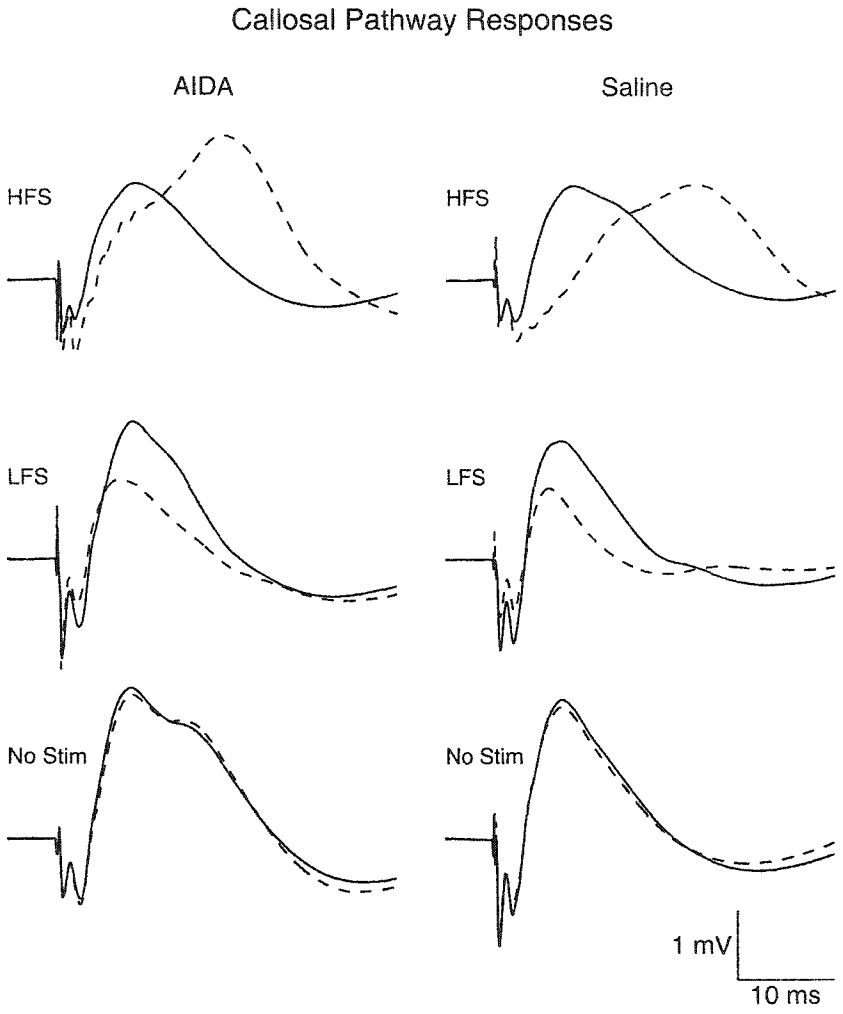

Figure 4.2: Callosal pathway raw data. Representative sweeps from the different groups are shown. The left column shows the different stimulation conditions for the animals injected with the mGluR blocker (AIDA). The right column shows the stimulation conditions for animals injected with saline. Solid lines are baseline responses, dashed lines are 24 hours after the last conditioning session.

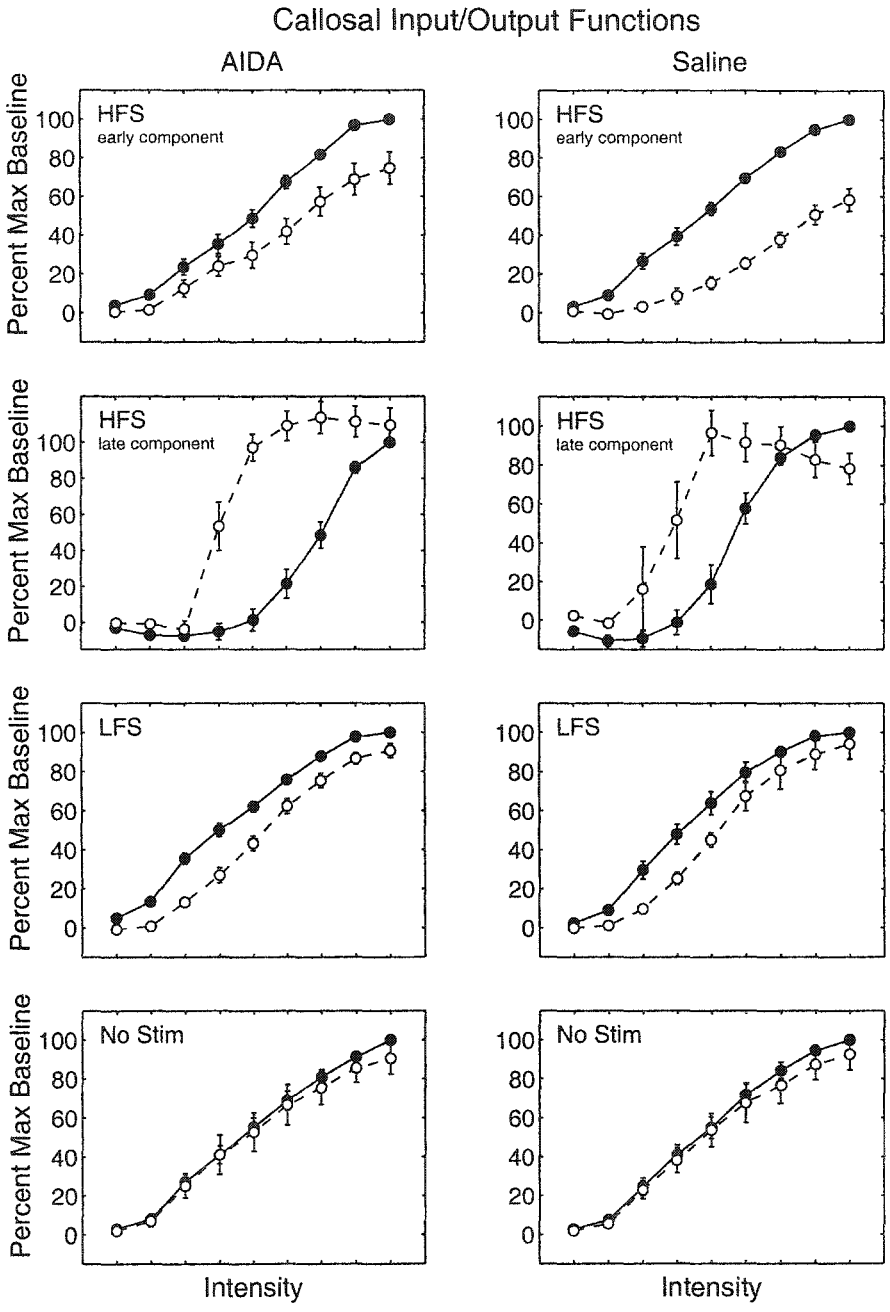

Figure 4.3: Callosal pathway input/output functions. Solid lines and filled symbols are baseline measures, dashed lines and open symbols are 24 hours following the final conditioning session. The functions in the left column are from animals treated with the mGluR blocker (AIDA), the right column are saline controls. Stimulation condition is indicated at the top left of each panel.

appeared to be a transient effect noticeable during the first few days of administration (see Figure 4.4).

Induction and Decay. The group data for the callosal pathway is shown in Figures 4.4 and 4.5. Antagonizing mGluRs partially blocked the induction of LTP in the early component. The saline animals developed a marked potentiation over the 10 days of conditioning ($F_{9,72} = 14.66$, $p < 0.001$). However, HFS did not potentiate the responses of the AIDA rats at the same rate. For the first 4 to 5 days of conditioning their responses remained stable. During the remaining 5 days of conditioning their responses did begin to potentiate, but at the end of the 10 days, the change in their responses were only about half that of the saline rats. The difference between the conditioned AIDA rats and the control AIDA rats was significant ($F_{9,81} = 2.72$, $p < 0.01$). The difference between the conditioned AIDA rats and the conditioned saline rats was also significant, supporting the conclusion that blocking mGluRs partially blocked LTP in the early component ($F_{9,72} = 2.94$, $p < 0.01$).

There appeared to be a temporary effect of drug administration alone. The responses of the AIDA rats that did not receive conditioning stimulation showed a small depression effect during the first 4 or 5 days. After this period of time the responses returned to baseline levels. This effect did not prove to be significantly different from the saline control rats ($F_{9,81} = 1.38$, $p = 0.21$). It is interesting that this depression occurred at the same time as the strongest block of LTP in the conditioned rats. When the depression in the control rats began to reverse, the responses of the conditioned rats began to potentiate.

Administration of the mGluR blocker did not affect induction of LTP in the late component. Daily tetanization strongly potentiated the responses of both the saline and AIDA rats ($F_{9,72} = 18.31$, $p < 0.001$ and $F_{9,81} = 2.50$, $p < 0.001$ respectively). The amount of potentiation did not differ significantly between the two groups ($F_{9,72} =$

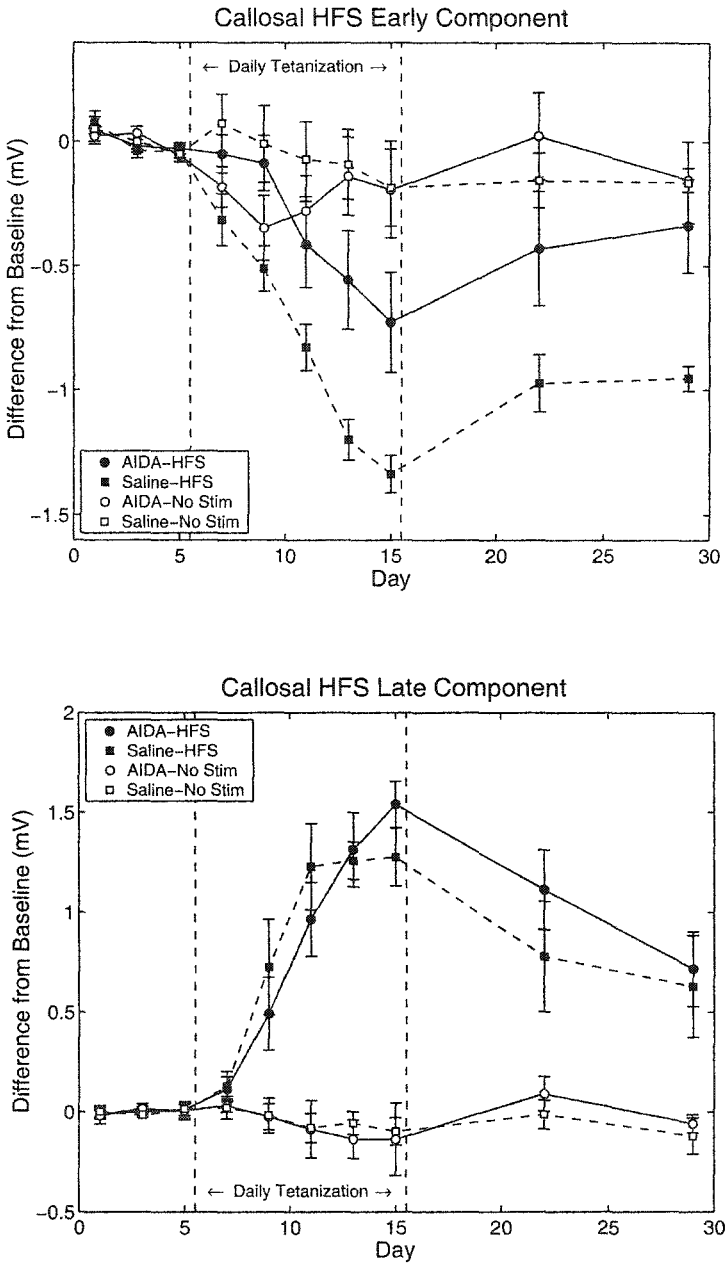

Figure 4.4: Callosal group data for the early and late components showing the effects of high-frequency stimulation and mGluR antagonism. Data are standardized to the average of the 3 baseline measures

1.06, $p = 0.40$).

Drug administration alone did not affect the callosal late component. The responses of the saline and AIDA control rats remained relatively stable over the course of the experiment, and there was no significant difference between these groups ($F_{9,81} = 0.32$, $p = 0.97$).

The group data showing changes in the callosal pathway during LFS is shown in Figure 4.5. Surprisingly, blocking mGluRs did not block the induction of LTD. The

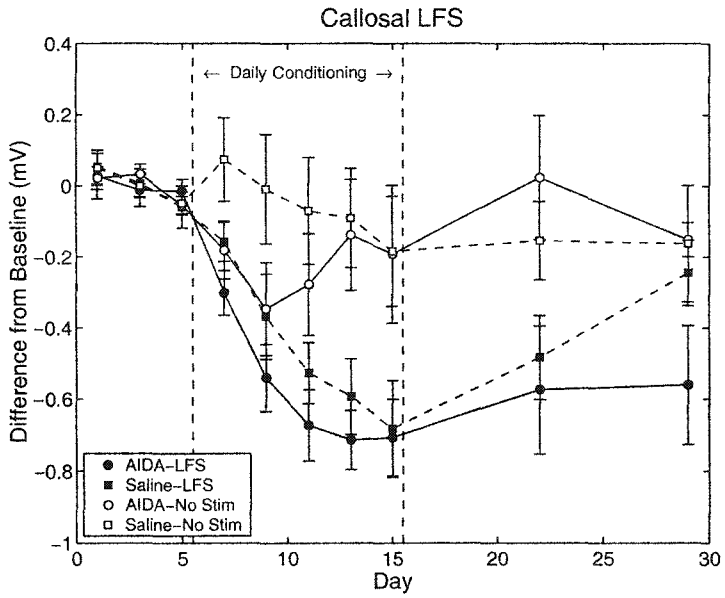

Figure 4.5: Callosal group data showing the effects of low-frequency stimulation and mGluR antagonism. Data are standardized to the average of the 3 baseline measures

responses of both the saline group and the AIDA group depressed progressively over the course of the 10 days of conditioning. Both saline and AIDA groups that received LFS were significantly different from their respective control groups ($F_{9,99} = 3.67$, $p < 0.001$ and $F_{9,90} = 3.32$, $p < 0.01$ respectively). There was no difference in the amount of depression induced in the saline and AIDA groups ($F_{1,12} = 1.36$, $p =$

0.27). During the two week period following conditioning, there was a trend for the depression effect to decay faster in the saline animals. The responses of the AIDA group remained depressed, but the saline responses decayed gradually back to near control values.

4.3.3 Thalamic Pathway

Raw Data. Blocking mGluRs did not affect any of the plasticity measures in the thalamic pathway. Representative responses from the different groups are shown in Figure 4.6. In saline animals, both HFS and LFS caused a depression of the evoked response that was approximately equal in magnitude. Neither of these effects was affected by mGluR antagonism. The responses of rats injected with AIDA were depressed for both the HFS condition and the LFS condition and at a magnitude similar to that seen in the saline rats.

Input/ Output Functions. The input/output functions do not reveal any differences between the saline and AIDA rats (Fig. 4.7. Following HFS, the responses of saline animals were depressed at all intensities ($F_{8,80} = 3.36$, $p < 0.01$). The responses of AIDA rats were also depressed following HFS ($F_{8,80} = 6.61$, $p < 0.001$).

Following LFS, the responses of the saline and AIDA rats were also significantly depressed ($F_{8,64} = 8.20$, $p < 0.001$ and $F_{8,96} = 2.89$, $p < 0.01$ respectively).

Induction and Decay. The group data, showing the effects of HFS and LFS across days, is shown in Figure 4.8. HFS caused a progressive depression of the field potential in both the saline and AIDA rats. The effect was significant for both groups (saline: $F_{9,90} = 4.07$, $p < 0.001$; AIDA: $F_{9,81} = 2.28$, $p < 0.05$). The magnitude of the depression was approximately the same for the saline and AIDA rats ($F_{1,10} = 1.84$, $p = 0.20$).

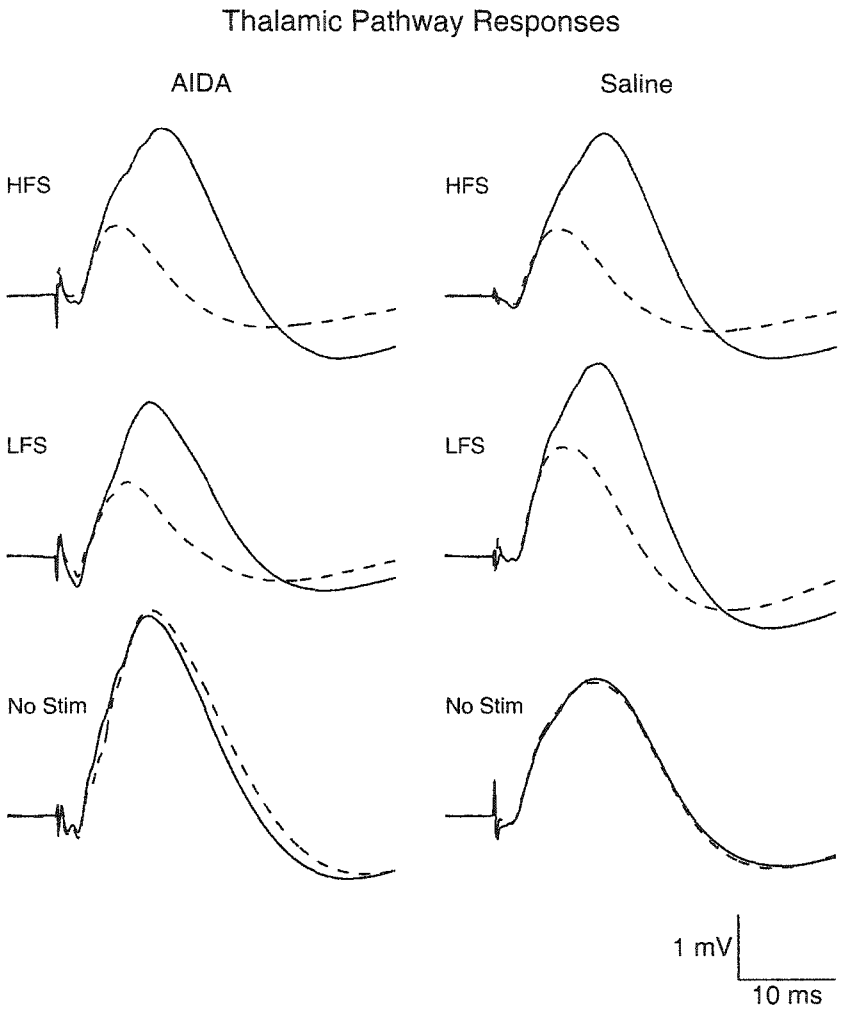

Figure 4.6: Thalamic pathway raw data. Representative sweeps from the different groups are shown. The left column shows the different stimulation conditions for the animals injected with the mGluR blocker (AIDA). The right column shows the stimulation conditions for animals injected with saline. Solid lines are baseline responses, dashed lines are 24 hours after the last conditioning session.

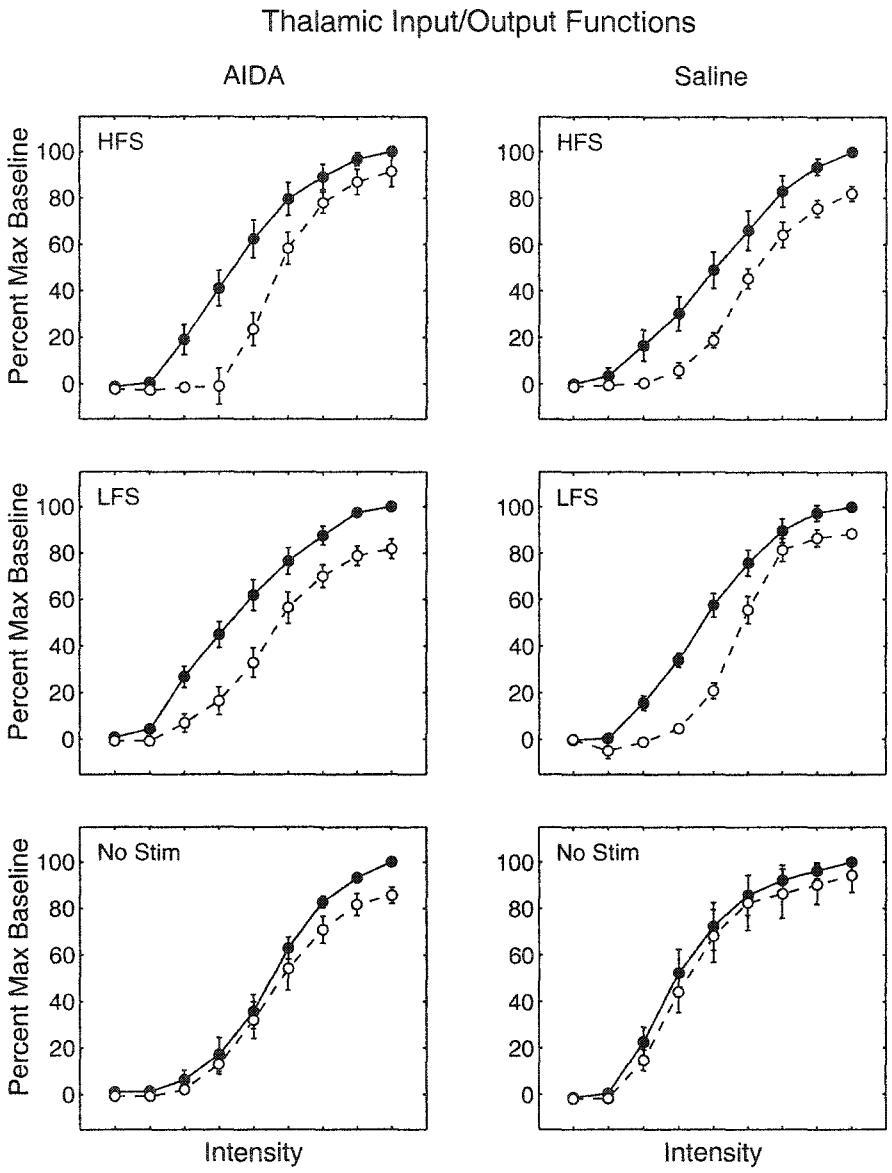

Figure 4.7: Thalamic pathway input/output functions. Solid lines and filled symbols are baseline measures, dashed lines and open symbols are 24 hours following the final conditioning session. The functions in the left column are from animals treated with the mGluR blocker (AIDA), the right column are saline controls. Stimulation condition is indicated at the top left of each panel.

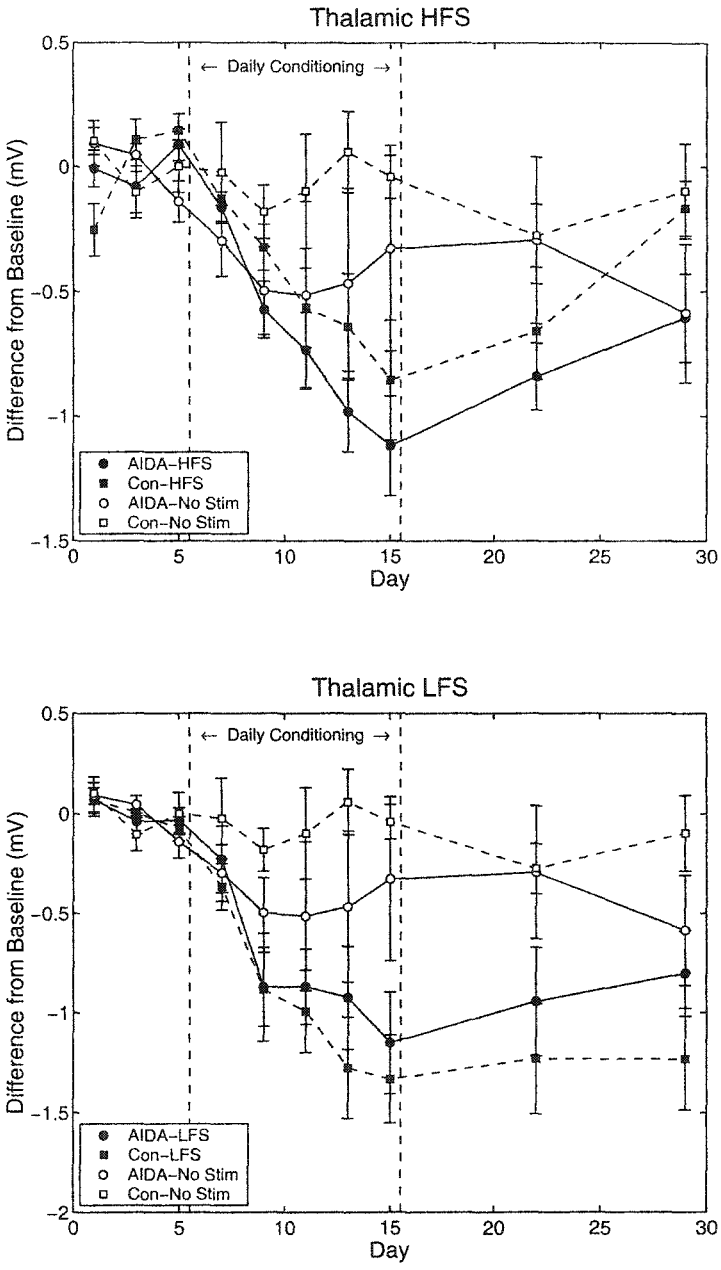

Figure 4.8: Thalamic group data showing the effects of high- and low-frequency stimulation and mGluR antagonism. Data are standardized to the average of the 3 baseline measures

As observed in the callosal pathway, there appeared to be an effect of administering the mGluR blocker by itself. The responses of the rats that received AIDA but no conditioning stimulation depressed slightly during the first few days of the conditioning period. The effect did not last and the responses returned to near control values. Although this depression was consistant with that seen in the HFS controls, the responses were more variable in this group and the effect did not reach statistical significance ($F_{9,81} = 1.35$, $p = 0.22$).

Blocking mGluRs had no significant effects on the induction of LTD in the thalamic pathway. The saline rats developed a significant depression over the course of the conditioning period ($F_{9,81} = 12.80$, $p < 0.001$). The amplitude of the responses of the AIDA rats also depressed over the course of the conditioning period and there was no significant difference between the groups in the level of LTD induced ($F_{1,10} = 0.50$, $p = 0.49$). However, administration of the mGluR blocker alone caused a small depression, and, although the stimulation plus drug produced an apparently larger LTD than drug alone, the two groups were not significantly different ($F_{9,90} = 1.74$, $p = 0.09$). Thus, part of the depression effect observed in the conditioned AIDA rats was likely due to the effects of drug administration alone.

4.4 Discussion

The purpose of this experiment was to investigate the contribution of group 1 mGluRs to LTP and LTD induction in two afferent pathways to the neocortex. In the callosal pathway, blocking mGluRs partially blocked the induction of LTP in the early but not late component and there was no effect on the induction of LTD. In the thalamic pathway, mGluR antagonism partially blocked the effects of LFS but not HFS.

4.4.1 Callosal Plasticity

Previous work in our lab has shown that neocortical LTP in the freely behaving animal depends on NMDA receptor activation (Trepel and Racine, 1998). If NMDA receptors are blocked during conditioning, then LTP is blocked in both the early and late components. The results of the present experiment show that group 1 mGluRs also contribute to the induction of LTP in the early component, but they do not contribute to LTP in the late component.

From the limited evidence available, the role of mGluRs in hippocampal LTP is controversial (Anwyl, 1999). There has been even less work done to investigate the role of mGluRs in neocortical LTP. Two studies in visual cortex slices provided conflicting results. In one study, application of MCPG prior to conditioning did not block the induction of LTP (but did block LTD; Haruta et al., 1994). In the other study, a non-NMDA-dependent form of LTP was induced using weak theta-burst stimulation. Application of MCPG prior to conditioning blocked the induction of this form of LTP. The discrepancy between the findings of the two studies could be due to the fact that they were examining slightly different forms of LTP. However, it could also be due to the fact that MCPG has been shown to be an ineffective antagonist of group 1 mGluRs (Huber et al., 1998). The results presented in this chapter provide clear evidence that blocking mGluRs with the selective group 1 antagonist AIDA partially blocks LTP in the early component.

Blocking mGluRs failed to block the induction of LTP in the callosal late component. This finding provides further evidence of mechanistic differences between the two components. The results of the first two chapters showed that the late component was more susceptible to associative conditioning paradigms than the early component. The results from the current experiment show that LTP induction in the

late component does not depend on mGluR activation, whereas LTP induction of the early component does depend partly on group 1 mGluR activation. Taken together, the results from the 3 chapters provide strong evidence for the different properties of the two neocortical field potential components.

Blocking mGluRs did not affect the induction of LTD in the neocortex. This was somewhat surprising given that several studies in the hippocampus have shown a role of mGluRs in LTD (Anwyl, 1999). Results from neocortical studies have been mixed. Some early reports using the broad spectrum antagonist MCPG showed that LTD in the neocortical slices was blocked following mGluR antagonism (Haruta et al., 1994; Hensch and Stryker, 1996). However, a more recent study using the group 1 selective antagonist LY341494 showed that LTD was not blocked following LFS (Sawtell et al., 1997). The results from the current experiment, which also used a selective group 1 mGluR antagonist, support the more recent finding. Given that MCPG is a broad spectrum antagonist, but only weakly antagonizes group 1 mGluRs, it is possible that group 2 or 3 mGluRs play more of a role in neocortical LTD. The findings from a recent study support this hypothesis. Using a neocortical slice preparation, Otani et al. (2002) found that bath application of a group 2 mGluR *agonist* was sufficient to induce LTD. Furthermore, application of a group 2 selective *antagonist* blocked the induction of LTD following conditioning stimulation. Thus, it could be that groups 2 and 3 mGluRs are more important in neocortical LTD, whereas group 1 mGluRs are more important in hippocampal LTD.

4.4.2 Thalamocortical Plasticity

The effects of mGluR antagonism on thalamocortical plasticity are less clear. Both HFS and LFS induced a lasting depression of the field potential. However, the appli-

cation of the antagonist alone produced a temporary small depression. After taking this into account, only the effect of HFS was significant. Thus, blocking mGluRs partially occluded the effects of LFS but not HFS.

In the saline rats, LFS induced a reliable depression. This is a notable difference from the results of the previous chapter. The LFS protocol used in the previous chapter only produced a small depression—an effect that was slightly smaller than the depression produced by HFS. The 1 Hz LFS protocol used in the previous chapter consisted of 600 pulses at an intensity of 500 μA, whereas in this experiment it consisted of 900 pulses at an intensity of 1000 μA. There is recent evidence that the induction of homosynaptic LTD involves metaplastic changes (Mockett et al., 2002). That is, pulses early in the conditioning procedure prime the pathway for LTD induction by pulses later in the conditioning procedure. Mockett et al. (2002) found little LTD in the hippocampus following 600 or 900 pulses, but significant LTD following 1200 pulses. The 1200 pulses were equally effective if they were delivered during a single session or as two sessions of 600 pulses. Thus, it could be that the 600 pulse protocol used in the previous chapter was not long enough to induce robust LTD.

Thus far in this thesis, the properties of thalamocortical plasticity have been difficult to interpret. The effects of AIDA administration on LTD induction do not suggest a clear role for group 1 mGluRs in thalamocortical LTD. Blocking mGluRs did not prevent the induction of LTD following LFS. However, there did appear to be a partial occlusion of LTD following drug administration. Drug administration alone induced a transient depression, and little further depression was induced in the drug + conditioning group. Thus, mGluRs are not necessary for LTD induction in the neocortex, but the effects of the drug alone group suggest that they might play a role maintaining a balance of activity. It could be that the homeostatic state of a glutamatergic synapse depends on activation of both ionotropic and metabotropic

receptors. Because activation of both types of recpeptor can increase intracellular calcium, blocking mGluRs could reduced the amount of calcium in the postsynaptic cell. The reduced level of calcium could cause a depression (Lisman, 1989).

The HFS-induced plasticity in the thalamocortical pathway remains difficult to interpret. The effects of HFS were not blocked by the group1 mGluR antagonist, suggesting that the effects are mediated by a different substrate. The effects of thalamic HFS share some properties with neocortical *LTP*, although the effect is opposite in direction. They both show an incremental induction over several days and the magnitude of the effect is increased following associative conditioning. LTP in the neocortex of freely behaving rats has been shown to depend on NMDA receptor activation (Trepel and Racine, 1998). It could be that HFS-induced plasticity in the thalamocortical pathway also depends on NMDA receptor activation. This possibility remains to be tested.

Chapter 5

General Discussion

Changes in synaptic strength as a result of experience are thought to mediate information storage in the brain (Martin and Morris, 2002). Long-term potentiation of synaptic transmission is a model of activity-dependent changes in synaptic strength that allows the study of the mechanisms of these changes. A significant amount of research on LTP over the years has made considerable progress toward characterizing these mechanisms. However, most of the research so far has been done in hippocampal slice preparations. In order to validate LTP as a model of memory, experiments investigating mechanisms must be undertaken in the neocortex of awake, freely behaving animals.

This thesis consisted of a series of three experiments designed to test mechanisms of LTP and LTD in two input pathways to the neocortex in freely behaving rats. The first two chapters tested the effects of associative conditioning of both pathways on the induction of LTP and LTD. The third chapter tested the effects of blocking group 1 metabotropic glutamate receptors on the induction of LTP and LTD. The significance of the findings will be discussed under four themes: associative conditioning, callosal

plasticity, thalamic plasticity, and molecular mechanisms.

5.1 Associative Conditioning

The experiments on associative conditioning were undertaken for three main reasons. First, classic experiments by Levy and Steward (1979) established *associativity* as a key property of LTP in the hippocampus. It is important to know if this property holds for neocortical LTP in freely behaving animals. Second, one theoretical prediction that resulted from the early associative LTP experiments was that anticorrelated activity administered on two pathways should induce LTD (e.g., Stanton and Sejnowski, 1989). Experimental support for this prediction has been mixed. Finally, associative conditioning has implications for learning and memory in the context of classical conditioning.

The effects of associative conditioning on both LTP and LTD were examined. In this thesis, the term 'associative' has been used to indicate the general condition where two input pathways are stimulated. When attempting to induce LTP, a strong pathway and a weak pathway were coactivated to test if the weak pathway would potentiate (Levy and Steward, 1979). When stimulated alone, the weak pathway does not potentiate, presumably becuase it does not drive the postsynaptic neuron sufficiently. Without the postsynaptic activation, the pre- and postsynaptic activity is anticorrelated and no LTP is induced. Coactivation of the strong pathway provides the necessary postsynaptic activity and the weak pathway can potentiate. When attempting to induce LTD, the purpose of the experiment was to anticorrelate stimulation on two pathways. Under these conditions, an associative LTD would be one that was greater in magnitude than either a homosynaptic or heterosynaptic LTD.

The results presented in the chapter on associative LTP demonstrated effects

of associative conditioning that were greater than those produced by homosynaptic stimulation. However, the results were not as straightforward as those reported in the hippocampus (Levy and Steward, 1979). LTP of the callosal late component was facilitated but the early component was not affected. The thalamocortical pathway, a 'weak' pathway, was expected to potentiate when coactivated with the strong callosal pathway. A significant *depression* of the thalamocortical pathway occurred under these conditions. Strictly speaking, the effect still qualifies as an associative effect, because the magnitude of depression was greater than that following homosynaptic tetanization of the thalamic pathway or heterosynaptic tetanization of the callosal pathway.

The results presented in the chapter on associative LTD did not support the prediction that associative conditioning would produce a larger LTD than homo- or heterosyanptic conditioning. On both the callosal and thalamic pathways, associative conditioning produced depressions approximately equal in magnitude to homosynaptic conditioning. Evidence for associative LTD has been mixed (e.g., Bear and Abraham, 1996) and the current results argue against an associative LTD effect in the neocortex.

Previous work in our lab has shown that LTD induction in the neocortex does not follow the same rules as LTP induction (Froc et al., 2000). LTP induction in the neocortex of the freely behaving animal requires spaced, repeated activation over several days (Trepel and Racine, 1998). While LTD induction requires multiple stimulation sessions, the timing of the sessions is not as critical. Ten stimulation sessions over the course of 10 hours will produce an LTD equal in magnitude to 10 stimulation sessions over the course of 10 days (Froc et al., 2000). A lack of an associative effect for LTD may be another difference between the two phenomena.

In relation to learning and memory, the associative stimulation paradigm most

closely models classical conditioning (Kim and Thompson, 1997; Blair et al., 2001). For example in fear conditioning, an auditory tone (the CS) weakly activates neurons in the lateral amygdala whereas a foot-shock (the US) strongly activates an overlapping population of neurons. Following repeated pairings of the CS and US, the response to the CS is potentiated, suggesting that associative LTP has taken place (Blair et al., 2001). The two pathways studied in this thesis were chosen partly because the neocortex receives patterned activation from both of these pathways during normal behaviour, likely satisfying the requirements for associative LTP (or LTD) mechanisms. The resultant changes in synaptic strength in the motor cortex would have obvious consequences for behaviour. The results clearly demonstrate that the two pathways interact to produce associative changes at neocortical synapses. However, further experiments are needed to understand the complex nature of the associative changes observed.

5.2 Callosal Plasticity

One unexpected finding to emerge out of these experiments is the independence of the callosal early and late components. Regression analysis of the early and late components showed that the amount of LTP induced in the early component was a poor predictor of the amount of LTP in the late component. Following associative high-frequency stimulation, the late component showed a facilitation of LTP, but the early component did not. If HFS on the callosal pathway was paired with LFS on the thalamic pathway, a reduction in the amount of late component LTP, but not early component LTP, was observed. Finally, administration of a group 1 mGluR antagonist partially blocked LTP in the early component, but had no effect on the late component. All these lines of evidence suggest strong physiological and molecular

differences between the two components.

Current source density analyses from our lab indicate that both the early and late components are the result of current sinks located in upper layer V, but they are associated with different current sources (Chapman et al., 1998). This indicates that the terminal fields of the projections driving each component overlap spatially. Because of this overlap, the authors suggested that the late component might be driven by local horizontal connections. This hypothesis could be tested using some of the results from this thesis to establish criteria. If one stimulating electrode was placed in the thalamus and one in the horizontal connections, the monosynaptic component of the horizontal fibers could be tested to see if it behaved like the callosal late component. Specifically, associative HFS on the thalamocortical and horizontal pathways should facilitate LTP induction in the horizontal pathway. Furthermore, HFS to the horizontal pathway combined with LFS to the thalamic pathway should reduce the amount of LTP induced in the late component. Finally, administration of an NMDA antagonist should block LTP in the horizontal pathway (Trepel and Racine, 1998), but administration of a mGluR antagonist should not.

One of the properties of the callosal late component is that it was influenced more by associative conditioning procedures. If it could be shown that the late component is driven mainly by horizontal fibers, then the associativity of this pathway could augment our understanding of behavioural data implicating horizontal connections in motor skill learning. Rioult-Pedotti et al. (1998) trained animals on a unilateral skilled reaching task and found that horizontal connections were stronger in the contralateral hemisphere compared to those in the ipsilateral (untrained) hemisphere. They later showed that the strengthening of connections was due to an LTP-like process because attempts to induce further LTP in the contralateral hemisphere were unsuccessful (Rioult-Pedotti et al., 2000). The skilled reaching task probably invokes pathways

similar to those studied in this thesis—a combination of thalamocortical, callosal and horizontal. Thus it could be that the strengthening of horizontal connections following skill learning is due to an associative interaction between these pathways.

5.3 Thalamocortical Plasticity

In the first chapter, the homosynaptic HFS to the thalamocortical pathway produced an unexpected long-term depression. The magnitude of the depression increased following associative HFS with the callosal pathway. Induction of a true LTD following HFS is rare, although possible (e.g., Trepel and Racine, 1998). Throughout the experiments described in the thesis, there was a trend for the responses in the thalamocortical pathway to depress, regardless of the type of conditioning. This property of the thalamocortical pathway raises questions about its possible functional significance.

One possibility is that the depression is a form of synaptic strength normalization. Connectionist models of neural systems that use a simple Hebbian learning rule will only increase connection strengths. In order to prevent uncontrolled growth in the connection strengths, some method for decreasing strengths must be employed. This can be achieved by using a modified Hebbian update rule that allows for decreases as well as increases in synaptic strength (van Ooyen, 2001). It can also be achieved by enforcing a rule that places a limit on the total amount of connection strength a neuron can support. Such a rule is known as weight normalization (Goodhill and Barrow, 1994). Typically the constraint placed on the learning maintains a constant sum of squares of the connection strengths. Thus, after one iteration in the model, some increases in synaptic strength will occur at some of the connections on the neuron. After the appropriate connection strengths are updated, the entire set of connection strengths are divided by a value that enforces the constraint (Goodhill

and Barrow, 1994). This procedure allows learning to take place, but maintains the connection strengths within reasonable limits.

It is possible that a similar process occurs in the motor cortex. As mentioned earlier (Section 5.2), Rioult-Pedotti et al. (1998, 2000) showed that acquisition of a motor skill is accompanied by an increase in the strengths of horizontal connections. This finding was surprising because it implies that learning the motor skill occurs primarily through increases in synaptic strength. If learning occurred via a bidirectional learning rule, then some synapses would increase in strength and some synapse strengths would decrease in strength. Since field potentials represent an average of the activity occurring in the underlying neurons (Mitzdorf, 1985), it is likely that no change would occur in the field potential following bidirectional learning. However, Rioult-Pedotti et al. (1998) did show an increase in the amplitude of the field potential evoked by stimulation of horizontal connections which is consistent with a large, net increase in synaptic strength as a result of learning the motor skill. The finding raises the same problem posed to connectionist models using a simple Hebbian learning rule: how is the uncontrolled growth of synaptic strength prevented? The tendency of the thalamocortical pathway to depress following both HFS and LFS suggests that thalamocortical depression might contribute to a temporary normalization of synaptic strength in the motor cortex.

The depression in the thalamocortical pathway might be a way of balancing the potentiation of the horizontal pathway. Under normal conditions, this could maintain a constant total synaptic strength in motor cortex neurons. This hypothesis makes predictions that could be tested. First, induction of LTP in horizontal connections might also cause a heterosynaptic depression at thalamocortical synapses. There is partial evidence for this prediction in this thesis. Some heterosynaptic effects were observed following conditioning of the callosal pathway. If the callosal late component

is driven by horizontal fibers, as proposed by Chapman et al. (1998), then this would support the hypothesis. However, a more direct test is necessary (i.e., inducing LTP in horizontal fibers). A heterosynaptic depression of thalamocortical responses should also occur following motor skill acquisition. This would have to be tested either in acute or chronic preparations as a slice preparation of the thalamocortical pathway to the motor cortex is not feasible. Finally, this hypothesis could be implemented in a connectionist model. Instead of applying weight normalization by forcing a decrease in connection weight at all synapses, it could be linked to the activity in a heterosynaptic pathway. A demonstration of learning in this network would demonstrate that a heterosynaptic weight normalization rule might support learning. However, this would likely be a temporary solution—a total lack of normalization in the horizontal pathways would eventually lead to saturation. Perhaps the thalamocortical depression provides a rapid means to maintain a constant set of connection strengths, and a more gradual normalization of both pathways occurs over a longer period of time.

5.4 Molecular Mechanisms

5.4.1 LTP

The induction of most forms of LTP requires the activation of NMDA receptors (Bliss and Collingridge, 1993). This is also true of neocortical LTP in freely behaving animals (Trepel and Racine, 1998). The results presented in this thesis indicate that group 1 mGluRs also play a role in neocortical LTP. Administration of the mGluR antagonist partially blocked LTP induction in the callosal early component, but not the late component. However, blocking mGluRs did not block the effects of HFS on the thalamocortical pathway. Given the limited effects of mGluR antagonism on

LTP induction, coupled with the complete block of LTP following NMDA receptor antagonism, it seems likely that mGluRs play a limited role in neocortical LTP in freely behaving animals.

5.4.2 LTD

Group 1 mGluRs have been shown to be involved in hippocampal LTD (Oliet et al., 1997), but their role in neocortical LTD has been more controversial. The results presented here indicate that group 1 mGluRs are not involved in callosal LTD. This supports recent findings from visual cortex slices (Sawtell et al., 1997). This raises the question of what molecular mechanisms do contribute to callosal LTD. Induction of LTD in the hippocampus is believed to follow a rise in intracellular calcium and activation of protein phosphatases (Lisman, 1989). These properties have been shown to hold for LTD in slices of visual cortex (Kirkwood and Bear, 1994). Thus, it is likely that LTD in the motor cortex is induced by a similar mechanism. However, recent work in our lab indicates that callosal LTD does not depend on NMDA receptor activation (Froc, 2002). If callosal LTD is independent of both NMDA and group 1 mGlu receptor activation, then what is causing the rise in intracellular calcium? Or is a rise in intracellular calcium even necessary?

One possible source of Ca^{2+} is the voltage-dependent calcium channel (VDCC). Support for a role of VDCCs in LTD has been mixed (Bear and Abraham, 1996). A recent study examined the role of VDCCs in one form of LTD in hippocampal slices (Normann et al., 2000). Using explicitly anticorrelated pre- and postsynaptic activity, they reliably induced LTD in the CA1 region of the hippocampus. If VDCCs were blocked prior to the conditioning procedure, LTD induction was unsuccessful. It could be that VDCCs contribute to LTD in the motor cortex. This hypothesis

remains to be tested.

Another possible source of Ca^{2+} is group 2 mGluRs. The results of a recent study provide strong support that group 2 mGluRs are involved in neocortical LTD (Otani et al., 2002). Using a frontal cortex slice preparation, Otani et al. (2002) found that bath application of an mGluR *agonist* was sufficient to induce reliable LTD. Furthermore, they showed that activation of group 2 mGluRs was associated with a rise in intracellular Ca^{2+}. These results suggest that group 2 mGluRs might play a role in neocortical LTD in the freely behaving animal. The relatively new mGluR antagonist, LY341495, could be used to investigate this possibility. It has been shown to block all mGluR subtypes and it can be administered systemically.

mGluR antagonism did partially occlude the effects of LFS on the thalamocortical pathway, but only because the antagonist itself induced a partial depression effect. A necessary next step in the investigation of the thalamocortical pathway is the investigation of the role of the NMDA receptor. The depression of thalamocortical responses following HFS, LFS, and associative conditioning is a curious phenomenon, but it provides further evidence for the complexity of the interactions among the cortical pathways and the need for additional work in awake, freely behaving animals.

Appendix A

Software Development

This appendix describes the development and implementation of the software used to collect and analyze the data in this thesis. The first section describes software written in LabVIEW used to collect the data. The second section describes software written in Matlab used to analyze the data.

A.1 Data Acquisition

LabVIEW is a graphical programming environment that can be used to control and measure a variety of systems. LabVIEW code is graphical—functions are represented as icons and loops as boxes. Writing a LabVIEW program involves selecting the appropriate function icons and connecting them to one another (passing arguments between them) via 'wires'. This is quite different from the common programming language construct, although the programming logic is similar. An example piece of the LabVIEW code used in the data acquisition program is shown in Figure A.1.

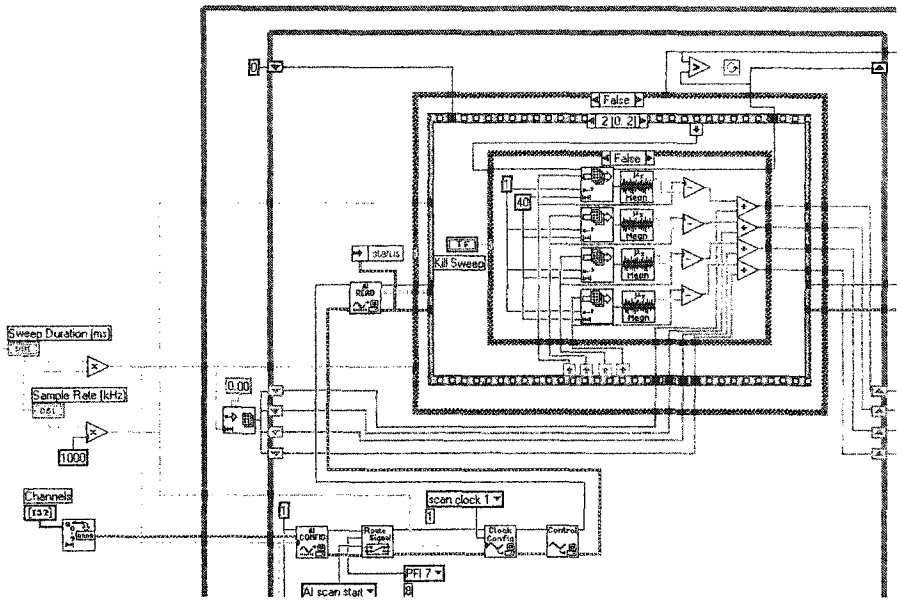

Figure A.1: Excerpt of the LabVIEW code used in the data acquisition program. Functions are represented as icons and the values passed between them are represented as the lines or 'wires'.

A.1.1 Evolution of the Program

The main purpose of the program is to read the digitized neural activity and synchronize this operation with the delivery of the stimulation pulses. For a typical input/output function, evoked activity from a number of stimulation intensities is recorded. At each intensity of stimulation, a number of responses must be averaged together because the responses vary slightly from pulse to pulse. Once all intensities have been run, the data must be stored to files. Thus, the secondary functions of the program include averaging the data and writing the data to text files. Implementing the secondary functions of the program proved to be straightforward, but the task of synchronizing the data collection with delivery of the stimulation pulses proved to be difficult.

The initial implementation of the synchronization was crude and relied on the timing of the operating system. This proved to be unacceptable because the timing in an operating system typically has an error margin of $\pm$ 3–5 ms. This is a significant amount of time in a 50ms sweep. The resultant variability in the relative timing of the data collection and pulse delivery operations prevented the sweeps from being averaged properly.

The second implementation of synchronization was more reliable, but it relied on the stimulator for timing. The stimulator was set to deliver pulses at a regular interval and to send out a trigger pulse at the same time. The program was written so that the start of the data collection was triggered by the pulse sent from the stimulator. This resulted in reliable synchronization of the two events and this is the version of the program that was used initially. The short-coming of this implementation is that the pulse output operation of the stimulator is limited, so the data collection procedures were necessarily restricted to more basic operations.

The final implementation of the program is close to the ideal. The timing of the stimulation pulses is controlled by the program and this operation has been synchronized with the data collection operation. The next section describes the implementation in more detail.

A.1.2 Current Version

The graphical interface of the program is divided into two screens: the first controls the data acquisition (Fig. A.2), and the second controls the trigger pulse output (Fig. A.3).

Most of the default settings in the program are designed for input/output function collection: a 50 ms sweep duration, sampled at 10 kHz, with a single trigger pulse sent

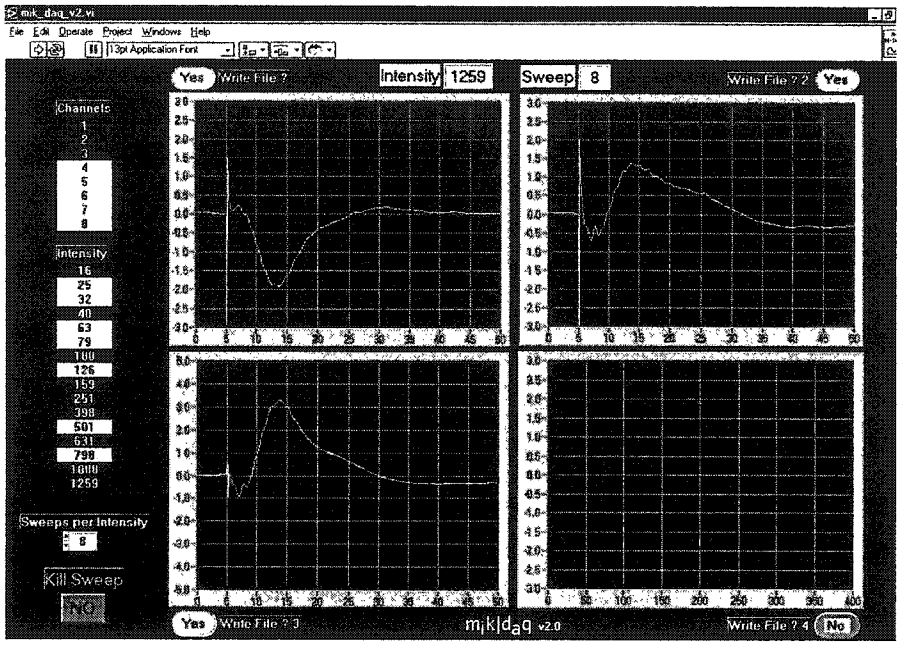

Figure A.2: Screen capture of the front panel that controls the data acquisition process.

to the stimulator 5 ms into the sweep. To collect an input/output function, the user must select which amplifier channels to record, the intensities to include, and the number of sweeps to average at each intensity. The appropriate `Write File` buttons must be depressed as well, if the data are to be stored.

When the program runs, the following steps occur. First, the trigger pulses are generated. The parameters set by the user are used to generate a vector of voltage values that will be used for the digital to analog conversion. For example, in the case of the single trigger pulse used for input/output functions, the value is held at 0 V for 5 ms, when it changes briefly to 10 V, and then returns to 0 V for the remainder of the sweep. This vector is stored in a buffer until the data acquisition begins.

Next, the parameters for the data acquisition are read and used to allocate sufficient memory for the process. Once the memory has been allocated, the main core of

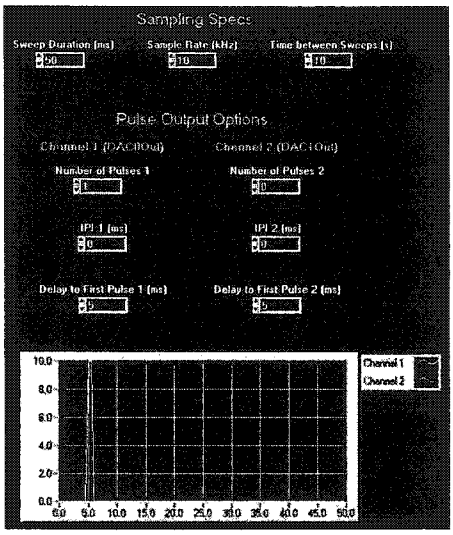

Figure A.3: Screen capture of the pulse output options. There are two channels for delivering trigger pulses, so two stimulators can be controlled independently.

the program, which consists of two nested loops, begins. The first loop is controlled by the number of selected intensities. The first operation in this loop is to set the appropriate intensity amplitude. This is done by writing an 8-bit word through the digital output line to the programmable intensity selector. The purpose of the programmable intensity selector is to adjust the voltage of the pulse generated by the stimulator before it is converted into a current pulse and delivered to the animal.

Once the intensity is set, the program enters the second loop, which is controlled by the number of sweeps to average at that intensity. Each iteration of this loop consists of synchronizing the trigger pulse delivery (analog out operation) with the data acquisition process (analog in operation). The start of the analog out operation is triggered by the start of the analog in operation. In this way the two processes always occur at the same time. Once the sweep has been collected, the data is displayed in the appropriate windows. The program then enters a wait cycle for the remainder of

the interval between successive pulses (usually 10 s). During the wait cycle, the user has the option to hit the `Kill Sweep` button. If the sweep is corrupted in some way (e.g., noisy), then depressing this button will prevent the sweep from being included in the average for that intensity. At the end of the wait cycle, the program checks the status of the `Kill Sweep` button and either discards the sweep or stores it in a buffer. Once the correct number of successful sweeps have been collected, they are averaged together and passed to the outer loop. This is the end of the inner loop, and the next iteration of the outer loops begins by setting the next intensity.

Once the averaged sweeps have been collected at each intensity they are written to a text file (if the `Write File` button is selected). This results in one data file per rat written for each input/output function.

A.2 Data Analysis

Our lab is primarily interested in how field potentials change over time. From the raw data, the most useful measures for this purpose are the amplitudes at the selected latencies of the field potential, measured across days. Thus, the main purpose of the program is to extract field potential amplitudes, for each animal in a group, from all of the input/output functions collected over the course of the experiment. This section describes the development of a graphical interface, programmed in Matlab, to accomplish this task.

A.2.1 Evolution of the Program

The first implementation of the program was crude at best. The user was able to select latencies graphically by mouse-clicking plotted sweeps and the resultant data was written to text files. Beyond that, the implementation left much to be desired. There

was little interaction with the user, so a number of variables had to be changed in the code before each analysis. Variables set in this fashion are known as 'magic numbers' because failure to change all of them correctly will result in irregular performance. This was one of the main issues addressed in the second implementation.

For the second implementation, the program prompted the user to input a number of variables. This corrected the 'magic numbers' problem, but it wasn't very sophisticated. The interaction with the user consisted of questions at the command prompt:

```
≫ How many animals to analyze ?  8
≫ How many IO's ?  12|
```

This version of the program was used for some time because it functioned well and was stable. The one drawback to this version is that the command prompt interface is intimidating for new users and, in this age of sophisticated graphics, it isn't very aesthetically pleasing. The final version of the program includes a GUI interface, making it very user friendly.

A.2.2 Current Version

The current version builds significantly on the core of the first two versions. The most significant change is the addition of a graphical interface (Fig. A.4). This eliminates the need to use the command prompt. All of the program operations are controlled with selection menus and push-buttons.

Initially the user selects a number of options, such as number of animals etc., via several drop down menus. Once the options are set, the analysis begins. The main loop of the program is controlled by the number of animals. The loop starts by prompting the user to select the first input/output function data file for the first

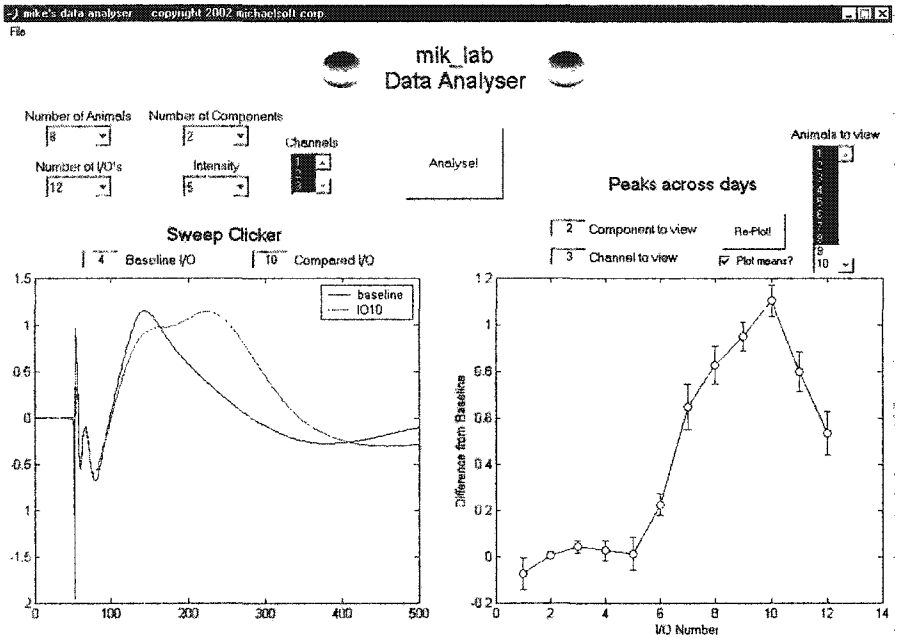

Figure A.4: Screen capture of the Matlab data analysis GUI. On the left are the controls for measuring the amplitudes across days. On the right are the visualization options.

rat. This file name is dissected to extract the rat number, channel number etc. Next, a baseline sweep and a post-stimulation sweep are plotted on the left axes. The user selects the latencies to measure with mouse clicks. When the latencies have been selected, the program cycles through all of the input/output functions for that animal and extracts the field potential amplitudes using the chosen latencies. The cycle then repeats until all rats have been analyzed.

Following the initial analysis, the user has the option to visualize the analyzed data. By default, the animals are plotted on an individual basis to make sure there are no inconsistencies in the data. The data can also be plotted as means $\pm$ SEM. These figures can be printed, or stored as postscript files.

To save the data, the program writes the amplitudes to a text file for each latency

and channel combination. In addition to the raw data, the latencies for each rat are also written to a file.

Programming the GUI was challenging, at first. The program is controlled by one master function. Within this function, each element of the GUI (push-buttons, selection menus, etc.) is represented by a separate sub-function. When the user interacts with one of the elements, the corresponding sub-function runs. For a simple element, such as a numeric selection menu, the function simply updates the value of a variable. The challenge was to make sure these values were available to the other sub-functions. One large data structure was used for this purpose. When the user updates a variable, the updated value gets stored in the master data structure. When the main function of the program is called (when the user depresses the `Analyse!` button), the values of the variables are retrieved from this data structure before the rest of the code executes.

A.3 Summary

In addition to the experiments in this thesis, the author developed a new data acquisition system. First, new data acquisition hardware was installed and configured. Second, data acquisition software was written using the graphical programming environment LabVIEW. Finally, a graphical interface for data analysis was written in Matlab.

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