ACTIVITY DEPENDENT PLASTICITY IN PATHWAYS BETWEEN SUBCORTICAL AND CORTICAL SITES

By

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ABSTRACT

Information storage between subcortical and cortical structures may include the occurrence of synaptic modifications such as those expressed following the induction of long-term potentiation, or LTP. It is well known that the hippocampus is important for memory, but certain adjacent and anatomically related cortical structures in the temporal lobe, especially entorhinal, perirhinal, and parahippocampal cortex also participate. It seems likely that the hippocampal system influences memory and consolidation primarily through an extensive set of reciprocal connections within the system and between this system and widespread areas of the neocortex. LTP has been extensively studied in the intrinsic connections of both the hippocampus and the neocortex. LTP in the pathways and structures thought to convey information between the hippocampus and the neocortex, however, have received little attention. It is unlikely that these structures simply relay information passively. Considerable additional processing must occur within them. Are they also involved in information storage? If so, and if LTP reflects a general synaptic encoding mechanism, then these systems should support LTP. Another focus of this thesis is the reciprocal thalamo-cortical system. Some of these pathways may also utilize LTP as a mechanism for information storage. Although much is known about the transmission of information from thalamus to cortex, little is known about the information transmitted along the reciprocal pathways back to the thalamus. In most cases these pathways are as large as,

or larger than, those running from the thalamus to the cortex. The current studies were designed to investigate LTP in the reciprocal projections in both the hippocampal-neocortical and thalamo-neocortical systems. Whereas the latter is often viewed as a hardwired system, the former system is believed to remain plastic throughout the lifetime of all mammals. One of the goals of this thesis was to test this hypothesis, using the parameters required for induction of LTP in cortico-cortical pathways in the chronically implanted rat. LTP was found in all pathways in the hippocampal-neocortical system that were examined. There were, however, differences in induction and decay rates that are of particular interest. These results are discussed in relation to the presumed memory functions of the medial temporal lobe. None of the thalamocortical pathways sustained LTP with multiple stimulation sessions, but the corticothalamic pathway, showed a lasting potentiation. This finding is of great interest. These results are discussed in relation to a filtering mechanism and the response of thalamo-cortical systems to mismatch information.

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General Introduction

1.0 MEMORY

The behaviour of many organisms can be modified by experience. In vertebrates, much of the adjustment in behaviour is due to the ability to learn, remember, and retrieve enduring representations of objects and events that the organism has experienced. Here, learning refers to the formation of an experience-dependent internal representation that is relatively long lasting. Memory is the retention of some or all of these representations over time. The time period over which these representations remain available can range from seconds to many years, depending on the organism, the experience represented, and the memory system engaged. Retrieval refers to the ability to recall and make use of the stored representations.

Although several conceptual frameworks have been developed to describe memory, it remains a difficult phenomenon to study. It has been assumed that memories are represented, in part, at the level of the cortex in a distributed array of cell assemblies. Little is known, however, about how the information is initially encoded, where it is encoded, or how the memories are reactivated. The highly distributed nature of the memory representations presumably accounts for many failed attempts to localize the engram (e.g. Lashley, 1950).

Model systems or model phenomena aid in the investigation of the physiology of memory. Model systems are simpler systems that show at least some of the features of the phenomenon of interest. For example, organisms such as aplysia, nematodes, or even drosophila have very simple nervous systems that are more amenable to experimental investigation. The components of the aplysia nervous system can actually be dissected out of the organism for study. The risk that is taken with this approach is that some of the processes and mechanisms present in more complex systems will be missing in the simple model systems. To get around this problem, researchers often study simpler *phenomena* in the more complex systems. A useful model phenomenon will show characteristics that are similar to those of the phenomena of interest, but will be more amenable to experimental investigation. The phenomenon that was utilized in the research outlined in this thesis is long-term potentiation (LTP). Before reviewing the research in this area, however, I will review the memory phenomena that we are striving to understand.

1.1 HUMAN MEMORY

Neuropsychological evaluation of the amnesic patient H.M. established, for the first time, that the ability to acquire new memories was a function distinguishable from other cognitive and perceptual abilities (Penfield & Milner, 1958; Squire, 1992; Squire & Zola-Morgan, 1991). H.M. became severely amnesic following the bilateral removal of his medial temporal lobes, including the anterior two-thirds of the hippocampus, the posthippocampal gyrus, and the amygdala. The experimental operation was performed in an attempt to relieve him from intractable epileptic seizures that had appeared to originate in the medial temporal

lobes. Following the operation H.M. exhibited an anterograde amnesia, specifically for facts and events, and a retrograde amnesia, for events in the decade preceding the operation (Corkin, 1984). More remote memories were not effected, suggesting that they were stored in an area other than the medial temporal lobes.

Other individuals with extensive medial temporal lobe damage have been found to show memory impairments similar to those seen in H.M.. Less extensive damage, however, tends to produce less severe memory loss (Milner, 1972; Penfield & Milner, 1958; Rempel-Clower, Zola, Squire & Amaral, 1996; Scoville & Milner, 1957; Squire, 1992). An interesting case in the literature is that of R.B.. R.B. had an ischemic episode secondary to a coronary bypass operation (Zola-Morgan, Squire & Amaral, 1986; Squire & Zola-Morgan, 1991). He also exhibited a moderately severe anterograde amnesia, with a mild retrograde amnesia, extending not more than a year or two (Rempel-Clower et al, 1996). R.B.'s memory deficit was significantly milder than that of H.M. (Corkin, 1984; Squire, Shimamura & Amaral, 1989). R.B. died from cardiac arrest approximately 5 years following the onset of his amnesia. A postmortem examination revealed a bilateral loss of all cells in area CA1, a subfield of the hippocampus. Only small pathological findings were detected in other brain areas and these were deemed to have been of little clinical significance (Squire, et al, 1989). These results strongly indicated that the amnesia seen in R.B. was specifically a result of a bilateral lesion confined to a subfield of the hippocampus. The more severe memory deficits of patients such as H.M., however, indicate that it is likely that non-hippocampal structures are also important for memory function.

Deficits In Declarative Memory. In general, amnesia resulting from bilateral damage

to the medial temporal lobes impairs the ability to acquire and store information about facts and events, or what is termed 'declarative' information (McClelland, McNaughton, & O'Reilly, 1995; Shimamura & Squire, 1987; Squire & Zola-Morgan, 1991). Declarative memory is not restricted to those memories that can be declared verbally. Declarative memory includes memory for faces, spatial layouts and other things that are really declared only in the sense that the image or fact is brought to conscious awareness (Squire, 1992). Whereas declarative memory is thought to be accessible to conscious recollection and available to various response systems, non-declarative memory is thought to involve an unconscious retrieval process that is expressed specifically through performance such as skill learning, conditioning, habit learning and the priming phenomena (Squire & Zola-Morgan, 1991). Declarative memory is apparently completely lost following extensive bilateral damage to the medial temporal lobes and patients are described as being unable to form any *new* memories. The capacity for non-declarative memory, however, is spared. H.M. was able to learn motor tasks following his operation, for example, but could not remember having ever done them.

Testing For Deficits In Declarative Memory. Given that some types of memories remain intact in subjects with temporal lobe damage, it is important that tests used to evaluate memory deficits be chosen carefully. Deficits in new learning are most often evaluated with tests of delayed recall or with tests of paired associate learning (Rempel-Clower, et al., 1996; Squire, et al., 1989), which would indicate the severity of the anterograde amnesia. Other tests include diagram recall, word recognition and face recognition tests. In a test of delayed recall, individuals are read a small passage of prose.

Recall is then tested immediately and after a delay that can extend for several minutes. Amnesics tend to be able to perform at near normal levels when tested immediately, provided the amount of information that they need to remember is not too large, but they fail when tested after the delay (Squire, et al., 1989). To examine memory using the paired associate learning test, amnesics are asked to learn pairs of unrelated words. The number of pairs, which generally number 10 or more, exceeds the capacity of short term memory. After presentation of the study list, a single word from the pair is presented and the subject is asked to produce the second word. Amnesics find this task very difficult, and they remember few if any of the pairs of words presented to them (see Rempel-Clower, et al., 1996 for comprehensive examination of amnesic patients). These findings are interpreted to mean that immediate, or short-term, memory is intact, while long-term memory is disrupted by medial temporal lobe damage (Squire, et al., 1989). The lesion-based separation of declarative and non-declarative memory systems, then, applies only to long term memory.

While it seems likely that the hippocampal system influences memory primarily through an extensive set of reciprocal connections with cortical areas (Squire, 1992; Otto & Eichenbaum, 1994), details of this system cannot be ascertained from amnesic patients alone. Experiments aimed at the study of animals, particularly rats and non-human primates, have provided a means for some refinement of the theories on multiple memory systems and the identification of some of the contributing circuitry.

1.2 ANIMAL MODELS OF TEMPORAL LOBE AMNESIA

Initial attempts to characterize the effects of temporal lobe damage in animals

provided few parallels with the human condition. Lesions to the hippocampus had the ability to produce deficits on spatial learning tasks in rats (Morris, Garrud, Rawlins, & O'Keefe, 1992; Nadel, 1991; O'Keefe & Nadel, 1978; Squire, 1992) and on non-spatial stimulus-stimulus associations such as transmission of food preferences (Bunsey & Eichenbaum, 1995). Ischemic episodes in rats also produce a circumscribed bilateral lesion involving the entire extent of the CA1 neurons in the hippocampus, much like the lesion seen in R.B. (Squire & Zola-Morgan, 1991). While there were behavioural deficits, they were difficult to describe in terms of impaired memory and even more difficult to describe in terms of amnesia. Rats were able to complete certain types of tasks normally and did not appear to suffer from global memory deficits. In order to validate the animal models, with respect to temporal lobe amnesia, it was necessary to reconsider the relationships between the memory tests that were being used to evaluate animals and humans.

Model of Human Amnesia in Monkeys. Mishkin and collegues (Mishkin, 1978; Mishkin & Delacour, 1975) developed an animal model of human amnesia in the nonhuman primate and this development set the stage for a series of elegant studies on temporal lobe systems. Evidence from monkeys, together with findings from rats and amnesic patients, has resulted in the identification of structures and connections that seem to be important for declarative memory (Squire, Knowlton, & Musen, 1993). To produce the model of human amnesia, monkeys received large bilateral lesions of the medial temporal lobe that approximated the damage received by H.M. (Mishkin, 1978; Squire & Zola-Morgan, 1991). The lesion was produced surgically and the damaged area included the hippocampal formation, the subicular complex, the entorhinal cortex, the amygdala, and surrounding

perirhinal and parahippocampal cortex. This lesion has been termed the H+A+ lesion as it included the hippocampus (H), the amygdala (A), and cortical regions (+) adjacent to the hippocampus and the amygdala (Mishkin, 1978; Mishkin & Delacour, 1975; Squire & Zola-Morgan, 1991). The lesion produced deficits similar to those seen in human patients. Especially interesting was the finding that, like amnesic patients, the monkeys were severely impaired on a number of memory tasks, but were quite normal at acquiring and retaining skills (Squire & Zola-Morgan, 1991).

Delayed Nonmatching To Sample Task. The task most commonly used to test monkeys is the delayed nonmatching to sample task (Squire & Zola-Morgan, 1991). This test of recognition memory appears to be comparable to the word association or recognition tasks used to test humans. A single object is presented initially. After a delay, two objects are presented, the original object and a novel one. The animal must correctly choose the novel object to obtain a food reward. Unique pairs of objects are used on every trial. Following lesions to the hippocampus and amygdala, and the cortical areas surrounding both structures, monkeys show greater performance deficits as the latency is increased between the reference and choice trials (Squire & Zola-Morgan, 1991). Monkeys exhibited normal performance at shorter retention delays. Also, monkeys were entirely normal at *relearning* postoperatively the rules of the task, as long as the retention intervals were kept short and there was a sufficient delay between the initial learning of the task and the operation (Mishkin & Delacour, 1975). Some of the information about how to perform the task must have been stored in areas untouched by the lesion.

The Role of Structures Within the Medial Temporal Lobe. In an attempt to determine

the function of the separate components of medial temporal lobe systems smaller and more specific lesions have been utilized (see Squire, 1992; Squire & Zola-Morgan, 1991; Alvarez, Zola-Morgan & Squire, 1994, 1995; Zola-Morgan, Squire & Ramus, 1994; Suzuki, Zola-Morgan, Squire & Amaral, 1993). Lesions that include only the hippocampus and surrounding cortex (H+) produce a smaller memory impairment than that seen following an H+A+ lesion (Squire and Zola-Morgan, 1991). The affected tasks include object discriminations, eight-pair concurrent discrimination learning and delayed response learning with delays up to 30 seconds (Squire, 1992; Zola-Morgan & Squire, 1990). There were no short term memory deficits with the H+ lesions (Alvarez, et al., 1994), and the long-term memory deficits were similar to those seen in the clinical population.

As lesions to the hippocampus and surrounding cortex were extended to include the amygdala, the cortex surrounding the amygdala, or a combination of the amygdala and surrounding cortex, the memory impairment became more severe (Squire & Zola-Morgan, 1991). Monkeys with a lesion to the amygdala alone (an A lesion), however, performed normally on the delayed nonmatching to sample task, as well as other memory tasks that appear to be sensitive to the H+A+ lesions and H+ lesions (Squire & Zola-Morgan, 1991; Zola-Morgan, Squire & Amaral, 1989). The evidence is consistent with the theory that the hippocampus and surrounding cortex are important for memory functions. The evidence also indicates that the amygdala is not intimately involved in declarative memory.

Alvarez, et al. (1995) used magnetic resonance imaging techniques and stereotaxic techniques to produce specific bilateral lesions limited to the hippocampus in the monkey and found a significant impairment in the delayed nonmatching to sample task. Monkeys

performed as well as normals with the 15 and 60 second delays, but exhibited memory deficits with the 10 minute delays. Although they performed better than monkeys with H+ lesions, their performance was similar to monkeys that had an ischemic hippocampal lesion (Alvarez, et al., 1995). Thus, like R.B., these monkeys had less damage, and suffered a less severe deficit than did monkeys with damage approximating that of H.M.. These results indicate an important memory function for the tissue adjacent to the hippocampus.

Lesions in the monkey that include the hippocampus and extend forward to include the anterior entorhinal cortex and perirhinal cortex, but not the amygdala (also called the H++ lesions) result in a memory impairment nearly as severe as that following the H+A+ lesions (Squire, 1992; Zola-Morgan, et al., 1994). In fact, lesions that extend into the anterior entorhinal cortex and the perirhinal cortex, but spare the amygdala *and* the hippocampus, produce a deficit similar to that produced by the H+ lesion (Gaffan & Murray, 1992; Squire, 1992; Zola-Morgan, Squire, Amaral & Suzuki, 1989). Lesions limited to the entorhinal and perirhinal cortex also result in memory impairments in rats (Mumby & Pinel, 1994; Otto & Eichenbaum, 1992).

There is a more robust memory impairment on the delayed nonmatching to sample task following damage to the perirhinal and parahippocampal cortices than following entorhinal cortex damage alone (Suzuki & Amaral, 1994b; Meunier, Bachevalier, Mishkin & Murray, 1993). These monkeys also show some behavioural recovery following the lesion, indicating that the entorhinal cortex may be utilized, but that it may not be essential for the delayed nonmatching to sample task (Leonard, Amaral, Squire, & Zola-Morgan, 1995; Squire & Zola, 1996). Much of the input to the entorhinal cortex is provided by the

perirhinal and parahippocampal cortices. Damage specific to the perirhinal and parahippocampal cortices produces deficits in delayed nonmatching to sample, as well as concurrent discrimination tasks (Squire, 1992). Although the hippocampus, amygdala, and entorhinal cortex were spared in these investigations, the impairments were still as severe as those seen following H+A+ and H++ lesions (Squire, 1992; Zola-Morgan. et al., 1989).

In summary, evidence from lesion studies indicates that the hippocampus is important for memory in rats, non-human primates and humans. It also indicates that adjacent and anatomically related structures are involved in memory functions. These sites are not critical for all memory functions, but play an essential role in what has been termed declarative memory, or memory for facts and events. There does, however, seem to be some time limit to the memory functions of this system. Permanent memories appear to be established independently of the medial temporal lobe system.

1.3 PARALLEL MEMORY SYSTEMS

Memory systems can be distinguished according to a series of attributes or criteria. These include the following: 1) the nature of the information stored, 2) the storage and retrieval mechanisms utilized, 3) the storage capacity, 4) the persistence, and 5) the susceptibility to interference (see O'Keefe & Nadel, 1978 for review). Much of what we know about these criteria has come from lesion studies, and the interpretation of the results of these studies has led to several proposals about complementary characteristics among memory systems.

Complementary Memory Systems. Descriptions of multiple memory systems are based on differences between these systems in the attributes outlined above. Distinctions based on the type of information processed and stored include episodic versus semantic (Tulving, 1972), local versus taxon (O'Keefe & Nadel, 1978), working versus reference (Olton, Becker & Handelmann, 1979), and procedural (non-declarative) versus declarative memory (Cohen & Squire, 1980). More recently, it was proposed that the brain was utilizing *complementary* memory systems (McClelland, et al., 1995; McClelland & Goddard, 1996), with a division of labours between systems that exploit the advantages of each.

McClelland and collegues (McClelland, et al., 1995; McClelland & Goddard ,1996) suggest that memories are first stored via synaptic changes in a fast learning hippocampal system and that these changes provide the basis for the reinstatement of recent memories in a slow learning neocortical system. Rapid learning rates, as seen in the hippocampus, are appropriate to systems designed for the rapid acquisition of critical information. This is done, however, at the cost of increased susceptibility to interference between input patterns. Rapid learning decreases capacity; sparse encoding can reduce this problem and reduce interference, at the expense of the ability to extract common structure. Once the hippocampus has stored information about a single episode, it can be used as a template for reinstating representations in the neocortex until such time that the information has become consolidated.

The representations in the hippocampus, according to the theory, are reduced sketches of the cortical patterns (McClelland, et al., 1995; McClelland & Goddard, 1996). Repetitions of the experience, or hippocampal reinstatements of the cortical representations, can serve

to establish a permanent and distributed trace in the slower learning neocortical system. McClelland, et al. (1995) propose that the action of reinstatement can occur during active rehearsal and reminiscence, as well as during other inactive states such as sleep, an idea originally proposed by Marr (1971). The number of reinstatements will determine the degree to which the pattern will be degraded by various other events that will interfere with it and cause decay.

Retrograde Amnesia is Associated With Medial Temporal Lobe Damage. One obvious component of the memory deficit produced by bilateral lesions to the medial temporal lobes is the temporally graded retrograde amnesia (Corkin, 1984; McClelland, et al., 1995; Squire, 1992; Warrington, 1996). In patients with retrograde amnesia, performance is impaired if damage to the medial temporal lobe memory system occurs within a window of time following an experience. Following such damage, humans perform much worse on more recent material than on older material, suggesting that the more recent memories are stored in a hippocampal or medial temporal lobe dependent form. Correspondingly, older material is stored in a hippocampal or medial temporal lobe independent form.

In order to determine the extent of the retrograde amnesia, memory for past events must be tested. Patients are asked about personalities, television shows and significant media events in the years preceding their memory deficit (The Public Events and Famous Faces Tests). Patients with temporal lobe damage show a temporally graded performance deficit on this kind of task, with more remote memories being less effected than more recent ones. It has been suggested that information is first rapidly stored in the medial temporal lobe and then over time transferred to another system, likely the neocortex. If the damage to the

medial temporal lobe system occurs before this transfer, all information is lost, but if the damage occurs during or after the transfer then some or all of the information will be maintained in the other structure. The change in dependence from the medial temporal lobe system to the other one has been termed 'consolidation'. Consolidation appears to be a slow and gradual process and can, in some cases, take up to 15 years (McClelland, et al., 1995). Retrograde amnesia gradients may reflect the gradual incorporation of new reinstatements into the neocortex and the loss of information represented by too few reinstatements.

Consolidation is a Component of Long-term Memory. Animal models have been used to more thoroughly investigate retrograde amnesia gradients. Zola-Morgan and Squire (1990) trained monkeys on 100 object pairs prior to bilateral removal of the hippocampal formation. Twenty object pairs were learned at each of 5 different times before the operation (16, 12, 8, 4, and 2 weeks). Following the surgery, memory for all 100 object pairs was tested. Whereas normal monkeys remembered the more recently presented pairs better than the ones learned earlier, monkeys with hippocampal lesions remembered the items learned early on in the training much better than the items presented most recently. Similar, but shorter, temporal gradients have also been demonstrated in rats when acquiring a context-dependent fear response at various times prior to hippocampal damage (Kim & Faneslow, 1992) and when acquiring a food preference prior to hippocampal damage (Winocur, 1990). In each of the experiments there was a time period during which the memory was completely or partially susceptible to hippocampal damage.

Interleaved Versus Focussed Learning. McClelland, et al. (1995) argue that the cortical system requires a strategy of interleaved learning in order to extract common

structure among multiple representations. Interleaved learning refers to the mixed presentation of information to be learned. Presentation of new information is interleaved with re-exposure to old material. The process of interleaved learning allows the cortical networks to take advantage of the slow learning rates to find a set of connection weights that can accommodate both old and new information as well as to extract the relationships between new and old information (McClelland. et al., 1995). The alternative is focussed learning where information is presented until some criterion of learning has been met. Only after the criterion is met, is a second set of material presented. Learning is accomplished rapidly with focused learning, but the memories are highly susceptible to interference. These interference effects can be avoided if the system continues to be exposed to the old information while acquiring new information.

Neural network models have been used to test the focussed versus interleaved protocols. A phenomenon called catastrophic interference is exhibited by the models when non-orthogonal patterns are presented in the focussed manner (McClelland, et al., 1995). This interference effect is so profound that retrieval of old information can drop to zero.

The advantage of possessing two interacting learning systems, then, is that critical information can be acquired and stored rapidly, while common structure can still be extracted from related input patterns, albeit more slowly. Catastrophic interference-like effects can, thus, be avoided. The medial temporal lobe memory system not only acquires information rapidly, it also participates in the reinstatement of representations in the neocortex. This is part of the interleaving process, and consequently, critical to the consolidation of information into the neocortex.

1.4 RECIPROCAL CONNECTIONS BETWEEN THE HIPPOCAMPUS AND THE NEOCORTEX.

To use the hippocampal system as an initial storage site and the neocortex as a final repository of memories, there must be routes by which activity can be propagated both into and out of the hippocampus during information processing. In addition, both the hippocampal and neocortical systems must utilize mechanisms for altering synaptic connections (McClelland, et al., 1995; Otto & Eichenbaum, 1994). Information is thought to be carried between the hippocampus and the neocortex via a series of bidirectional neuronal pathways. In fact, it is generally the case that projections between related brain areas are bidirectional (Maunsell & VanEssen, 1983; Felleman & VanEssen, 1991).

A survey of the literature indicates an extensive set of reciprocal connections between the hippocampus and neocortex that may play a role in short and long term storage and information transfer. Evidence from the monkey is quite detailed, but numerous similarities have been reported for the rat brain.

Anatomy and Afferents of the Entorhinal Cortex. It is well known that the entorhinal cortex provides much of the input to the hippocampus, but it also appears to be the primary structure involved in the routing of information *back* to the neocortex (McClelland, et al., 1995; Otto & Eichenbaum, 1994; Squire, 1992). The entorhinal cortex is comprised of six layers (Insausti, Tunlon, Sobreviela, Insausti & Gonzalo, 1995). Layer I , the plexiform layer, is compact with numerous branching apical dendrites, but few cell bodies. Layer II contains densely packed islands of large darkly staining cells, which are primarily stellate cells, and layer III is thicker, containing medium sized pyramidal cells. There is no granular

layer in the entorhinal cortex, but in place of a granular layer is an acellular region that is usually called layer IV. Layer V is comprised of large pyramidal cells and is only about 5-6 cell layers thick. Layer VI is difficult to distinguish from layer V, but there is an identifiable border in the more caudal portions of entorhinal cortex. Layer VI contains primarily polymorphic non-pyramidal cells (Amara, Insausti, & Cowan, 1987). The entorhinal cortex receives direct inputs from various cortical sites, including the cingulate, parietal, and insular cortices (Burwell, et al., 1995). Frontal areas send direct projections to the entorhinal cortex, but these make up only about 10% of the inputs (Burwell, personal communication). The major inputs to the entorhinal cortex, however, are from the perirhinal cortex and the parahippocampal cortex. It has been estimated that approximately 2/3 of the input to the entorhinal cortex originates in these sites (Burwell, Witter, & Amaral, 1995; Insausti, Amaral, & Cowan, 1987; Squire, 1992; Suzuki & Amaral, 1994a, 1994b).

Anatomy and Afferents of the Perirhinal and Parahippocampal Cortices.. The perirhinal cortex in both the monkey and rat has two distinct areas that are cytoarchitechtonically different. The more medially situated area 35 is somewhat smaller than the more laterally situated area 36 (Burwell, et al., 1995; Suzuki & Amaral, 1994a, 1994b). Area 35 is agranular and is characterized by a relatively thick layer I. In area 35, layer II is populated by small round cells, layer III is poorly populated, and layer V is distinguished by large, darkly staining pyramidal cells. Area 36 is characterized by a distinct layer II containing round cells in patches. Area 36 has a very sparse granular layer, but the granule cells are intermixed with the cells that make up layers III and V, making it difficult to separate the layers based on cytorarchitecture.

The parahippocampal cortex of the monkey is also made up of two distinct areas (Burwell, et al., 1995; Suzuki & Amaral, 1994a, 1994b). Area TH is agranular and bilaminar. In this area, layer V/VI is characterized by large darkly staining cells and layer II/III is thin. Area TF is more granular and has a very distinct layer V, which is made up of large darkly staining pyramidal cells that are intermixed with layer VI. Although the monkey has a distinct parahippocampal area, the rat does not. Burwell, et al. (1995) suggest that a cytoarchitechtonically similar area in the rat, that they call the postrhinal cortex, shares many features with the parahippocampal area. Detailed investigations of this area, however, have not been completed.

The perirhinal cortex receives inputs from both the visual areas of the temporal lobe and the cingulate cortex (Suzuki & Amaral, 1994b). Cells in the perirhinal cortex have a strong response to visual and memory related stimuli. The parahippocampal cortex, on the other hand, receives inputs from superior temporal, retrosplenial, and posterior parietal cortices (Suzuki & Amaral, 1994b). There is some evidence that the parahippocampal cortex receives visuospatial information from the posterior parietal cortex. Suzuki and Amaral (1994a) injected a retrograde tracer into the perirhinal cortex in monkeys and found that visual areas in temporal cortex (TE and TEO) provided the main projections to the perirhinal cortex. Other projections to the perirhinal cortex had origins in frontal, insular, precentral, agranular retrosplenial, and cingulate cortices and in the subiculum and presubiculum (Deacon, Eichenbaum, Rosenberg & Eckmann, 1983; Suzuki & Amaral, 1989). Burwell and colleagues (Burwell, et al., 1995) determined that the perirhinal cortex of the monkey also received projections from medial prefrontal cortex, auditory association

areas, piriform cortex, and somatosensory cortex. It seems likely that almost every area of cortex sends projections to the perirhinal cortex (Kolb, 1990). The inputs to the perirhinal cortex suggest that it may act to synthesize diverse sensory information and interact with other memory structures.

The finding of retrogradely labelled projections from the visual area TE and TEO are consistent with the data suggesting that the perirhinal cortex is involved in visual recognition memory (Zola-Morgan & Squire, 1989). The delayed nonmatching to sample task is thought to rely on this kind of memory. Monkeys with perirhinal cortex lesions perform poorly on the task and the deficit seems to be more severe than damage to any other component of the medial temporal lobe system (Squire & Zola, 1996).

The parahippocampal cortex may be more involved in spatial memory than is the perirhinal cortex (Squire & Zola, 1996). While no specific effects of selective parahippocampal lesions has been evaluated, damage to the parahippocampal region in humans results in a disorder known as topographical disorientation (Habib & Sirigu, 1987). As the name of the disorder implies, patients exhibit extreme difficulties with navigating through spatial environments. They have difficulties with surface features of places or objects, the generation of mental movement of objects, and visually guided movements.

Anatomy and Afferents of the Hippocampus. The entorhinal cortex is provided with the appropriate inputs to inform the hippocampus about much of the processing that is occurring in virtually all cortical areas. It also provides the major input, the perforant path, to the hippocampal dentate gyrus granule cells. These cells give rise to the mossy fibres, which make synaptic contact with the CA3 field of the hippocampus. The pyramidal neurons

of CA3 send projections (the Schaffer collaterals) to the pyramidal cells of CA1. A kind of 'loop circuit' is completed when CA1 neurons send projections to both the subiculum and back to the entorhinal cortex. This circuit appears to provide a unidirectional flow of information through the hippocampus and back to the entorhinal cortex (Squire, et al., 1989). The importance of this circuit in learning and memory is reflected in the deficits seen in patient R.B., whose lesion to CA1 essentially opened the circuit and disrupted processing that was critical for the formation of new memories.

There are also direct projections from the perirhinal cortex to the hippocampus. Liu and Bilkey (1996a, 1996b) found that stimulation of the perirhinal cortex triggered a monosynaptic evoked potential in the hippocampus. The authors also observed that the evoked potential recorded in CA1 followed a 100 Hz train, which is generally accepted as evidence for a monosynaptic connection (e.g. Berry & Penreath, 1976; Laroche, Jay & Thierry, 1990).

Efferents of the Hippocampus and Entorhinal Cortex. The fate of the information returning from hippocampus to entorhinal cortex is still not completely understood (Squire, et al., 1989). There are bidirectional projections between the entorhinal cortex and both the perirhinal and parahippocampal cortices, but the two systems are organized very differently. The rostral 2/3 of the entorhinal cortex projects to all regions of the perirhinal cortex. The degree of reciprocity between the perirhinal and entorhinal cortex, however, depends on the mediolateral position within the perirhinal cortex, as more medial portions demonstrate more reciprocity (Suzuki & Amaral, 1994b). The connections between the entorhinal cortex and the parahippocampal cortex, on the other hand, show a high degree of reciprocity (Suzuki &

Amaral, 1994b). It seems likely that the entorhinal cortex does send projections to the polyassociation areas that it receives projections from, but the mapping has yet to be examined in detail.

Efferents of the Perirhinal and Parahippocampal Cortices. The efferents of the perirhinal and parahippocampal cortices originate in layer III and layer V, but terminate in layers II and III of the entorhinal cortex. Suzuki and Amaral (1994b) argue that this represents a feedforward organization. The projections from the entorhinal cortex originate in layer V and project primarily to layer I, which, according to the authors, reflects a feedback organization.

There are extensive reciprocal projections between the perirhinal cortex and the frontal cortex in the rat (McIntyre, Kelly & Staines, 1996). Labelling was seen in all frontal areas (Fr1, Fr2 and Fr3), much of which has been reclassified as primary motor cortex, or M1 (Paxinos & Watson, 1997) following a PhAL injection to the perirhinal cortex. There was heavy labelling of cells in layers I, II, and VI in Fr2 and Fr1, while layers I and II in anterior Fr3 show a sparse labelling following the PhAL injection. Heavy labelling was also found in parietal cortex (Par1 and Par2), which is also classified as primary somatosensory, or S1 (Paxinos & Watson, 1997). An injection of the retrograde tracer Fluorogold (FG) into the Fr1 and Fr2 area results in a dense labelling of cells in layer V of perirhinal cortex.

These results confirm a reciprocal relationship between the frontal cortex and perirhinal cortex previously described by Deacon, et al. (1983). Interestingly, Deacon, et al. (1983) also reported that it was layer II neocortical cells that projected to the perirhinal cortex. McIntyre, et al. (1996) note that most other cortical areas are innervated by layer V

and VI neurons, indicating that the projection from the frontal cortex to the perirhinal cortex is somewhat unique.

Following PhAL injections into the perirhinal cortex, labelling was also found in the agranular insular, infralimbic, orbital, parietal, and entorhinal cortices (McIntyre, et al., 1996). Although some subcortical sites did show labelling, it was sparse or absent in the hippocampus or subiculum. Only when the PhAL injections were restricted to perirhinal layers III/V was there labelling in the hippocampus and subiculum.

Reciprocal Connections Between Frontal And Perirhinal Cortices. The FG injection into the frontal cortex in rats resulted in the filling of layer V cells along the longitudinal axis of the entire perirhinal cortex (McIntyre, et al., 1996). Discrete PhAL injections into the perirhinal cortex resulted in a divergent labelling throughout the neocortex. Similarly, discrete Fluorogold injections to the frontal cortex resulted in a labelling throughout the perirhinal cortex. The convergence-divergence in these connections suggests that the perirhinal cortex could exert a broad influence over all of the frontal motor cortex (McIntyre, et al., 1996). This kind of circuitry may be useful in a system using interleaved learning to teach the cortex about patterns of activation within the hippocampus. Even small localized activation at the level of the perirhinal cortex could result in an activation along the entire extent of the frontal cortex. This circuitry would also provide the appropriate connections for creating a distributed representation at the level of the neocortex.

Anatomy of Frontal Cortex. Motor areas of the rat cortex were first identified as regions that could elicit movement when a low intensity electrical stimulation was applied, but it has also been suggested that motor areas be defined based on a lack of a well defined

granular layer IV. Frontal cortex, and specifically area M1, lacks a prominent inner granular layer IV, which allows it to be delineated from the somatosensory and parietal cortices (Donoghue & Wise, 1982). There are some very small perikarya that could be considered to be comparable with the neurons in the adjacent layer IV of parietal cortex that are found above layer V, but the term agranular cortex is used to describe the entire frontal cortex (Zilles, 1990). A predominant inner pyramidal layer of large and densely packed pyramidal cells can also be used to separate the frontal areas from the adjacent parietal areas (Zilles, 1990). At least three divisions (Fr1, Fr2, Fr3) of the frontal area have been discerned based on cytoarchitecture. The medial area, or Fr1, has a pale-staining layer III and a small layer II. Fr3, or the more lateral agranular field, has a slightly higher cell packing density than Fr1 in the lower range of layer III (Zilles & Wree, 1995). Fr3 also has a thick layer V, which contains large, densely staining cells. At the most lateral portions of M1 there seems to be some overlap with part of the adjacent sensory cortex (SI) representation (Donoghue & Wise, 1982). Motor areas represent specific body areas in roughly a somatotopic manner and electrical stimulation of some sensory areas can also result in motor behaviours (Zilles, 1990). This overlap is not all that surprising given the close proximity of frontal and parietal areas; parietal areas are surrounded medially and rostrally by primary motor cortex.

1.5 TRANSFER OF INFORMATION BETWEEN THE HIPPOCAMPUS AND THE NEOCORTEX.

The structured relationships of inputs and outputs of the hippocampal-cortical networks are believed to subserve the transfer of information into a long term storage storage

site in the neocortex.

Compression and Decompression. It is unlikely that the hippocampal system receives a direct copy of the neocortical activation patterns. There are many more neurons in the neocortex (McClelland, et al., 1995). The hippocampal representation has presumably been compressed and distributed over a smaller number of neurons. The compressed version can be utilized with little loss of information if there is some redundancy in the inital representation at the cortical level and/or the hippocampus serves to tie together components of memory that are already stored in the neocortex. McClelland, et al. (1995) suggest that compression is carried out prior to, or within, the entorhinal cortex. It is this compressed representation that is subsequently stored in the hippocampus. When it is necessary that the pattern be reinstated at the level of the neocortex, the efferents of the hippocampus participate in a decompression process. The connections from the entorhinal cortex, and the connections within the neocortex itself, would also participate in this decompression.

Intermediary Structures and Learning Rates. The entorhinal, perirhinal and parahippocampal areas are not simply relay sites; they contribute to the processing of information, at least some of which is conveyed to the hippocampus. It is also likely that these sites store information, as well, and it has been proposed that their learning rates would show parallels with their location along the chain of information flow (Eichenbaum, Otto, & Cohen, 1994; McClelland, et al., 1995). For example, whereas the hippocampus and neocortex represent fast and slow learning systems, respectively, the regions between the hippocampus and the neocortex might show intermediate learning rates. Alternatively, since these structures are heavily involved in declarative memory, they may show rates that are

similar to those found in the hippocampus.

It is also possible that learning rates vary over the lifespan of the organism. There may be relatively fast neocortical learning rates at the beginning of life in order to extract structure from experience relatively quickly (McClelland, et al., 1995). As experience accumulates the rates would be reduced to accommodate the increased load. Such changes could account for the critical periods that exist in young animals. Learning rates in animals with short lifespans may also remain faster than those with long lifespans (McClelland, et al., 1995; Squire, 1992).

1.6 PUTATIVE MECHANISM FOR INFORMATION STORAGE

Long-term Potentiation and the Hebb Rule. It is widely accepted that information is stored as a change in strength of synaptic connections. Hebb proposed a rule for such changes in 1949. He proposed that "When an axon of cell A is near enough to excite cell B, and repeatedly or persistantly takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (p.62). There was, however, no firm experimental evidence for such an activity dependent modification for over 20 years. The first demonstrations of a phenomenon that would subsequently be shown to have Hebb-like properties were provided by Bliss and colleagues (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973). They were able to induce long-lasting increases in the strength of synaptic responses in the hippocampal formation of both anaesthetized and freely moving rabbits. Evoked responses in the dentate gyrus were found to be markedly enhanced following an application of brief, high frequency stimulation

to the perforant path. This strengthening, or enhancement of the evoked response, by electrical stimulation has become widely known as long-term potentiation, or LTP.

There are a number of features of LTP that make it attractive as a memory mechanism. First, it is long-lasting. LTP has been shown to last up to 5-6 weeks in the non-neocortical areas tested (Racine, Milgram, & Hafner, 1983), and longer in neocortical areas (Racine, Chapman, Trepel, Teskey, & Milgram, 1995). LTP can also be induced rapidly in non-neocortical areas, reaching asymptotic levels within a single session of multiple stimulations. Perhaps the most important features of LTP are that it is specific to the activated pathway and it shows associative properties. Associativity refers to the interaction between co-active pathways. Synapses that are normally unable to elicit LTP on their own, are capable of showing LTP when their activation is paired with the activation of stronger inputs.

LTP Induction. The associative properties of LTP appear to be linked with the activation requirements of the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptor contributes minimally to basal levels of neurotransmission, but plays a major role when the postsynaptic depolarization exceeds a critical threshold level (Bliss & Collingridge, 1993). The ion channel associated with the NMDA receptor is normally blocked by a magnesium ion, even in the open state. The ion is too large to actually pass through the channel, but effectively blocks other ions from entering the channel.

The initial depolarization evoked at a glutamatergic synapse is a result of AMPA receptor activation. The AMPA receptor contains a sodium channel that opens with activation, producing a fast depolarization. If the postsynaptic depolarization is sufficiently

strong, the magnesium ion is dislodged from ion channels associated with the NMDA receptor. Other cations, particularly calcium, are now free to pass through the channel (Cotman and Iversen, 1987). The resulting influx of calcium is responsible for the activation of many of the enzymes involved in plasticity, including the plasticity associated with LTP induction. Similar calcium influxes may play a role in memory formation.

The maintenance of LTP has been less well described than the induction of LTP. The possible mechanisms include everything from an increase in neurotransmitter release from existing synapses to an increase in the number of synapses (synaptogenesis).

Relations Between Learning and LTP. In attempts to establish relationships between LTP phenomena and learning, researchers have examined the effects of LTP on learning, the effects of learning on LTP, and the general effects of learning on synaptic strength. Bruce McNaughton and Carol Barnes have been instrumental in exploring the tradeoffs between learning and the LTP phenomenon. Castro, Silbert, McNaughton, and Barnes (1989) tried to saturate LTP in the hippocampal synapses by providing a massive amount of electrical stimulation. Their assumption was that saturation of LTP would make subsequent learning impossible. This assumption suggested that plasticity would be *used up* until LTP had decayed. This logic is clearly flawed if the synaptic plasticity mechanism is bidirectional. Indeed, subsequent investigations by Barnes (Barnes, Jung, McNaughton, Korol, Andreason, Worley, 1994; Castro, Silbert, McNaughton, & Barnes, 1993; Korol, Abel, Church, Barnes, & McNaughton, 1993), Morris (Jefferey & Morris, 1993) and others (Cain, Hargreaves, Boon, & Dennison, 1993; McNamara, Kirkby, dePape, Skelton, & Corcoran, 1993; Sutherland, Dringenberg, & Hoesing, 1993) indicated that the initial findings could not be

replicated. While Korol, et al. (1994) did find that LTP and performance in spatial learning tasks could be blocked by maximal electroconvulsive shock (MECS), which probably results in the saturation of LTP in more synapses, there is still no guarantee that saturation has been produced in *all* the synapses by this procedure. The MECS protocol is also quite extreme and it is likely to have effects extending beyond the hippocampus, which is the structure of interest. Reduced performance in spatial tasks and LTP may be secondary to lasting effects in other areas as a result of MECS.

Barnes and McNaughton (1985) tested old rats and found that they show a much faster rate of forgetting of spatial information than do younger animals. Evidence indicated that, while induction of LTP is normal, old animals show a more rapid decay of LTP than do younger animals providing some parallels between memory decay and LTP.

Morris has conducted studies with the drug AP5, which blocks NMDA receptor activation, to determine correlates between learning and memory (Morris, Anderson, Lynch, & Baudry, 1986; Morris, 1989). Doses of AP5 that were effective in inhibiting LTP also impaired learning of the spatial morris water maze task. Cain and colleagues (Cain, Saucier, & Boon, 1997; Saucier & Cain, 1995) found that an NMDA receptor antagonist caused disrupted acquisition of the water maze and blocked dentate gyrus LTP. It also appeared to cause sensorimotor disturbances. Furthermore, performance in the water maze was improved if the animals were familiarized with the task before being tested. The findings indicate that NMDA antagonists cause visual and sensorimotor impairments that result in poor performance in naive animals that may have been interpreted as learning deficits in other studies. While such studies suggest that Morris' original work is confounded by training

effects, a number of other experiments have been published showing a disruption of performance on learning tasks with NMDA antagonists. The conflicting results indicate that the links between LTP and memory are not yet firmly established.

LTP in The Neocortex. Although most of the LTP research has focussed on the intrinsic pathways of the hippocampus, there has been an increase in the number of experiments aimed at investigating LTP in other structures in the mammalian brain, especially the neocortex. High frequency stimulation delivered at relatively high intensities reliably produces LTP in many limbic areas in the brain of the behaving rat (see Racine, et al., 1983). Until recently, however, demonstrations of LTP in the cortex of the chronic preparation have been lacking, with neocortical LTP reported only in slice or acute preparations. Racine, et al. (1995) found that LTP could be reliably induced in the cortico-cortical field potential in chronically implanted rats with a multi-session stimulation protocol. In this procedure animals receive a series of brief, high frequency trains each day, for a number of days.

Neocortical LTP shows some properties that might be expected of a neocortical memory mechanism. Potentiation is not evident for a few days and does not reach asymptotic levels until 10 or more sessions of stimulation. Both monosynaptic and polysynaptic components of the evoked response are potentiated and the LTP is much longer lasting than the LTP seen in the hippocampus. Some components of the response remain potentiated for almost 1 year (Froc, personal communication).

Theory suggests that the hippocampus learns rapidly, but has a transient storage and limited capacity. The neocortex, on the other hand, is thought to learn more slowly, but the

capacity is large and the memories long-lasting. The neocortical potentiation effect of Racine, et al. (1995) may be the first direct evidence that an LTP-like mechanism may be involved in the *long-term* storage of information in the neocortex. Additional research from Racine and co-workers indicates that almost any area of neocortex may be potentiated, so long as the stimulations are spaced and repeated.

Chapter 2

Thalamocortical and Corticothalamic Connectivity

2.0 THALAMOCORTICAL RECIPROCITY AND PLASTICITY

A dominant view in the neurosciences is that the adult sensory systems are stable, or non-plastic, especially when contrasted with the prevalent plasticity characterizing development (Weinberger, 1995). The assumption is that sensory systems, once developed, should remain stable in order to provide a consistent output and that plasticity in these systems might actually disrupt the ability to function within a stable environment. A growing literature, however, indicates that there may be a large degree of both short- and long-term modification in the adult sensory areas following learning, sensory stimulation, electrical stimulation, and deafferentation (see Fregnac, Shuz, Thorp & Bienenstock, 1988; Nudo, Milliken, Jenkins & Merzenich, 1996; Weinberger, 1995). Some of this plasticity has been demonstrated in the thalamocortical systems (i.e. Iriki, Pavlides, Keller, & Asanuma, 1991).

General Thalamocortical Organization. The thalamus is part of the diencephalon and forebrain. Thalamic nuclei receive their major input from the body's sensory systems, but also receive substantial input from other areas of the brain (Jones, 1985; Kolb & Whishaw, 1990; Salt & Eaton, 1996). The most thoroughly studied areas of the thalamus are the lateral geniculate nucleus (LGN), which receives visual projections, the medial geniculate nucleus

(MGN), which receives auditory projections, and the ventral-posterior lateral nuclei (VPL), which receives touch pressure, pain, and temperature projections from the body (Kolb & Whishaw, 1990). In turn, the LGN projects to visual cortex, the MGN projects to auditory cortex, and the VPL projects to primary somatosensory cortex. Whereas the LGN, MGN, and VPL receive relatively direct inputs from sensory systems, the mediodorsal nucleus (MD), receives projections from other parts of the brain including the amygdala, temporal neocortex, and the caudate. The MD sends projections to the frontal lobe, piriform lobule and the hippocampal formation (Jones, 1985). The general organization of all sensory thalamic systems is similar. A peripheral sensory neuron (ie. mechanoreceptor, photoreceptor, etc.) transduces stimulus energy into a neural signal that is subsequently carried via a polysynaptic pathway to the thalamus. From the thalamus, sensory information is transmitted to the cortex. The only sensory system that does not utilize a thalamic nucleus directly is the olfactory system.

The organization of the cortical areas to which the thalamic areas project is also quite similar. The sensory cortex is multilaminate and can be thought of as having both input and output areas (Kolb & Whishaw, 1990). The two main cell types that have been distinguished based on their shape are pyramidal cells and stellate cells. Pyramidal cells represent the major efferents of the cortex. They are primarily found in cortical layers II, III and V. Stellate cells provide the interconnections between cortical efferents and afferents. Stellate cells are found in all cortical layers, but the heaviest concentration is in layer IV. Layer IV is often called the granular layer due to the large concentration of small, stellate granular cells. The middle layers of primary sensory cortex, especially layer IV, can be thought of as

a zone of sensory analysis. Layer IV receives most of the projections from the thalamus. The cells in layers V and VI, on the other hand, can be thought of as an output zone. These cells project largely to other cortical areas and back to the thalamus. In secondary sensory cortices, however, thalamic fibres avoid layer IV and tend to terminate almost completely in the deeper portions of layer III (Burton & Jones, 1976). The layer III projections show a lower density than those targeting layer IV of the primary cortical areas (Jones & Burton, 1976). It is also the case that the secondary sensory cortices have a reduced number of granule cells in layer IV. As mentioned, the corticothalamic axons arise from pyramidal cells in layers V and VI of the cortex. Those that arise in layer VI project back to the principle thalamic relay nucleus (Jones, 1985).

Corticothalamic and Thalamocortical Loops. The principle of corticothalamic reciprocity is well established anatomically (see Winer & Larue, 1987). While much is known about the information that the thalamus transmits to the cortex, little is known about the nature of the information transmitted from the cortex back to the thalamus (Jones, 1985). Pathways that are this dense and highly organized presumably have a function. Jones (1985) and Mumford (1991) both argue that the anatomical precision of these pathways reflects their functional importance. Every dorsal thalamic nucleus receives fibres back from all the cortical areas to which it projects and there is precise mapping within the thalamic target.

The fact that thalamocortical systems have developed in the form of loops rather than chains may indicate a high degree of on-line filtering of input. Templates of common or generalized patterns may be stored as connection patterns within the thalamus. The thalamocortical system would keep a record of information that passes through the system

and determines whether the current information matches a *prototype*, thus, maintaining a sensitivity to new information. In order to maintain an adaptive response system it is expected that there is a filtering out of old information and an optimization of neural activation conveying new information to update the *prototypes*. This optimization likely involves changes in cortico-cortical, thalamocortical, and corticothalamic connections.

Mumford (1991) has described the thalamus as a storage site for something like a prototype. In Mumford's description, for example, the thalamus is an *active blackboard* that is continually erased and updated. Mumford argues that the neocortex is required for higher order processing of sensory information, but that it can only complete this higher order processing vis a vis connections with the thalamus.

2.1 THE AUDITORY SYSTEM

Anatomy of the Auditory Thalamus. Winer and Larue (1987) examined the medial geniculate in the adult rat following horseradish peroxidase and tritiated leucine injections into the primary auditory cortex. Based on the pattern of organization, cytoarchitecture, fibre architecture and reciprocal connectivity, they suggested that the medial geniculate complex of the rat could be divided into three substructures: medial, ventral, and dorsal. The medial portion has also been called the magnocellular division of the medial geniculate because of its large cells. Jones (1985) suggests that those animals with more primitive brains may not contain the large cells, and that the term medial is more appropriate than the term magnocellular, overall. The medial and dorsal/ventral subdivisions of the medial geniculate show a different connectivity and physiological properties (Weinberger & Diamond, 1987).

The medial portion (MGm) demonstrates a number of unique properties including a sensitivity to a broad range of frequencies, extensive dendritic arborization, and a sensitivity to somatosensory stimuli (Weinberger & Diamond, 1987). A tonotopic organization has recently been found in the medial portion of the MGN in cats (Rouiller, Rodrigues-Dagaeff, Simm, DeRibaupierre, Villa and DeRibaupierre, 1989). Although this region receives ascending auditory, medial lemniscal, spinothalamic, and vestibular fibres, single cell recordings show that most cells respond primarily to auditory signals (Jones, 1985). There are increased responses to tactile and vibratory stimuli in the anterior part of the MGm. The MGm projects to layer I and IV throughout the auditory cortex and cortical projections back to the MGm originate in layer V in all fields of auditory cortex (Jones, 1985; Weinberger & Diamond, 1987). The MGm also projects to somatosensory cortex (Weinberger & Diamond, 1987).

The ventral nucleus (MGv) usually consists of medium sized, tightly packed, darkly staining cells that form relatively vertical rows (Jones, 1985). The dorsal nucleus (MGd), which sits just above the ventral portion of the MGN, contains an array of small and medium sized cells that are pale staining and less densely packed that those in the ventral portion. The thalamocortical axons from the ventral and dorsal nuclei show a typical termination pattern within layers III and IV of the cortex (Jones, 1985). The projections from the cortex to the ventral portion are more dense than those to the MGd and MGm, indicating that each subdivision could receive a different pattern of activation from the cortex (Winer & Larue, 1987).

Anatomy of the Auditory Cortex. The primary auditory area is designated as Te1 by Zilles (1990), area 41 by Krieg (1946), and Au1 by Paxinos and Watson (1997). Paxinos and Watson (1997) also distinguish the more dorsal and ventral portions of the primary auditory cortex. The area is characterized by a granular layer (layer IV) and a high density of myelinated fibres terminating within the middle cortical layers (Kelly, 1990). Surrounding this area is a belt of more agranular and less myelinated areas that include Te2 and Te3, as described by Zilles (1990), areas 20, 36, and 39, as described by Krieg (1946), and TeA (temporal association area) by Paxinos and Watson (1997). The auditory cortex can also be divided into the core and belt area on the basis of the pattern of thalamocortical projections (Kelly, 1990). MGm projects primarily to layers I and IV of the core and belt areas. Fibres from the MGv and MGd project to layer IV in the core area and the lower part of layer III in the belt area, respectively.

Sally and Kelly (1988) examined the responses of neurons in the auditory cortex of the rat using a microelectrode mapping technique. Although there had been several reports of deficits following the ablation of auditory cortex, a detailed study of unit responses in the rat auditory cortex was lacking. The results indicated that the auditory cortex of the rat had low levels of spontaneous activity and responded to a stimulus onset with a transient burst of action potentials. The strongest responses were recorded at a depth of approximately 500 microns, within layer III or IV. The general organization of the tonotopic maps in the rat is consistent with those found in other mammals. An area was found that was characterized by short-latency responses. It showed a strictly tonotopic organization, with the higher frequencies represented rostrally and the lower frequencies represented caudally. The

frequency sensitivity ranged between about 0.42 kHz and 47 kHz, with most of the cortical area devoted to frequencies between 8 kHz and 40 kHz. These are also the frequencies to which the rat is most sensitive. An area dorsal to the primary area appears to be characterized by responses with longer latencies and less precise frequency tuning.

Plasticity in the Adult Auditory Thalamus. There is increasing evidence that the thalamus is plastic. Gerren and Weinberger (1983) found that high frequency stimulation of the brachium of the inferior colliculus produced a long-term potentiation (LTP) of responses evoked in the MGm. This potentiation was also seen in unit responses (Weinberger & Diamond, 1987; Rouiller, Rodrigues-Dagaeff, Sim, DeRibaupierre, Villa & DeRibaupierre, 1989). Although the medial portion of the auditory thalamus appears to support potentiation, there is little evidence for a potentiation effect in auditory cortex. There is, however, evidence for response modifications based on conditioning procedures.

Plasticity in the Adult Auditory Cortex. Weinberger and Diamond, (1987) paired an auditory stimulus with foot-shock. The paradigm led to changes in the firing rates in neurons in primary and secondary auditory cortex. These changes occurred within 5 to 17 trials, so they were likely restricted to modification in existing pathways; the change was too rapid to be accounted for by synaptogenesis. Receptive fields were determined before and after training, and the enhanced responses were found only in response to the conditional stimuli (Lennartz & Weinberger, 1992; Weinberger, 1995). Responses to other frequencies were decreased or showed little change. There was also a retuning of responses in some cells away from the *best frequency* (the frequency that the neurons responded to best prior to the onset of training) towards the frequency of the conditional stimulus.

Within subject recordings obtained before and at intervals up to eight weeks following conditioning suggest that the receptive field plasticity develops quite rapidly and is long lasting (Edeline, Pham & Weinberger, 1993). The animals in this study were trained while awake, but the receptive fields were examined under anaesthesia. Given that the conditioning effect is robust enough to be expressed under anaesthesia, it is not likely an artifact of an elevated arousal during the receptive field determinations (Weinberger, 1995).

Although cells in the MGv were affected little by conditioning procedures (Edeline & Weinberger, 1991; Weinberger & Diamond, 1987), the cells in the MGm developed an increased responsiveness to conditioning stimuli and their receptive fields were retuned towards the conditioning stimulus frequency (Edeline & Weinberger, 1992). Whereas it seems unlikely that the cortical receptive field changes are simply driven by the MGm changes, or vice versa, the retuning results probably depend upon interactions *between* the two sites.

2.3 THE VISUAL SYSTEM

Anatomy of the Visual Thalamus. The main relay station for visual information in the mammalian brain is the lateral geniculate nucleus (LGN). According to Jones (1985), the structure of the lateral geniculate is quite stable across mammals. Even moles, which have congenitally absent or incredibly small eyes, show a familiar LGN organization. The LGN is composed of two or more components. The dorso-lateral, but not the ventral, portion of the LGN projects to the cerebral cortex.

In a number of species the structure is laminated (Coleman & Clerici, 1980; Warton,

Dyson & Harvey, 1988, Dean, 1990). In the human and primate brain the LGN is characterized by six layers of cell bodies, separated by layers of axons and dendrites (Kandel, Schwartz & Jessell, 1995). The layers are numbered in the ventral to dorsal direction from 1 to 6. The two most ventral layers are called the magnocellular layers due to their characteristic large cells and their main inputs from the M cells of the retina. Layers 1 to 4 are the parvocellular layers, which receive input from the P cells in the retina. Each of the layers contains a representation from one eye only. Fibres from the contralateral nasal hemiretina make synaptic contact in layers 1, 4, and 6 and fibres from the ipsilateral temporal hemiretina make synaptic contact in layers 2, 3, and 5.

In a Nissl prepared section, the LGN contains a relatively homogenous cell population. An alteration of the left and right eyes, however, can be revealed in the nucleus by a selective staining of retinal axon terminals (Jones, 1985). In the rodent there is also a separation of ipsilateral and contralateral terminations. Generally, only a small number (10-20%) of the inputs to the lateral geniculate are actually from the retina (Kandel, et al., 1995). The other inputs come from other areas of the brain, including feedback inputs from the visual cortex.

Most of the afferents of the lateral geniculate terminate in layer IV of the visual cortex with some minor inputs to layer I and VI. Layer IV of visual cortex can be further subdivided into four sublamimae based on its cytoarchitecture. The parvocellular and magnocellular axons of the LGN terminate in different sublaminae of layer IV (Dean, 1990).

Anatomy of The Visual Cortex. Primary visual cortex has been given many different labels. It is appropriate to call it striate cortex, Oc1 (Zilles, 1990), or area 17 (Krieg, 1946).

To describe the segregation of visual cortex, Oc1 is the easier of the terms to use. It has been shown that Oc1 can be further divided into a monocular and binocular part, Oc1M and Oc1B, respectively (Zilles, 1990). Whereas Oc1M receives input from the contralateral eye, Oc1B receives input from both eyes. These subareas can be examined following tritiated amino acid injections into a single eye. They are also visible following Nissl and myelin staining, as there is a higher cell density and lower myelin density in Oc1M, compared with Oc1B. Recently, Paxinos and Watson (1997) changed their atlas terminology to V1 and subdivided areas have been relabelled V1B and V1M.

On the lateral border of Oc1 is Oc2L, or lateral peristriate cortex. On the medial side, Oc1 is bordered by Oc2M. While the cell density of layer IV in Oc2L and Oc2M is much smaller than that of Oc1, the reverse is true for layer V (Zilles, 1990). The secondary visual areas, Oc2M and Oc2L, both receive inputs from primary visual cortex. Zilles (1990) states that the primary visual area Oc1 is the most thoroughly investigated neocortical area with respect to cell types and laminar distributions and that this area could be used as a model for most of the cortex. The majority of neurons in the rat's visual cortex are pyramidal cells (as many as 92% of all cells between layer II and VI). Spiny stellate cells make up much of the rest of the population of cells in layer II through VI. Layer I is characterized by spine-rich multiangular, or bipolar, cells. All other nonpyramidal cells (double bouquet, chandelier, and Martinotti cells) account for only about 6 percent of the cortical neurons.

Burne, Parnavelas & Lin (1984) found that 90 percent of the cells recorded in area 17 of the rat were responsive to visual stimuli. Of these, 95 percent responded to moving images, whereas only 5 percent responded to stationary images. Cells were further divided

on the basis of their optimum stimuli: forty-four percent were classified as simple cells; complex cells, which respond best to a bar of light oriented in a specific direction, accounted for 27 percent of the cells recorded; hypercomplex cells fire only to moving lines of a specific length or to moving angles, and accounted for 13 percent of the sampled cells; and the remaining 16 percent of the cells recorded from were classified as non-oriented cells.

Plasticity in The Adult Visual Cortex. Fregnac, et al. (1988) used iontophoresis to manipulate thresholds in area 17 neurons. Thresholds were first adjusted to increase the response to one visual stimulus and then to decrease the response to a second stimulus in a conditioning-like paradigm. This procedure allowed thresholds to be increased to normally optimal stimuli, and decreased to non-optimal stimuli. A long-term modification was induced in both occular dominance and orientation selectivity in 33 and 43 percent of the cells recorded, respectively. The results are consistent with the theory that the efficiency of transmission at preexisting synapses in the adult visual cortex is dependent on the covariance between pre- and post-synaptic activities (Fregnac, et al., 1988).

2.3 THE SOMATOSENSORY SYSTEM

As some of the terminology used to describe somatosensory thalamic areas and input is based on somatosensory cortex organization, the cortical anatomy is presented first.

Anatomy of The Somatosensory Cortex. In a series of pioneer studies, Woolsey used electrophysiological mapping techniques to map out the topographic organization of the somatosensory cortex (Chapin & Lin, 1990). The somatotopic mapping was found to be relatively similar in many mammals. In the rat, the ratunculus faces in the forward direction,

with its paws and nose being represented more rostrally. The cortex of all rodents has an extremely large representation of the facial whiskers, or mystacial vibrissae. In this cortical region, each vibrissa occupies a distinct area. The somatosensory cortex is divided into three areas using Zilles' nomenclature, Par1, HL and FL. The first area, Par1 contains much of the head representation and a portion of the forelimb representation (Chapin & Lin, 1990). The representations for the hindpaw, hindlimb, and back are included in the HL area, or hindlimb area. The third area, FL, or forelimb area, contains most of the forelimb and forepaw representations. Whereas the Par1 area is primarily considered to be a granular zone, the HL and FL areas are primarily dysgranular. Much of the study of the somatosensory cortex in rodents has focussed on the granular regions of the cortex, specifically the area occupied by the vibrissae.

In the caudomedial portion of the face area each vibrissa on the mystacial pad is represented within layer IV by a large 'barrel' (Chapin & Lin, 1990; Chmielowska, Carvell & Simons, 1989; Woolsey & Van der Loos, 1970). The barrel is a morphological parallel to a single functional column, extending through the entire cortex (Chmielowska, et al., 1989; Woolsey & Van Der Loos, 1970). A dense cylinder of cell bodies makes up the sides of the barrel. Within the cylinder is a less cell dense area that is called the hollow. Each barrel is separated from the others by a septum this is virtually acellular. There is an apparent tapering at the top and bottom of the barrel. Areas of increased cell densities create apparent tops to the barrels. There is, however, no corresponding cell dense area on the bottom of the barrels.

Microelectrode studies of the cortical barrels of the SI have been used in an attempt

to explain some of the mechanisms of information processing in the cortex. Welker (1971, 1976) provided evidence for a direct one-barrel-to-one-vibrissa relationship. The electrophysiological data provided a background for other related studies on the importance of the barrel fields and vibrissa in the behaviours of the animals, especially tactile discriminations. Neurons within the columns respond to the activation, or movement, of a single whisker, and at different depths there are differential responses to different movements or deflections. These response properties are characterized by single whisker receptive fields that have very strong inhibitory surrounds (Chmielowska, et al., 1989).

The formation of barrels appears to be dependent on thalamocortical projections (Crandal, Mission & Butler, 1990). A major source of the afferents to the barrel fields of SI is the thalamic ventrobasal complex (Chmielowska, et al., 1989; Simons & Carvell, 1989). Following horseradish peroxidase injections into the ventrobasal complex dense anterograde labelling was found within the barrel centres. The acellular regions between barrels were found to receive more diverse inputs, including inputs from the posterior medial thalamus (Chmielowska, et al., 1989). The termination patterns suggest that there is communication both within the barrels and between the barrels and other brain regions.

Anatomy of the Thalamocortical Inputs. The ventrobasal complex, the anatomical correlate to the ventral posterior thalamus in primates (Chapin & Lin, 1990), also possesses a number of cells that predominantly respond to individual vibrissa. These are called 'barreloids' after the barrels (Simons & Carvell, 1989). Both anatomical and electrophysiological studies have established a relationship between individual barreloids, cortical barrels, and mystacial vibrissae. Projections from the ventrobasal area terminate

in the granular zones of the barrel fields. The terminations are primarily in layer IV in the centres of the barrels, but there are also terminations in layers I, II, and III within the same 'cortical column' (Chapin & Lin, 1990).

Lin and Chapin (1981) used retrograde tracers to confirm that the major source of inputs to the dysgranular and perigranular zones in SI originate in the posterior thalamus (c.f. Chapin & Lin, 1990). The two other primary thalamic areas that project to the SI are the ventromedial and ventrolateral nuclei. Whereas the ventromedial nuclei project to layer I throughout the somatosensory cortex, the ventrolateral nuclei send dense terminations to layers II and V throughout primary motor cortex and to an area of overlap between motor and somatosensory cortex (Chapin & Lin, 1990).

Plasticity in Somatosensory Cortex. Yun (1991) glued together the tips of a pair of adjacent whiskers for 4-8 days and found that there was a 5 to 6 fold increase in the cortical representation for the fused whiskers in the barrel fields (c.f. Weinberger, 1995). In a 2DG study, Welker, Rao, Dorfl, Melzer, and Van Der Loos (1992) found that continually stimulating the whiskers of mice for 1-4 days produced a decrease in activity in the contralateral cortex. While the habituation-like results of Welker, et al. (1992) are in opposition to those of Yun (1991), both studies demonstrate that the adult somatosensory cortex is plastic. Neither, however, rule out mediation of a plastic change by the thalamus.

Lee and Ebner (1992) simultaneously recorded unit activity from the ventral posterior lateral nucleus of the thalamus and layer IV cells of the somatosensory cortex. Following the direct stimulation of the thalamic neurons, there was an almost immediate increase in the responsiveness of layer IV cortical neurons to vibrissa stimulation. This increase was not

parallelled by an increase in the thalamic site. These results suggest that a potentiation was induced at the thalamocortical synapse. The authors propose that such shifts in responsiveness may occur during normal processing (Lee and Ebner, 1992).

2.4 THE MEDIODORSAL SYSTEM

One of the more reliable effects seen following mediodorsal (MD) thalamic lesions in rodents is perseveration, which leads to a disruption of performance on reversal tasks (Kolb, 1977). In patients with Korsakoffs disease, a disease associated with chronic alcoholism, there is severe damage to the mediodorsal nucleus and other thalamic nuclei, as well as to frontal areas (Kolb & Whishaw, 1990; Oscar-Berman & Zola-Morgan, 1980). These patients show rather severe anterograde *and* retrograde memory deficits. Similar behavioural and memory deficits have been seen following lesions of the frontal cortex. The role of the thalamus and neocortex cannot, unfortunately, be dissociated to look at thalamic damage alone. Relatively soon after the thalamus is damaged, atrophy in the neocortex is evident.

Anatomy of The Mediodorsal Nucleus. The mediodorsal nucleus in mammals is generally quite large, occupying up to two-thirds of the extent of the thalamus. It has a homogeneous cytoarchitecture, which consists primarily of small to medium sized cells (Jones, 1985). These cells are relatively pale staining and are rather well spaced within the nucleus, but evenly distributed. Ray and Price (1992) suggest that the MD of the rat can, however, be divided into three distinct areas based on cytoarchitecture, fibre staining and connectivity; the medial, lateral and central. Most of the afferents of the MD come from the

piriform lobule and adjacent areas of cortex. Many of these are GABAergic and appear to be inhibitory (Paxinos, 1995). The medial and central portions of the MD send efferents to the frontal cortex. These projections terminate mainly in layer III.

Anatomy of the Frontal Cortex. The cytoarchitecture of the frontal cortex was discussed in detail in the previous chapter (section 1.4). Matelli and Luppino (1996) found that in the monkey virtually all frontal areas receive projections from the mediodorsal nucleus. In the rat, corticothalamic fibres to MD appear to be glutamatergic and form terminals on distal segments of the thalamic neurons (Ray & Price, 1992).

Plasticity of the Frontal Cortex. Multiple sessions of brief, high frequency stimulation will produce a lasting potentiation in the frontal cortex (Racine, et al, 1995), as discussed in Section 1.5.

2.5 LTP IN THALAMOCORTICAL AND CORTICOTHALAMIC PATHWAYS

Critical Periods in the Brain. One of the most striking characteristics of the mature brain is the precision of the neuronal wiring and the conservation of patterns of connectivity across the sensory systems. The brains of many mammals have all the neurons that will be needed at birth, but the precise electrical circuitry actually develops a short time later. In the fine tuning of the neural connectivity that occurs during the 'critical period', neural activity plays an especially important role.

In the brain, neural development has been most thoroughly investigated in the visual system. During the segregation of retinothalamic inputs into their distinct layers, and the topographic mapping within the layers, there must be a temporal and spatial correlation in

the firing of the retinal ganglion cells within, but not between eyes. Wong, Meister and Shatz (1993) found a spontaneous correlated bursting in the ferret retinal ganglion cells during the critical period (P0 to P30) when retinogeniculate connections are known to segregate into layers in the lateral geniculate. This correlated bursting occurs before the eyes are open. Although the formation of ocular dominance columns in the visual cortex occurs subsequent to the formation of layers in the lateral geniculate, there is still a need for a synchronous pattern of firing of the retinal ganglion cells (reviewed in Shatz, 1992). At both the lateral geniculate and the cortex, the targets of cells that are spatially correlated at the retina remain strongly related and terminate in close proximity, as well.

Hebbian synapses and NMDA receptors are thought to aid in the process of wiring spatially correlated retinal cells in close proximity at the target (Artola & Singer, 1987; Shatz, 1992). A summation of excitation over time from a group of correlated neurons assures that the common target would become depolarized. Sufficient depolarization would remove the magnesium block from the NMDA receptors. It is possible that the resulting calcium-dependent second messenger cascade could be responsible for the organization of synaptic connections, stabilization of synapses and preservation of the retinotopic map in the lateral geniculate and cortex. Evidence for the role of NMDA receptors in map stabilization was found by Simon, Prusky, Oleary, & Constantine-Paton (1992). Rats chronically treated with NMDA antagonists during the first two postnatal weeks of life exhibited abnormal targeting and arborization of axons.

During development, activity-dependent changes are induced in overall morphology of presynaptic terminals (Shatz, 1990; Yen, Sibley & Constantine-Paton, 1993). While

some terminals are actively eliminated, others are just forming. NMDA appears to be necessary for these changes to occur. There is some evidence from the study of the frog visual system that NMDA is also involved in the refinement of the retinotectal pathway. Following the transplantation of a supernumery eye in a tadpole, the chronic application of NMDA to the doubly innervated frog tecta produced increases in eye specific segregation of retinotectal axons and stripe boundaries for each eye became more pronounced (Cline & Constantine-Paton, 1989; Constantine-Paton, Cline, & Debski, 1990). NMDA antagonists also disrupt map formation in the three eyed frog (Cline & Constantine-Paton, 1989).

Long-term Potentiation. Although a reduction in plasticity might be desirable after the system is fine tuned, the system must retain some ability to adapt in a dynamic environment. LTP might serve as the residual plasticity mechanism in the adult animal.

Age dependent LTP. Crair and Malenka (1995) investigated LTP in thalamocortical synapses in the developing rat. They used a novel slice preparation that allowed for the direct stimulation of the thalamus *and* the monitoring of responses in the barrel fields in SI. Barrel fields are susceptible to manipulations in the periphery (i.e. folicle removal) for approximately seven days after birth. The slice preparation included the ventrobasal thalamus (VB) and the afferents that innervate layer IV cells in the SI. High frequency stimulation delivered to the VB produced a potentiation in the slices of animals that were sacrificed at P3 to P7 only (3 to 7 days after birth). Older animals did not exhibit LTP. NMDA receptor-mediated synaptic currents were pharmacologically isolated and were found to decrease with age. The change was fivefold from P3-P7 to P8-P14.

LTP was also investigated in the developing rat visual cortex slice preparation by

Kirkwood, Lee and Bear (1995). In this preparation only the visual cortex and the white matter were retained for stimulation and recording. Theta burst stimulation delivered to the white matter elicited an increase in the EPSP amplitude recorded from layer III that appeared to be age dependent. The greatest increase was seen in animals approximately two weeks of age, which roughly corresponds to the peak of the critical period in the rat. By approximately 4 weeks of age, animals no longer exhibited significant potentiation, indicating that LTP induction was restricted to the critical period.

Kirkwood & Bear (1993) have found that an NMDA-dependent LTP *can* be induced in slices from adult cortex if the stimulation is applied to layer IV, rather than to the white matter. They suggest that an inhibitory 'gate' in layer IV normally keeps the plasticity thresholds high in layer III. By stimulating the layer IV cells directly, the gate is bypassed and potentiation results. These results suggest that levels of inhibition may also be a critical determinant of critical period plasticity.

Caveats of the Slice Preparation. There are risks in studying LTP exclusively in the slice preparation. There is some question about generalizeability to the intact preparation and, more specifically, to the intact, freely moving animal. The slice, for example, lacks some of the extra modulatory systems that are present in the intact system. Perhaps the major problem with the slice preparation is its short lifespan. In most slice experiments, LTP is rarely monitored for more than 30 minutes. Neocortical LTP in the chronically prepared animal is reported not to reach asymptotic levels until about 10 to 15 days of stimulation (see Racine, et al, 1995).

LTP in Corticothalamic Synapses. To date, there appears to be no literature on

attempts to induce LTP in the corticothalamic pathways, either in intact animals or the slice preparation. The thalamus contains many glutamate receptors, including receptors for NMDA (see review by Salt & Eaton, 1996). The corticothalamic pathways must have some functional significance. It has been proposed that these pathways are responsible for selective enhancement or gain of sensory transmission through the thalamus, a general theory proposed by a series of researchers (i.e. Mumford, 1991, 1992; Steriade, McCormick & Sejnowski, 1993; von Krosigk, Bal & McCormick, 1993). In the visual system, the pathway is approximately 13 times larger than the thalamocortical pathway and approximately 40 times larger than the retinogeniculate pathway (Mumford, 1991). While the precise conditions under which inputs to the thalamus are activated remains to be determined, an LTP-like mechanism in corticothalamic pathways is a putative mechanism contributing to their proposed filtering mechanism.

Chapter 3

Long-term Potentiation in the Efferents of the Perirhinal Cortex

While the hippocampus, along with the entorhinal, perirhinal, and parahippocampal cortices have been widely cited for their involvement in memory, LTP in the extensive reciprocal connections between the structures in this system and between this system and the neocortex have received little attention. If an LTP-like mechanism underlies information storage in this system, then the pathways connecting these sites should support LTP induction. Two different groups (Liu & Bilkey, 1996a, 1996b; Otto & Eichenbaum, 1994) have shown that LTP can be induced in area CA1 following high frequency stimulation delivered to perirhinal cortex. Liu and Bilkey (1996b) found similar effects in the dentate gyrus.

The following study was designed to investigate LTP in projections from the perirhinal cortex to the frontal cortex, the hippocampus and the entorhinal cortex in chronically implanted animals. The parameters of interest included the number of stimulations required to induce potentiation, the number of stimulation sessions required to reach asymptote, and the longevity of the LTP effect. The results of this experiment have been reported previously (Ivanco, Michelin, & Racine, 1996).

METHODS

Experiments were performed on male Long-Evans rats from the McMaster Breeding Colony weighing between 300 and 500 grams at the time of surgery. All rats were housed in pairs in plastic cages prior to surgery and individually in hanging wire cages following surgery. Food and water were provided *ad lib*. The colony was maintained on a 12 hr on/12 hr off light dark cycle.

Twisted bipolar electrodes were constructed from Teflon coated stainless steel wire, 200 μm in diameter, insulated except for the tips. All electrode tips were separated by approximately 500 μm, except for the electrodes placed in frontal cortex, which had a tip separation of approximately 1.0 mm. Animals were anaesthetized with sodium pentobarbitol (65mg/kg) and stereotaxically implanted with electrodes under electrophysiological control.

In all animals, a bipolar stimulating electrode was implanted into the perirhinal cortex and bipolar recording electrodes were implanted in the entorhinal cortex, dentate gyrus, and frontal cortex according to the atlas of Paxinos and Watson (1997). The coordinates were as follows: *perirhinal cortex*: 2.3 mm posterior to Bregma, 6.0 mm lateral to the midline, and 6.0 mm ventral to brain surface with an electrode angle of 15 degrees; *entorhinal cortex*: 7.0 mm posterior to Bregma, 5.2 mm lateral to the midline, and 8.0 mm ventral to brain surface; *dentate gyrus*: 3.5 mm posterior to Bregma, 2.2 mm lateral to the midline, and 3.3 mm ventral to brain surface; and *frontal cortex (area M1)*: 2.0 mm anterior to Bregma, 4.0 mm lateral to the midline, and 1.8 mm ventral to brain surface. Depths of placements were adjusted to maximize amplitudes of evoked responses.

Electrodes were connected to gold-plated male amphenol pins that were inserted into

a 9-pin connector plug. Four stainless steel jewellers screws were used to anchor and mount the plug to the skull with dental acrylic. One of the screws had a wire attached with a gold-plated pin that served as a ground electrode. Animals were given at least two weeks to recover before testing began.

Stimulation and Recording. Acquisition of evoked responses was completed using ASYST software on a Comptec 486, 33 MHz computer. Electrical stimuli were produced with a Grass S88 stimulator and photoelectric stimulation isolation units (Grass SIU6B). Signals were fed into Grass Model 12 EEG amplifiers and filtered (at half amplitude) at 0.3 Hz (high pass) and 3 kHz (low pass). The analog evoked responses were sampled at 10 kHz by a 12 bit analog-to-digital (A/D) convertor (Data Translation DT 2821) and stored on a computer hard drive for off-line analysis.

Baseline evoked field potentials were collected three times, once every 48 hours. To construct input/output curves (I/Os), biphasic stimulation pulses of increasing intensity were delivered to the perirhinal cortex electrode at a frequency of 0.1 Hz. Ten responses, 70 ms in duration, were evoked, amplified, digitized, and averaged at each of 12 logarithmically spaced intensities (63, 79, 100, 126, 159, 251, 398, 501, 631, 794, 1000, 1259 μA). Following the third baseline, animals were matched based on evoked response morphology and separated into control and experimental groups. Not all animals showed reliable evoked responses at each of the recording electrodes. In this and all other experiments, animals that were not included in the analysis were those animals that survived surgery, but showed a very weak response that required adding additional gain such that the noise also increased to levels that interfered with the evoked response, showed a flat baseline when recording was

begun, or showed an inappropriate increase in noise levels or flattening (overall flattening that is different than a depression effect) of the baseline prior to the completion of all the recording. In total, 19 animals were used to obtain reliable measures from each of the entorhinal cortex (experimental n=6; control n=4), hippocampus (experimental n=8; control n=6) and frontal cortex (experimental n=7; control n=4).

High frequency, 50 msec, stimulation trains were delivered to the perirhinal cortex in all experimental animals. The pulse intensity was set at a level that produced an 80% maximum response amplitude in at least one of the recording sites and pulse frequency was set at 300 Hz. Thirty trains were delivered per session at 0.1 Hz. This was repeated daily, immediately following an I/O test, for 15 days. I/O tests were given to the control animals, but no trains were delivered. Final follow-up I/Os were collected 24 hours, 48 hours and 1 week after the final set of trains in experimental animals and after comparable delays in control animals.

An additional 6 animals, which were not potentiated, were used to obtain paired pulse measures for the entorhinal cortex (n=4), dentate gyrus (n=4), and frontal cortex (n=4) sites. Biphasic stimulation pulses were delivered to the perirhinal cortex at 1259 mA. Ten responses were evoked, amplified, digitized and averaged for each of 10 inter-pulse intervals (50, 70, 100, 150, 200, 300, 500, 700, 1000, 2000 ms) and stored on a computer hard drive for off line analysis. Some of these animals were also used in a frequency-of-following test.

Analysis. Baseline was established by averaging the first three I/O tests. The evoked responses produced by the stimulation intensity that resulted in an evoked response of approximately 80% maximum amplitude in the baseline tests were used for analysis.

Latencies to an early monosynaptic and a polysynaptic peak in the field potential were determined using both the baseline and potentiated responses (24 hours after the last set of trains), as the polysynaptic component was not evident until after stimulation had been delivered. Changes from baseline amplitude were calculated at each of these two latencies for daily I/O curves to determine millivolt changes over time. Repeated measures ANOVAs were used to test for significant effects of stimulation on response amplitudes. Asymptotic levels of potentiation were determined by comparing means and standard errors of the mean (SEMs) with those representing the largest millivolt change following the experimental manipulation in all of the experiments reported in this thesis.

As an additional means to assess potentiation, the mean slope of the rising phase of the field potential was determined for each of the recording sites. An average of the first three I/O tests was again calculated to provide the baseline slope and this baseline was used to determine slope change over days. Repeated measures ANOVAs were used to test for significant effects of stimulation on the slope change. The slope changes were not signficant in any of the analysis done for this thesis. As the slope results do not add anything to the amplitude changes that were found, they are not reported in any of the experiments that follow.

Paired pulse measures were examined at the 50 ms latency to examine the facilitation effects. A t-test was used to determine if the response evoked by the second pulse was significantly different from the response evoked by the first pulse. The evoked responses from the frequency-of-following test were plotted on a computer screen and response failure was assessed at each of the frequencies used. The inputs were assumed to be monosynaptic

if the responses followed at frequencies up to 100 Hz. Inputs were assumed to be polysynaptic if the responses failed at frequencies below 100 Hz.

After completion of the electrophysiological experiments, the animals were perfused through the heart with 0.9% saline, followed by a formal-saline solution (4% formalin in 0.9% saline). Brains were removed and immersed in the formal-saline solution for at least 24 hours. Brains were then immersed in a 10% sucrose solution and placed in a 4 degree Celcius refrigerator for 24 hours. Each brain was sectioned in the coronal plane (40 μm) on a cryostat, mounted on gelatin coated slides, and stained with 1% Cresyl Violet. Slides were examined with a light microscope to confirm stimulating and recording placements.

RESULTS

Light microscope examination of brain sections provided verification that stimulating and recording electrodes were located within the appropriate target structures as defined by Paxinos and Watson (1997). For this study our definition of perirhinal cortex also included the area defined as ectorhinal cortex by Paxinos and Watson (1997) as this area corresponds to the perirhinal area 36 as defined by Burwell, and colleagues (Burwell, et al., 1995; Burwell & Amaral, 1996). Locations of stimulating and recording placements are shown in Figure 3.1.

High frequency stimulation of the perirhinal cortex induced a significant potentiation in all three recording sites. Although there were significant amplitude changes at each site, they occurred at a latency beyond the peak of the monosynaptic component. The peak of the monosynaptic component did not change following high frequency stimulation, indicating

a polysynaptic influence on the evoked response. Figure 3.2 shows an example of a baseline and an evoked response after trains had been delivered from each of the three recording sites. The small arrows at the peaks represent where the voltages are measured across days to determine millivolt changes. Test pulses delivered to the perirhinal cortex evoked a large surface positive field potential in or near the granular layer of the dentate gyrus. Following stimulation, the polysynaptic component became evident in the experimental group. Multisession stimulation delivered to the perirhinal cortex produced a significant increase ($p<0.001$) in the dentate gyrus field potential at the latency of the polysynaptic component. The amplitude of the evoked response 24 hours after the last stimulation had increased 1.1 mV +/- 0.4 mV (mean +/- SEM). The response evoked in the dentate gyrus by a single test pulse delivered to the perirhinal cortex is shown in figure 3.2a. The evoked response started to show small increases in amplitude relatively quickly, but did not reach an asymptotic level of potentiation until after 3 days of stimulation had been delivered to the perirhinal cortex (see Figure 3.3a). The increase in amplitude was long lasting and was present at least one week after the delivery of trains had ceased.

Perirhinal cortex stimulation also produced a potentiation in the entorhinal cortex response in the experimental group. Stimulation pulses evoked a surface positive potential in the entorhinal cortex. The amplitude of the polysynaptic component recorded 24 hours after the last stimulation session was increased by 0.32 mV +/- 0.04 mV (see Figure 3.2b). The increase in amplitude in the entorhinal cortex over days was significant ($p<0.001$), but relatively small (see Figure 3.3b). While the evoked response reached asymptote after approximately 4-5 days of stimulation, it appeared to be approaching baseline levels during

the final follow-up measures taken one week after trains had ceased.

Test pulses delivered to the perirhinal cortex evoked a surface negative potential in the frontal cortex. Although there was no potentiation seen following a single day of trains, potentiation was evident following the second set of trains. Potentiation did not appear to reach asymptote until after 5 to 6 days of trains (see Figure 3.3c). Stimulation delivered for 15 days produced a significant ($p<0.001$) increase in the amplitude of the polysynaptic component. Measures taken 24 hours after the last stimulation session indicate an increase of 0.78 mV +/- 0.17 mV in the peak amplitude of this component. Figure 3.2c shows the evoked response at the frontal site following perirhinal stimulation to induce LTP. The evoked response remained potentiated for at least one week following the last day of trains.

Paired pulse stimulation produced relatively small effects in both the frontal and entorhinal sites. In the dentate gyrus, the test response showed an increase of 3.05 mV +/- 1.32 mV when compared to the conditioning response (see Figure 3.4a). None of these changes, however, were significant.

The dentate gyrus evoked response appeared to follow at frequencies of 100 Hz delivered to the perirhinal cortex (see Figure 3.4b), but the frontal cortex and entorhinal cortex failed at about 50 Hz and 75 Hz, respectively. The dentate response, however, did show a decrease in amplitude with higher frequencies. It is likely that the field potentials, which have a component that has been labelled *monosynaptic* in the previous sections, are in fact a result of a combination of monosynaptic and polysynaptic influences, with the polysynaptic influences being especially prominent in the later portion of the evoked

response. This may account for the failure of responses to follow at frequencies up to 100 Hz, but still also account for the lack of failure of the dentate response.

DISCUSSION

There have been very few attempts to describe LTP effects in pathways to and from the perirhinal cortex, even though it is a major source of information conveyed to the hippocampus. The results presented here indicate that the pathways from the perirhinal cortex to the frontal cortex, dentate gyrus, and entorhinal cortex are capable of supporting LTP.

Due to a lack of significant change in the monosynaptic components of the evoked response, however, the potentiation effect must be cautiously interpreted. Changes in the monosynaptic component would allow for some certainty in suggesting that there is an increased level of plasticity in a specific pathway or the site we are recording from. Polysynaptic changes are difficult to interpret because we cannot be certain *where* the LTP is induced. Without additional experiments, we cannot confirm whether the potentiation in polysynaptic components represents plasticity within the structure of interest, which are not significant because of non-optimal electrode placements, or changes in intervening sites. We did find, however, that all potentiated groups contained a few animals that showed clear modifications of the monosynaptic component. An example can be seen in the dentate gyrus following stimulation delivered to the perirhinal cortex (see Figure 4.2a). In any case, the findings reported are of interest because they indicate that a modifiable pathway exists somewhere between the two sites.

Whereas pathways intrinsic to the hippocampus show a rapid induction of LTP, a rapid rise to asymptotic levels of potentiation, and a relatively rapid decay, cortico-cortical pathways show a slow induction of LTP, a slow rise to asymptotic levels of potentiation, and a slow decay. The efferents from the perirhinal cortex show properties that are *intermediate* between the hippocampal and neocortical systems. The perirhinal efferents, for example, require an intermediate number of stimulation sessions to reach asymptotic levels of potentiation. The dentate gyrus potentiates more *slowly* following perirhinal cortex stimulation than it does following entorhinal or perforant path stimulation and the neocortex expresses LTP more *rapidly* following perirhinal cortex stimulation than it does following callosal stimulation (see Racine, et al., 1995). If LTP is a memory mechanism, these results suggest that the perirhinal pathways may express intermediate storage rates. Intermediate storage rates were proposed by McClelland, et al. (1995), but they provided no speculation as to the functional implications of such rates.

The distribution of electrode tips of the stimulating electrodes in the perirhinal cortex indicate that the stimulation and test pulses were delivered in more superficial layers and not to the white matter of the external capsule. Also, the clear paired pulse facilitation seen in 2 of the 3 animals argues against a major contribution of fibres of passage, because these fibres do not produce a facilitation effect (Kanning, personal communication).

Previous studies have used the frequency-of-following test to determine if pathways are monosynaptic (e.g. Liu & Bilkey, 1996a; Laroche, Jay & Thierry, 1990). A pathway is thought to be monosynaptic if the evoked potential follows a high frequency train (i.e. 100 Hz) with a constant delay (Berry & Penreath, 1976). Whereas results of the frequency-of-

following test indicate that there is a monosynaptic component in the projections between the perirhinal cortex and dentate gyrus, the other two pathways tested appear to be polysynaptic.

Berry and Penreath (1976), however, have indicated that a direct connection is not ruled out by the absence of following. For example, some polysynaptic systems, although they cannot *follow* at high frequencies, might be recruited at high frequencies and block or distort the responses to monosynaptic inputs. Long-duration modulatory systems could easily affect each response in a brief, high frequency train.

Although unlikely, given the presence of both monosynaptic and polysynaptic connections, it is possible that information storage follows a sequence through the system. For example, changes may occur first at the synapses between the perirhinal cortex and entorhinal cortex. Subsequently, changes could occur at synapses between the entorhinal cortex and the dentate gyrus, and so on. For this reason, it was of interest to compare the rate at which LTP developed between the perirhinal cortex and entorhinal cortex *and* between the perirhinal cortex and the dentate gyrus. In fact, the entorhinal cortex required more stimulation sessions to potentiate than did the hippocampus, and the effect was weaker in the entorhinal cortex when compared to that seen in the hippocampus or the neocortex. Decay rates were only followed for one week, but it was apparent that the entorhinal cortex also showed a more rapid decay than either the dentate gyrus or the frontal cortex.

The results of the present study demonstrate that LTP can be induced in the pathways between the perirhinal cortex and other structures thought to be intrinsically involved in both memory formation and long term storage. There were, however, some interesting differences

that deserve further exploration. The efferents of the perirhinal cortex required sessions intermediate in number to those required for the hippocampus (perforant pathway) and neocortex (corpus callosum/cortico-cortical pathways), and the LTP effects expressed in the neocortex and dentate gyrus were more robust than those in the entorhinal cortex. These results do not indicate whether the reduced level of potentiation in the entorhinal cortex is due to less plasticity within the entorhinal cortex itself, or reduced plasticity in the pathways between the perirhinal cortex and entorhinal cortex. Other pathways with targets within the entorhinal cortex can be tested to determine which of these alternatives is true. In addition, it would be of interest to monitor potentiation effects in perirhinal afferents. This was done in the next set of experiments.

FIGURES and CAPTIONS

Chapter 3

Figure 3.1. The locations of stimulating electrodes in the perirhinal cortex (PRh) and recording electrodes in the dentate gyrus (DG), entorhinal cortex (EC), and primary motor/frontal cortex (M1) are shown on representative sections from the rat brain atlas of Paxinos and Watson (1997). Note that our definition of perirhinal cortex includes ectorhinal cortex as this area corresponds to the perirhinal area 36 (Burwell, et al., 1995; Burwell & Amaral, 1996). Locations for the lowest pole of the bipolar electrode for all experimental animals are represented with dots and for all control animals with squares. The stimulation site is demarked by an asterisk.

Figure 3.1

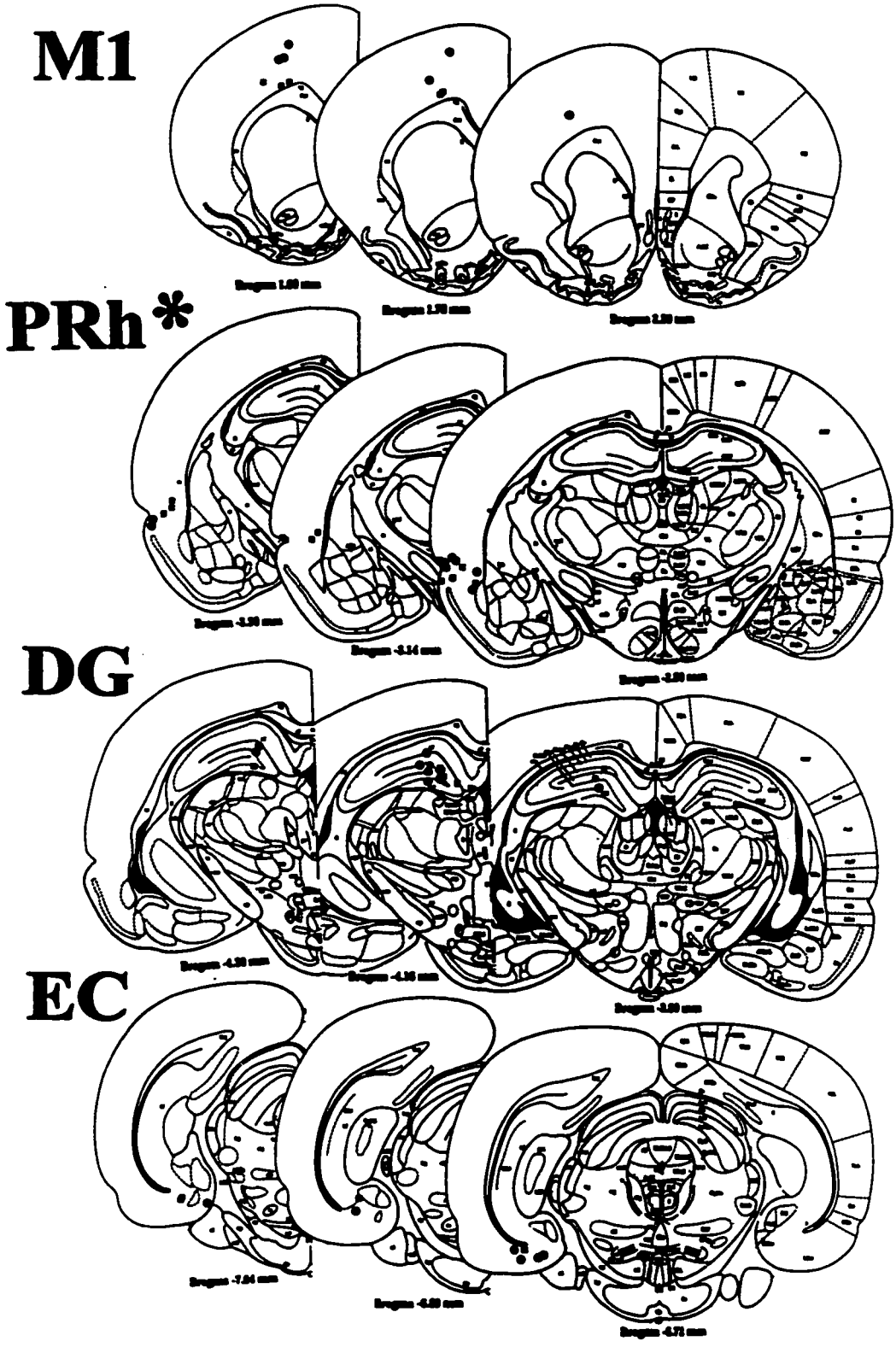


Figure 3.2. Field potentials evoked by perirhinal cortex stimulation in: A) the dentate gyrus (DG), B) the entorhinal cortex (EC), and C) the primary motor/frontal cortex (M1). Note that the solid lines represent the baseline recorded from each site, while the dashed line represents the evoked response recorded 24 hours after the delivery of trains. A thin arrow indicates the monosynaptic peak latency for the field potentials. A thick arrow indicates the polysynaptic peak latency for the field potentials. Horizontal calibration, 10 msec; vertical calibration, 0.5 mV, except those marked. Here, * = 2.0 mV and ** = 0.25 mV. Upward deflections indicate negativity in this and all subsequent figures, except where noted.

Figure 3.2

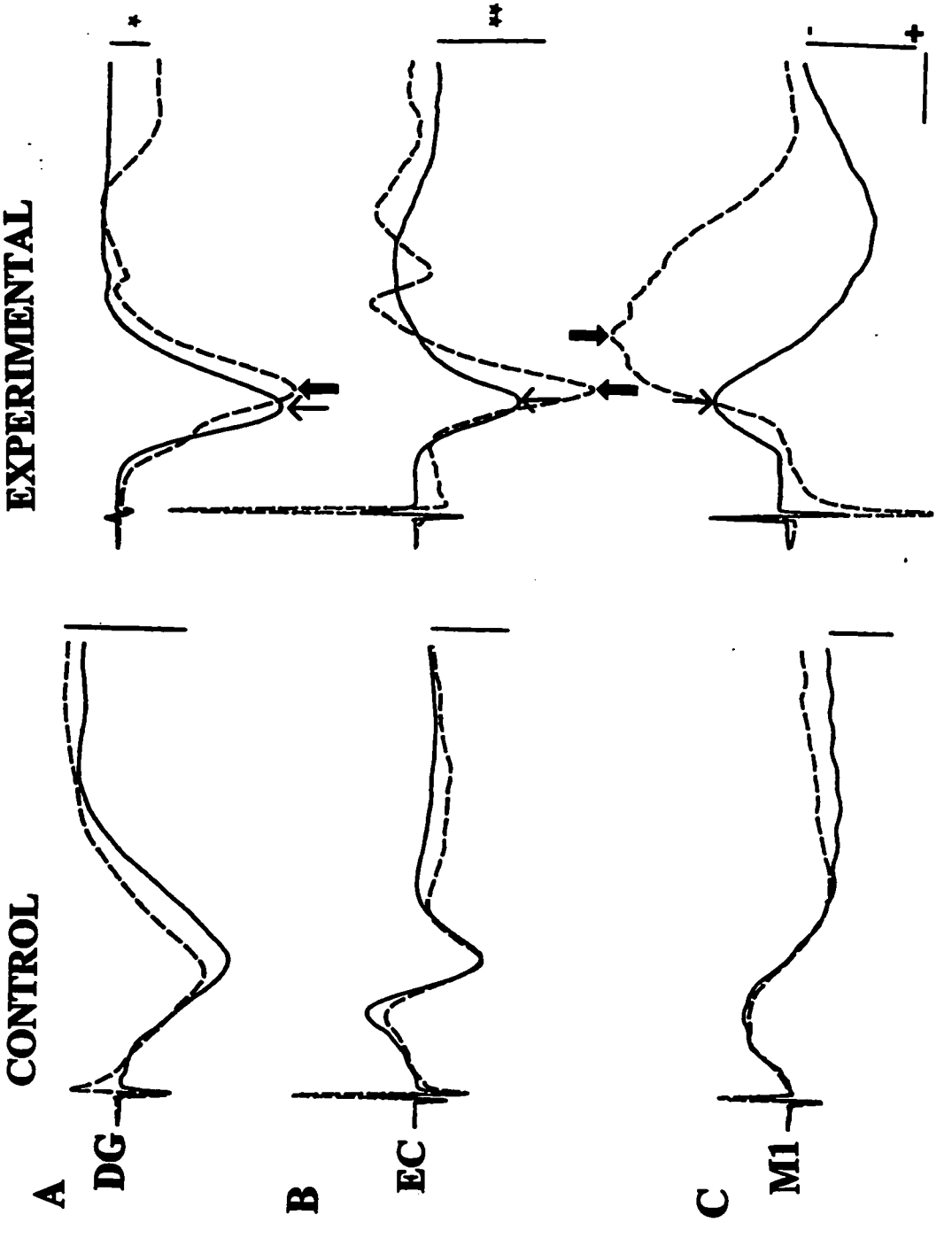


Figure 3.3. Evoked response amplitudes as a function of days. Amplitude changes from the average baseline are represented along the ordinate and days along the abscissa. The abscissa shows the pre-induction period (baseline), the induction period, and the follow-up period (post-LTP). Note that the ordinate is labelled differently in A) than in B) and C). A) The amplitude of the polysynaptic component in the dentate gyrus is shown to increase significantly (group x day interaction, $p<0.001$) over days. The amplitude of the polysynaptic component was also found to increase over days in the entorhinal cortex (B) and in the frontal cortex (C) (group x day interaction, $p<0.001$ and $p<0.001$, respectively).

POLYSYNAPTIC COMPONENT

Figure 3.3

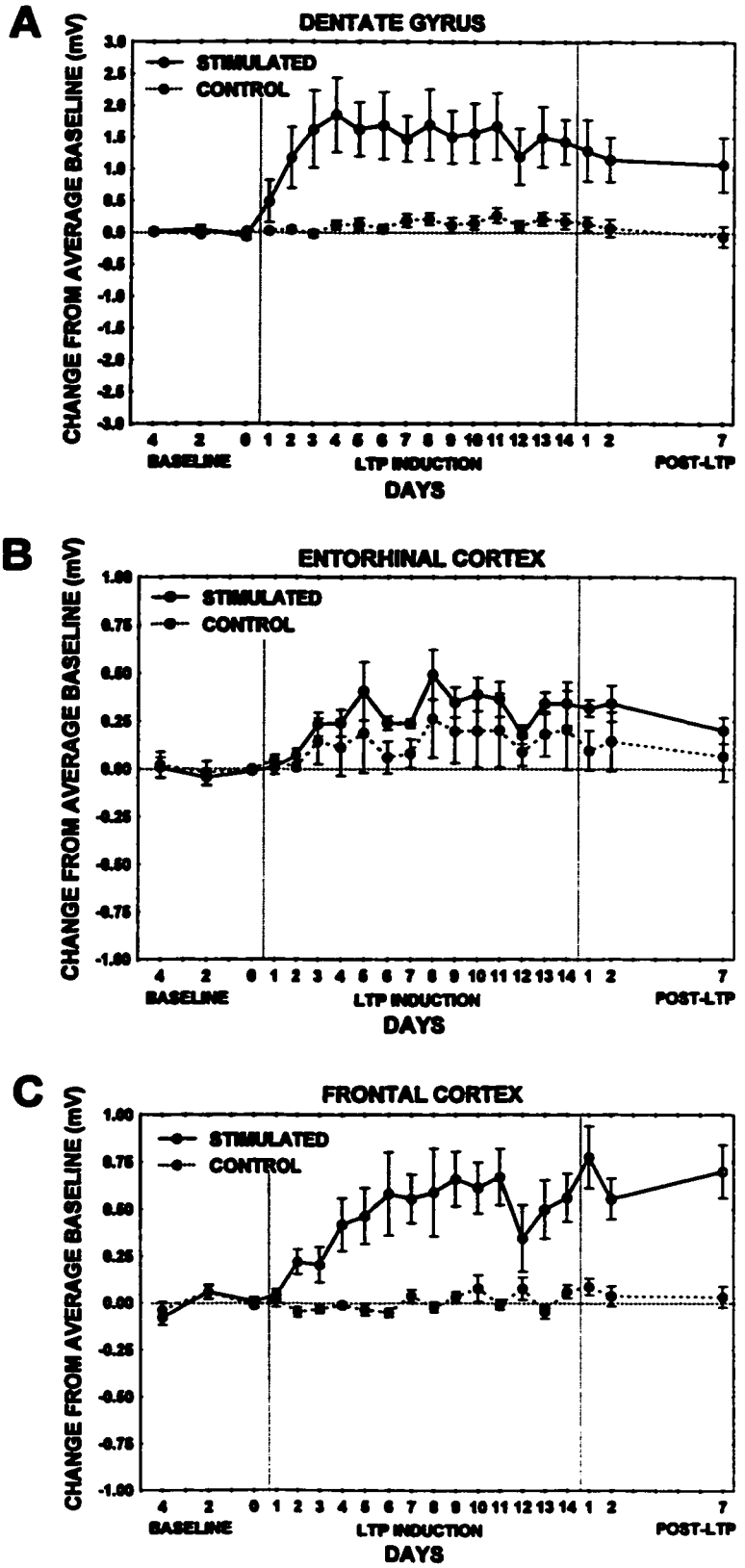
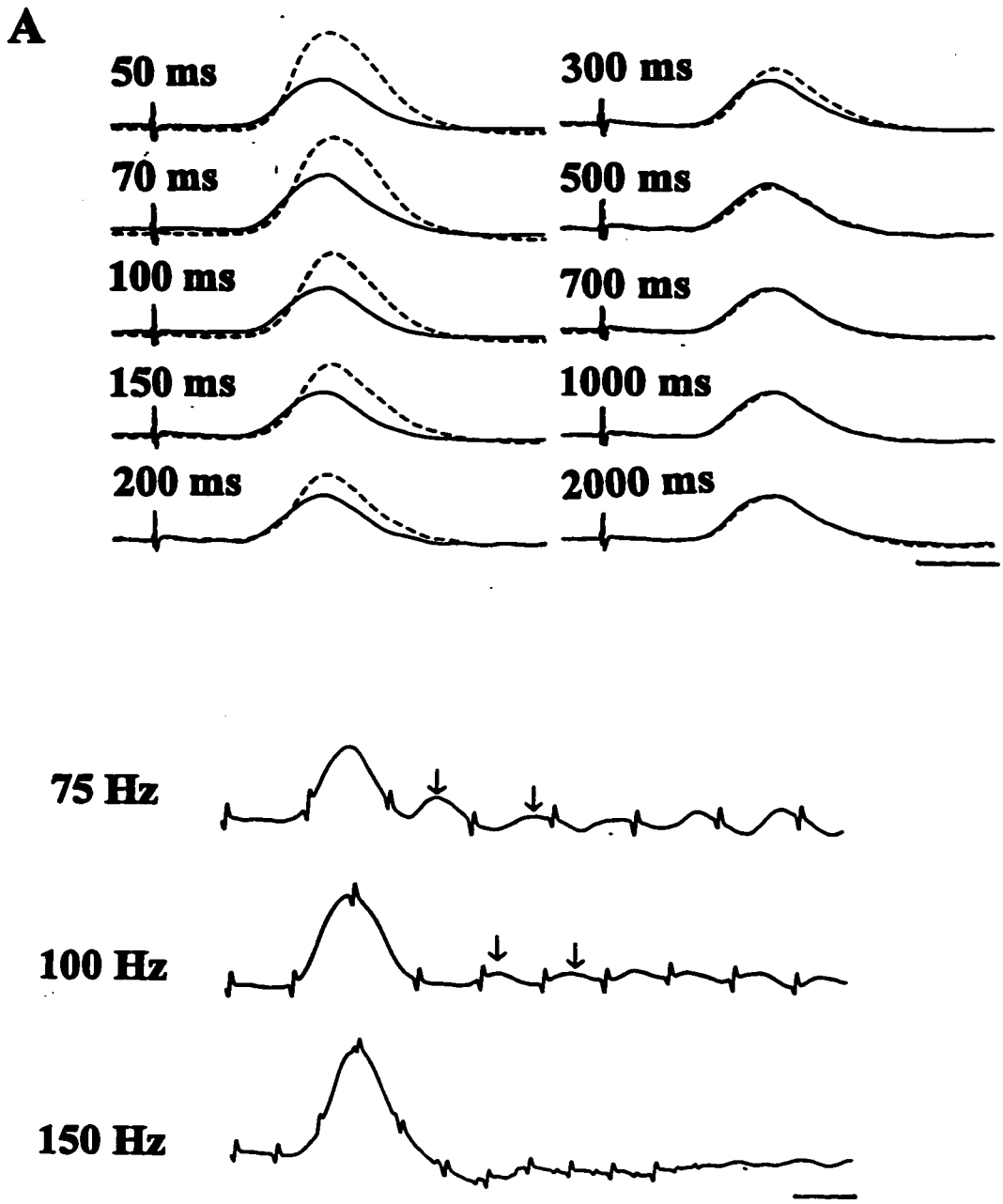


Figure 3.4. Paired pulse tests and frequency-of-following in the dentate gyrus. A) Dashed traces representing the evoked response to the second pulse have been superimposed on the solid trace representing the evoked response to the first pulse. Paired pulses delivered to the perirhinal cortex produced a slight facilitation of responses evoked in the dentate gyrus when the pulses were presented with a relatively short interpulse interval. B) The responses evoked in the dentate gyrus with high frequency stimulation delivered to the perirhinal cortex. The three frequencies represented can be used to determine whether a monosynaptic connection exists. The responses in the dentate gyrus appeared to follow at frequencies up to 100 Hz, indicating a monosynaptic pathway, but the responses were attenuated with the higher frequencies. Arrows point to some of the attenuated evoked responses in the 75 and 100 Hz traces. Note that in this figure, upward deflections indicate positivity. Horizontal calibration, 10 msec.

Figure 3.4


A
50 ms
300 ms
70 ms
500 ms
100 ms
700 ms
150 ms
1000 ms
200 ms
2000 ms
B
75 Hz
100 Hz
150 Hz

Chapter 4

Long-term Potentiation in the Hippocampal and Frontal Cortex Efferents

The hippocampus appears to have a relatively direct influence on long term storage of memories in the neocortex. In the past, it was assumed that hippocampal influences on the neocortex were mediated via the flow of information from the fornix to the cingulate gyrus and, from there, to other cortical areas (Rosene & Van Hoesen, 1977). Fornix lesions, however, do not result in the same loss of memory in humans or monkeys as seen following hippocampal damage (Markowska, Olton, Murray, & Gaffan, 1989; Woolsey & Nelson, 1975). Rosene and Van Hoesen (1977) examined hippocampal efferents reaching the cortex in monkeys and found that they originated primarily in the subiculum and travelled to the cortex without the use of subcortical relays. Input to the hippocampus passed unidirectionally through the dentate gyrus and ammonic subfields to the subiculum. Injections of tritiated amino acids, an anterograde label, into the CA1 resulted in a dense labelling in the subiculum. Using the same procedure the subiculum was found to send direct projections to the medial frontal cortex, cingulate and parahippocampal areas, including the cortex around the rhinal sulcus. There is also an organized input from the temporal half of CA1 to layers I and V of the prefrontal cortex in the rat (Swanson, 1981). In this study, a double labelling technique was used to determine that approximately 90% of the cells that projected to the prefrontal cortex also projected to the entorhinal cortex. There

appear to be synaptic connections between the hippocampus and prefrontal, entorhinal, and parahippocampal cortices. The connections between the hippocampus and prefrontal cortex are also likely to include perirhinal cortex.

Laroche and co-workers (Laroche, et al., 1990; Jay, Burette, & Laroche, 1995) examined unit activity in the prefrontal cortex following stimulation of the CA1/subicular region in anesthetized animals. Almost 50% of the units recorded in the prefrontal cortex exhibited excitatory responses. Paired pulse facilitation was found in both units responses and field potentials. High frequency stimulation delivered to the CA1/subiculum induced an NMDA-dependent LTP in prefrontal cortex. The authors suggest that hippocampal-neocortical LTP could serve as a useful model for the functional analysis of hippocampal-neocortical communication in learning and memory.

Experiment 1: LTP in the Hippocampal Efferents

This study was designed to investigate the responses evoked in the frontal, entorhinal, and perirhinal cortices following hippocampal stimulation in the chronically implanted rat. A preliminary mapping study indicated that electrodes could be lowered through the dorsal CA1 without discharge and that responses could be evoked in the frontal, perirhinal and entorhinal cortices with a biphasic test pulse delivered to the CA1 area. The parameters of interest included the number of sessions required to induce potentiation, the number of stimulation sessions to reach asymptote, and the longevity of the LTP effect.

METHODS

Experiments were performed on male Long-Evans rats from the McMaster Breeding Colony weighing between 300 and 500 grams at the time of surgery. All rats were housed in pairs in plastic cages prior to surgery and individually in hanging wire cages following surgery. Food and water were provided *ad lib*. The colony was maintained on a 12 hr on/12 hr off light dark cycle.

Twisted bipolar electrodes were constructed from Teflon coated stainless steel wire, 200 μm in diameter, insulated except for the tips. All electrode tips were separated by approximately 500 μm, except for the electrode implanted into the frontal cortex, which had a tip separation of approximately 1.0 mm. Animals were anaesthetized with sodium pentobarbitol (65mg/kg) and stereotaxically implanted with electrodes under electrophysiological control.

In all animals bipolar electrodes were implanted into the dorsal CA1, frontal cortex, perirhinal cortex, and entorhinal cortex, according to the atlas of Paxinos and Watson (1997). The coordinates were as follows: *dorsal CA1*: 3.5 mm posterior to Bregma, 2.2 mm lateral to the midline, and 2.8 mm ventral to brain surface; *frontal cortex (area M1)*: 2.0 mm anterior to Bregma, 4.0 mm lateral to the midline, and 1.8 mm ventral to brain surface; *perirhinal cortex*: 2.3 mm posterior to Bregma, 6.0 mm lateral to the midline, and 6.0 mm ventral to brain surface with an electrode angle of 15 degrees; and *entorhinal cortex*: 7.0 mm posterior to Bregma, 5.2 mm lateral to the midline, and 8.0 mm ventral to brain surface. Depths of placements were adjusted to maximize amplitudes of evoked responses.

Electrodes were connected to gold-plated male amphenol pins that were inserted into

a 9-pin connector plug. Four stainless steel jewellers screws were used to anchor and mount the plug to the skull with dental acrylic. One of the screws had a wire attached with a gold-plated pin that served as a ground electrode. Animals were given at least two weeks to recover before testing began.

Stimulation and Recording. Acquisition of evoked responses was completed using ASYST software on a Comptec 486, 33 MHz computer. Electrical stimuli were produced with a Grass S88 stimulator and photoelectric stimulation isolation units (Grass SIU6B). The signals were fed into a high impedence Grass Model 12 EEG amplifier and filtered (at half amplitude) at 0.3 Hz (high pass) and 3 kHz (low pass). The analog signals were sampled at 10 kHz by a 12 bit A/D convertor (Data Translation DT 2821) and stored on a computer hard drive for off-line analysis.

Baseline evoked field potentials were collected three times, once every 48 hours. To construct input/output curves (I/Os), biphasic stimulation pulses of increasing intensity were delivered to the dorsal CA1 electrode at a frequency of 0.1 Hz. Ten responses, 70 ms in duration, were evoked, amplified, digitized and averaged at each of 12 logarithmically spaced intensities (63, 79, 100, 126, 159, 251, 398, 501, 631, 794, 1000, 1259 μA). Following the third baseline measures animals were matched based on evoked response morphology and separated into control and experimental groups. Responses were recorded from the perirhinal cortex, entorhinal cortex and frontal cortex electrodes. Not all animals showed reliable evoked responses at each of the recording electrodes. In total, 15 animals were used to obtain reliable measures from each of the perirhinal (experimental n=7; control n= 4), entorhinal (experimental n=4; control n=4) and frontal (experimental n=4; control n=3)

cortices.

High frequency, 50 ms, stimulation trains were delivered to the anterior CA1 electrode. Pulse intensity was set at a level that produced an 80% maximum amplitude response in at least one of the sites being recorded from and pulse frequency was set at 300 Hz. Thirty trains were delivered per session at 0.1 Hz. EEG was monitored with an oscilloscope during the delivery of trains to ensure that there were no discharges. Trains were delivered daily, immediately following an I/O test, for 15 days. I/O tests were given to the control animals, but no trains were delivered. Final follow-up I/O measures were collected 24 hours, 48 hours and 1 week after the final set of trains in experimental animals and after comparable delays in control animals.

Analysis. Potentiation was assessed by comparing the amplitudes of the initial baseline evoked responses with those of responses evoked after trains had been delivered. The evoked responses produced by the stimulation intensity that resulted in an evoked response of approximately 80% maximum amplitude in the baseline tests were used for analysis. Baseline was established as the average of the first three I/O tests. All measures were compared against this baseline average to determine millivolt changes in amplitude over days. Repeated measures ANOVAs were used to test for significant effects of stimulation.

After completion of the electrophysiological experiments, the animals were perfused through the heart with 0.9% saline, followed by a formal-saline solution (4% formalin in 0.9% saline). Brains were removed and immersed in the formal-saline solution for at least 24 hours. Brains were then immersed in a 10% sucrose solution and placed in a 4 degree

Celcius refrigerator for 24 hours. Each of the brains were sectioned in the coronal plane (40 μm) on a cryostat, mounted on gelatin coated slides, and stained with 1% Cresyl Violet. Slides were examined with a light microscope to confirm stimulating and recording placements.

RESULTS

Light microscope examination of brain sections provided verification that stimulating and recording electrodes were located within the appropriate target structures as defined by Paxinos and Watson (1997). For this study our definition of perirhinal cortex also included the area defined as ectorhinal cortex by Paxinos and Watson (1997) as this area corresponds to the perirhinal area 36 as defined by Burwell, and colleagues (Burwell, et al., 1995; Burwell & Amaral, 1996). Locations of stimulating and recording electrode tips are shown in Figure 4.1.

High frequency stimulation delivered to the dorsal CA1 resulted in a potentiation of the evoked responses at all sites. Although there were significant amplitude changes in all sites, with one exception they occurred at a latency beyond the peak of the monosynaptic component following high frequency stimulation, indicating polysynaptic influences on the evoked response. Stimulation of the anterior CA1 evoked a potential in the entorhinal cortex that had a surface positive wave. Figure 4.2a shows an example of an entorhinal cortex evoked response before and after the delivery of trains. Stimulation delivered to the CA1 produced a potentiation that was evident after only two stimulation sessions, but did not reach asymptotic levels until after approximately ten stimulations (see Figure 4.3a). The

increase in the amplitude was significant ($p<0.02$) for the polysynaptic component. The amplitude was increased 0.37 mV +/- 0.04 mV (mean +/- SEM) 24 hours after the last stimulation. The polysynaptic component remained potentiated for at least one week following the last day of trains (see Figure 4.3a).

Test pulses produced a surface positive response in the perirhinal cortex (see Figure 4.2b). Once again, the polysynaptic component was most strongly affected following potentiation. This peak had significantly ($p<0.002$) increased by 0.15 mV +/- 0.06 mV 24 hours after the last stimulation. While the polysynaptic component was still potentiated 48 hours after stimulation ceased, it had returned to baseline levels one week later (see Figure 4.3b).

The frontal response had a surface negative peak. Figure 4.3 shows a representative example of an evoked response before and after trains were delivered to the dorsal CA1. Due to some upward drift in the control measures, potentiation of the polysynaptic component and an early monosynaptic component was not clearly evident until after 9 stimulation sessions, but asymptotic levels were reached shortly thereafter (see Figure 4.3c and 4.3d). The monosynaptic component showed a significant ($p<0.003$) surface positive shift in amplitude following the delivery of trains. Amplitude had shifted 0.26 mV +/- 0.05 mV in the positive direction 24 hours after the last stimulation. The amplitude increase of the polysynaptic component was highly significant ($p<0.000001$). This increase amounted to an average increase of 0.64 mV +/- 0.13 mV 24 hours after the last stimulation session. Both the early polysynaptic and monosynaptic potentiation effects remained for at least one week after stimulation had ceased (see Figure 4.3c and 4.3d, respectively).

DISCUSSION

As in the previous experiment, the results presented here indicate that the efferents of the CA1 to the entorhinal, perirhinal, and frontal cortices are capable of supporting LTP. Due to a lack of significant change in the monosynaptic components of some of the evoked responses, however, the potentiation effect must be cautiously interpreted. Changes in the monosynaptic component would allow for some certainty in suggesting that there is an increased level of plasticity in a specific pathway or the site we are recording from. Polysynaptic changes are difficult to interpret because we cannot be certain *where* the LTP is induced. Without additional experiments, we cannot confirm whether the potentiation in polysynaptic components represents plasticity within the structures of interest, which are not significant because of non-optimal electrode placements, or changes in intervening sites. A few animals, however, showed clear modifications of the monosynaptic component. In any case, the findings reported are of interest because they indicate that a modifiable pathway exists somewhere between the two sites.

In this experiment there was a long-lasting potentiation in entorhinal cortex. In a previous experiment (Ivanco, et al., 1996, reported in Chapter 3), we found a relatively short-lasting LTP in the entorhinal cortex following stimulation of the perirhinal cortex. In this experiment a significant potentiation was induced, albeit asymptotic levels were reached slowly, within the entorhinal cortex. The present results indicate that the entorhinal cortex is capable of supporting a long-lasting form of LTP in at least some of its input pathways. More work is required to determine if the pathways between the perirhinal and entorhinal cortices are truly less plastic than the pathways from the CA1 to entorhinal cortex.

The perirhinal cortex exhibited LTP, but it was not as long lasting as in the other sites of interest. This result indicates that the perirhinal cortex may be less plastic than other structures or, as in the case of the entorhinal cortex, different pathways may show different degrees of plasticity. These alternative hypotheses can be tested by stimulating another input into the perirhinal cortex.

The frontal cortex showed a strong potentiation effect. It appeared to develop early, but an upward drift in the control measures precluded an accurate determination of the precise time course. The evoked responses look somewhat similar to those elicited with cortico-cortical stimulation (Racine, 1995; Ivanco, personal observation) and the change in morphology is similar following LTP induction. The peak amplitude of the evoked response, however, is smaller than that elicited by white matter stimulation. In addition, the cortico-cortico evoked potential shows a somewhat more complex morphology than does the frontal response elicited by the dorsal CA1 morphology. While the bipolar electrode tips appeared to be positioned deeply within the hippocampus, so as to avoid stimulating neocortical white matter, the possibility of volume conducted currents to overlying parietal cortex cannot be ruled out. The results could be confirmed in animals with aspiration lesions of the immediately overlying cortex.

LTP had previously been demonstrated in the prefrontal cortex in anaesthetized animals following high frequency stimulation of the CA1/subicular region (Laroche, et al., 1990; Jay, et al., 1995). Their responses, however, lacked the polysynaptic components seen in the chronic preparation. Also, the longevity of potentiation cannot be determined in anaesthetized preparations.

Experiment 2: LTP in the Frontal Cortex Efferent System

Although reciprocity in the connections between various brain regions appears to be the rule (see Fellman & Van Essen, 1991; Winer & Larue, 1987), there is little evidence for reciprocal pathways between the frontal cortex and the hippocampus or entorhinal cortex. Burwell (personal communication) has recently found that approximately 10% of the inputs to the entorhinal cortex originate in the frontal cortex, but the laminar origins of this projection remain to be elucidated. There is, on the other hand, evidence of reciprocal connections between the frontal cortex and perirhinal cortex (McIntyre, et al., 1996).

In this study we were interested in determining whether stimulation delivered to the frontal cortex would evoke responses in the hippocampus, entorhinal cortex and perirhinal cortex and if evoked responses in these areas could be potentiated by a high frequency train delivered to the frontal cortex. It was assumed that monosynaptic connections would be represented by evoked responses with relatively short latencies to onset. Frequency-of-following tests appeared to give some ambiguous results in a previous experiment and we chose not to look at that measure in these experiments. Other parameters of interest included the number of stimulation sessions required to reach asymptote and the longevity of the LTP effect.

METHODS

The general materials and methods for surgery, data acquisition, analysis and histology used in this experiment were similar to those used in **Experiment 1** described

above.

In all animals, bipolar electrodes were implanted into the frontal, perirhinal, and entorhinal cortices, and the dorsal hippocampus according to the atlas of Paxinos and Watson (1997). The coordinates were as follows: *frontal cortex (area M1)*: 2.0 mm anterior to Bregma, 4.0 mm lateral to the midline, and 1.8 mm ventral to brain surface; *perirhinal cortex*: 2.3 mm posterior to Bregma, 6.0 mm lateral to the midline, and 6.0 mm ventral to brain surface with an electrode angle of 15 degrees; *entorhinal cortex*: 7.0 mm posterior to Bregma, 5.2 mm lateral to the midline, and 8.0 mm ventral to brain surface; and *dorsal CA1-DG*: 3.5 mm posterior to Bregma, 2.2 mm lateral to the midline, and 2.8-3.3 mm ventral to brain surface. Depths of placements were adjusted to maximize amplitudes of evoked responses. During this surgery, evoked responses could not be obtained from the entorhinal cortex. Electrodes were lowered to the appropriate depth, with the assumption that responses may be seen following recovery from the anaesthetic. These placements never showed a reliable evoked response following recovery. The number of animals used in this experiment was 13 to obtain reliable measures in the perirhinal cortex (experimental n=5; control n=5) and hippocampus (experimental n=4; control n=3).

RESULTS

Distributions of electrode tip locations are shown in figure 4.4. Light microscope examination of brain sections provided verification that stimulating and recording electrodes were located within the appropriate target structures as defined by Paxinos and Watson (1997). For this study our definition of perirhinal cortex also included the area defined as

ectorhinal cortex by Paxinos and Watson (1997) as this area corresponds to the perirhinal area 36 as defined by Burwell, and colleagues (Burwell, et al., 1995; Burwell & Amaral, 1996). The hippocampal placements were distributed near or within the dentate gyrus or CA1. Both of these locations were accepted for *hippocampal* placements as the evoked responses from each site was similar and the changes seen at each site following trains was similar. These animals had been divided into both the experimental and control conditions.

LTP was induced in both the perirhinal cortex and the hippocampus following high frequency stimulation delivered to the frontal cortex. Although there were significant amplitude changes in both sites, the changes occurred at a latency beyond the peak of the monosynaptic component following high frequency stimulation, indicating polysynaptic influences on the evoked response. Test pulses delivered to the frontal cortex generated a surface positive potential in the perirhinal cortex (see Figure 4.5a). The experimental group showed a significant increase ($p<0.005$), relative to the controls. Again there was a surface negative shift in the early monosynaptic component in some of the animals, but it was not significant. The polysynaptic peak increased significantly ($p<0.0001$) with multisession stimulation. The perirhinal cortex showed an immediate potentiation effect, and asymptotic levels were reached after only one stimulation session. The peak had increased by 0.16 mV +/- 0.04 mV 24 hours after the last stimulation, and remained potentiated for at least one week following the last day of trains (see Figure 4.6a).

Test pulses delivered to the frontal cortex produced a surface negative potential in CA1 and the granule layer of the dentate gyrus. The responses at both sites showed a similar potentiation effect. The amplitude shift in the early monosynaptic component did not reach

significance in this site, but the polysynaptic component showed a significant ($p<0.01$) potentiation. The evoked response in the hippocampus appeared to develop after approximately 4 stimulation sessions, but an upward drift in the control measures precluded an accurate determination of time course. The polysynaptic component, however, showed an increase of 0.44 mV +/- 0.13 mV 24 hours after the last stimulation session, and it remained potentiated one week following the last stimulation session (see Figure 4.6b).

DISCUSSION

The short latency to onset of the initial components of each of the evoked responses in the perirhinal cortex and the hippocampus were consistent with them being monosynaptically generated. These connections, however, need to be more thoroughly investigated using anatomical tracers. While there appears to be a reciprocal connectivity between the hippocampus and the neocortex, there are few descriptions of the functional role of such connections. Tyler and DiScenna (1986) proposed that the role of the hippocampus was to form and retain an index of neocortical areas that were activated by experiential events. This, they called the *hippocampal memory indexing theory*. The authors stated that cortical activation by experiential events could induce LTP in the corresponding hippocampal loci. LTP in the projections from the frontal cortex to the hippocampus could function to provide the hippocampus with an enduring representation of the patterns of frontal cortex activation. The results presented here are the first demonstration of LTP in these projections. The memory indexing theory proposes that LTP in the hippocampal-neocortical connection would not result from brief episodes of activation, but would require

activation over a more prolonged period (Tyeler & DiScenna, 1986). This LTP would be long lasting and reflect a capability for long term storage. The results here are consistent with the memory indexing theory, as LTP in the hippocampus did not occur after a single stimulation session, but required multiple stimulation sessions delivered over days, and the LTP was long lasting.

One of the more interesting findings in this investigation was the different rates of development of the potentiation effect. In Experiment 1 (stimulation delivered to the CA1), we found that the perirhinal cortex potentiation was not as long lasting as it was at the two other sites. Here (stimulation delivered to the frontal cortex) we found that the perirhinal cortex showed a very fast development of an LTP effect that was long lasting. The hippocampus, on the other hand, typically potentiates with a single stimulation delivered to the perforant pathway, but required multiple sessions before potentiation was evident. Examining the animals based on the location of electrode tips within the hippocampus does not provide additional information about differential plasticity rates in the hippocampus as both the dorsal CA1 and the dentate gyrus appear to show similar time courses of LTP induction.

A possible explanation for the fast potentiation in the perirhinal cortex is that the cortico-cortical synapses between the perirhinal and frontal cortices are much more plastic than the neocortical-subcortical synapses between the frontal cortex and the hippocampus. The potentiation effect in the perirhinal cortex following a single stimulation session, however, is much faster than the potentiation rates in other cortico-cortical connections that have been investigated. Other cortico-cortical investigations indicate that approximately 5

sessions are required before potentiation becomes evident and approximately 10 stimulation sessions are necessary to reach asymptotic levels (i.e. Froc & Racine, 1995). These different results indicate that there may be differential rates of plasticity in cortico-cortical synapses that are worthy of more investigation.

Another interesting finding in this experiment was the lack of a field potential induced in the entorhinal cortex by frontal cortex stimulation, indicating that most of the flow of information between frontal cortex and hippocampus is via the perirhinal cortex. In order to test the hypothesis that there is a long unidirectional loop between the cortex and the hippocampus, tracers could be used to map the pathways between the two areas. Tracers could be used to examine more thoroughly the relationship between the entorhinal and frontal cortices.

GENERAL DISCUSSION

It is likely that there are multiple routes for information flow between the hippocampus and the neocortex. These routes include the entorhinal and perirhinal cortices. Some of the evidence described above indicates that at least one pathway system, involving the perirhinal cortex, may be used for two-way communication between the hippocampus and the frontal cortex. Relationships between the perirhinal cortex and the frontal cortex and hippocampus could be further studied by using anatomical tracers to confirm pathways between these areas, and by the use of various recording and analysis techniques such as unit recording and coherence analysis to determine patterns of neuronal interaction. It might also be useful to investigate frontal/hippocampal interactions in animals with perirhinal cortex

lesions.

Hippocampal and neocortical LTP show parallels with proposed differences in processing and storage in those sites. The hippocampus is thought to function as a fast learning system that is transient and of a limited capacity (for review see Squire, 1992). The neocortex learns more slowly, but has a greater, more enduring capacity to store information. Further, it is proposed that the long term storage results from a direct influence of the hippocampus on the neocortex (i.e. via reinstatements of representations as described by McClelland, et al., 1995). The hippocampus shows a rapid induction of LTP and a rapid rise to asymptotic levels of potentiation. The cortico-cortical induction of neocortical LTP, on the other hand, requires multiple sessions and asymptotic potentiation takes many days to occur. While the results of Experiment 1 are consistent with these scheme, the results of Experiment 2 would appear not to be.

In Experiment 2 we report fast cortico-cortical processing and slow hippocampal processing. A potential explanation for the rapid induction of LTP in the perirhinal cortex following frontal cortex stimulation includes the possibility that pathways between frontal cortex and association areas, such as the perirhinal cortex, are more plastic than cortico-cortical pathways that link primary cortical areas. In Chapter 3 we (also Ivanco, et al., 1996) found that the frontal cortex potentiated with fewer stimulation sessions when the trains were delivered to the perirhinal cortex than was required when the callosum was stimulated (Racine, et al., 1995). As the hippocampus is easily potentiated by stimulation delivered to the perforant path or to the entorhinal cortex, it seems likely that the slower induction of LTP within the hippocampus reported above is a result of a reduced plasticity in the pathways

between the hippocampus and frontal cortex. The results reported in Chapter 3 (also Ivanco, et al., 1995) are also consistent with this hypothesis.

Taken together, the findings from Experiment 1 and Experiment 2 are consistent with the proposal that the hippocampus and neocortex affect each other directly, but the results indicate that this influence relies on neural signals that may only travel unidirectionally. The results also suggest that the level of plasticity is dependent upon the direction of the pathway within the loop.

FIGURES and CAPTIONS

Chapter 4

Figure 4.1. The locations of stimulating electrodes in the dorsal CA1 (CA1) and recording electrodes in the entorhinal cortex (EC), perirhinal cortex (PRh), and primary motor/frontal cortex (M1) are shown on representative sections from the rat brain atlas of Paxinos and Watson (1997). Note that our definition of perirhinal cortex includes ectorhinal cortex as this area corresponds to the perirhinal area 36 (Burwell, et al., 1995; Burwell & Amaral, 1996). Locations for the lowest pole of the bipolar electrode for all experimental animals are represented with dots and for all control animals with squares. One animal has the lowermost pole in the dentate gyrus area, but the upper pole was located within area CA1 and stimulation would have been delivered appropriately to that site. The stimulation site is demarked by an asterisk.

Figure 4.1

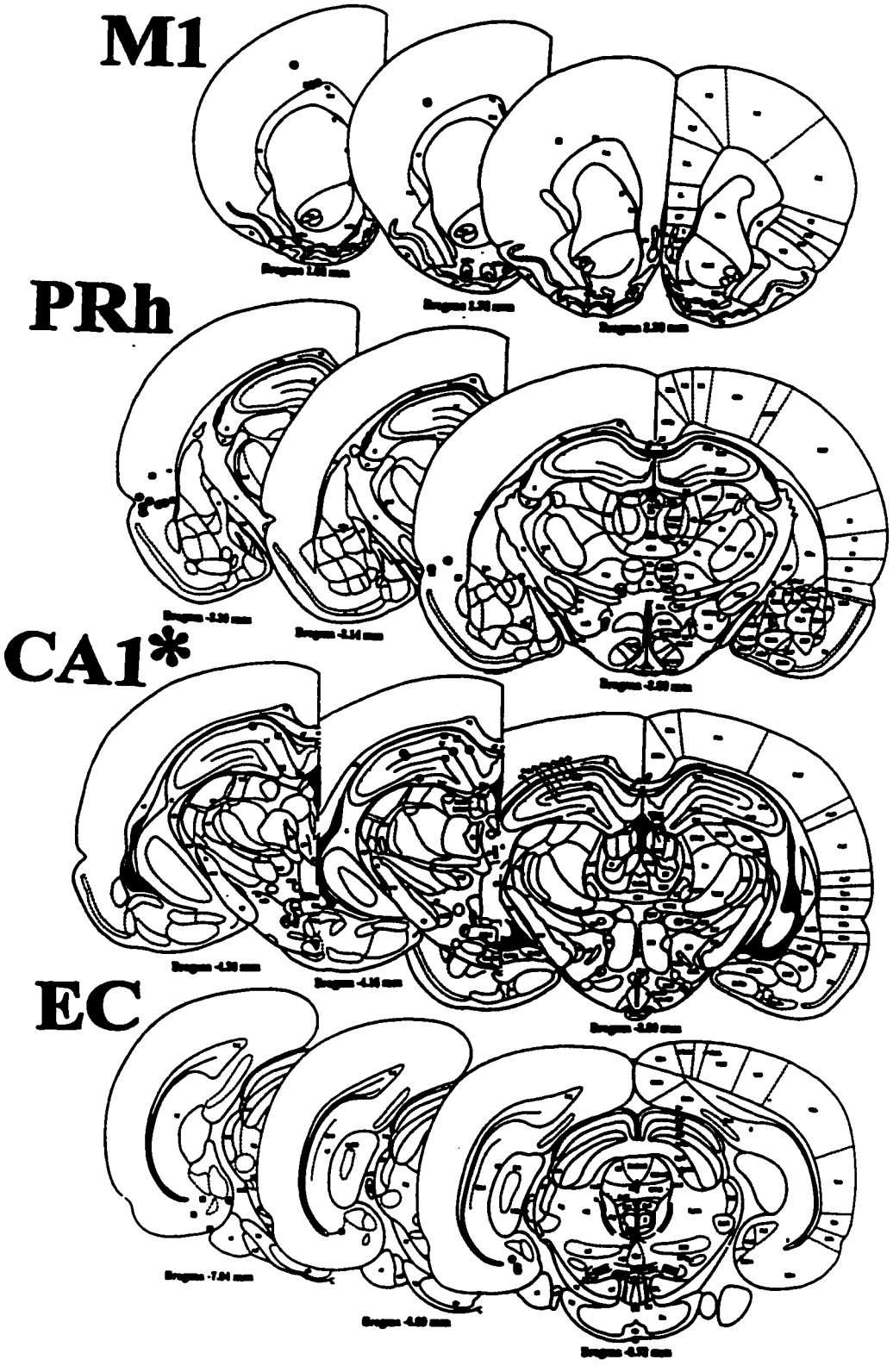


Figure 4.2. Field potentials evoked by dorsal CA1 stimulation in: A) the entorhinal cortex (EC), B) the perirhinal cortex (PRh), and C) the primary motor/frontal cortex (M1). Note that the solid lines represent the baseline recorded from each site, while the dashed line represents the evoked response recorded 24 hours after the delivery of trains. The open arrow indicates the early portion of the monosynaptic component that shows a reversal in amplitude with stimulation. The thin arrow indicates the monosynaptic peak latency for the field potentials. The thick arrow indicates the polysynaptic peak latency for the field potentials. Horizontal calibration, 10 msec; vertical calibration, 0.5 mV.

Figure 4.2

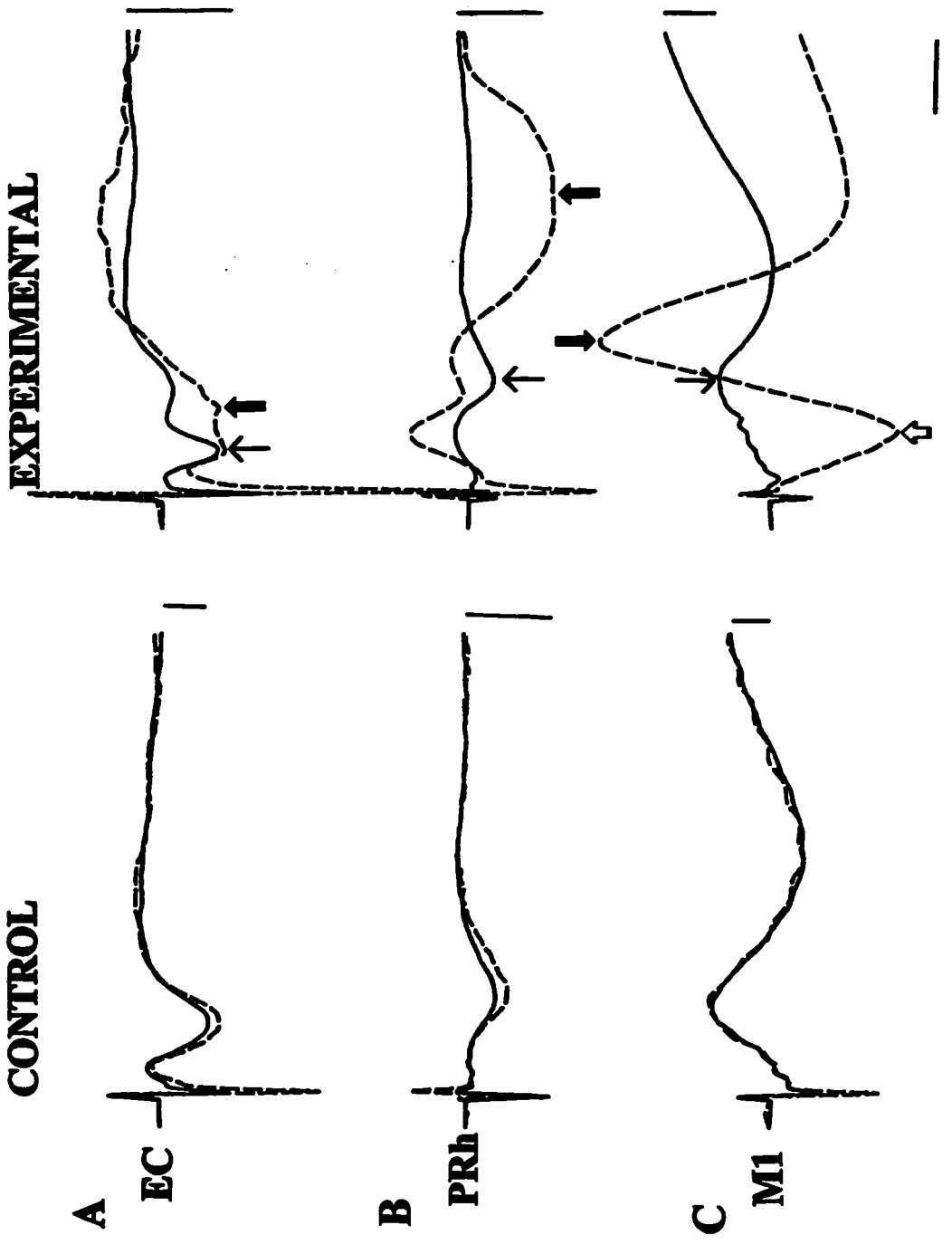
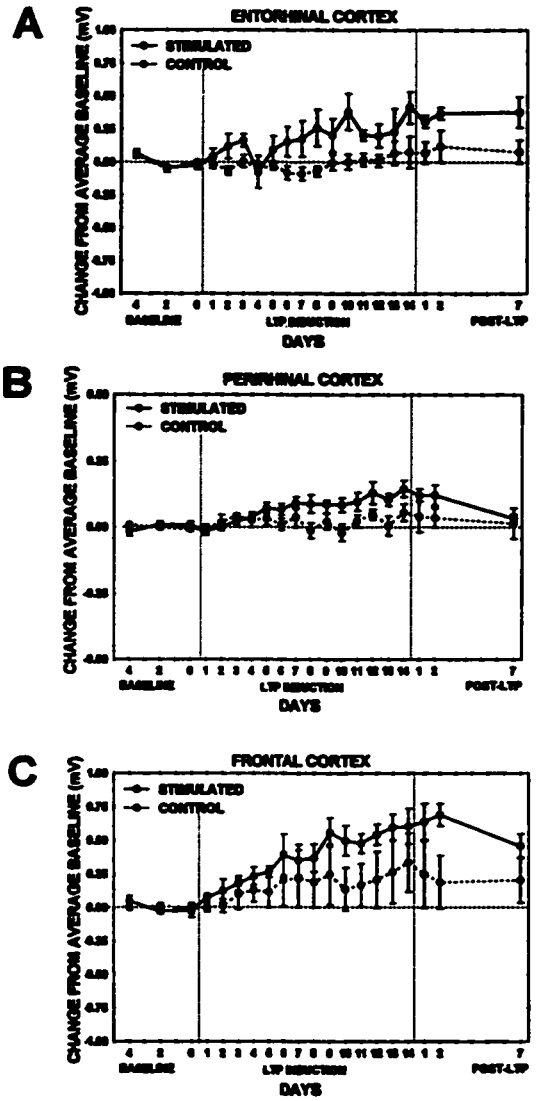


Figure 4.3. Evoked response amplitudes as a function of days following stimulation to the dorsal CA1. Amplitude changes from the average baseline are represented along the ordinate and days along the abscissa. The abscissa shows the pre-induction period (baseline), the induction period, and the follow-up period (post-LTP). Note that the ordinate is labelled differently in B) than in A), C), and D). A) The amplitude of the polysynaptic component in entorhinal is shown to increase slowly over days (group x day interaction, $p<0.02$). The amplitude of the polysynaptic component was also found to increase slowly over days in the perirhinal cortex (B) and in the frontal cortex (C) (group x day interaction, $p<0.002$ and $p<0.000001$, respectively). The amplitude of the early monosynaptic component in the frontal site was found to show a reversal (group x day interaction, $p<0.003$) in amplitude with mulisession stimulation. This reversal is shown in D).

Figure 4.3

POLYSYNAPTIC COMPONENT



MONOSYNAPTIC COMPONENT

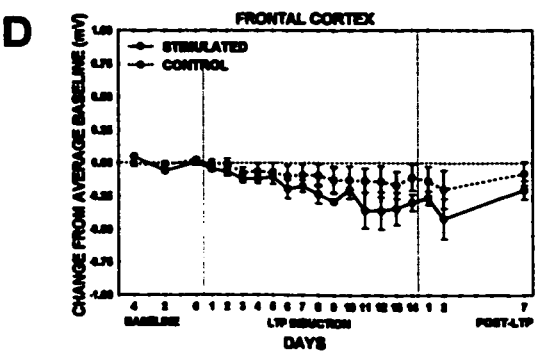


Figure 4.4. The locations of stimulating electrodes in the primary motor/frontal cortex (M1) and recording electrodes in the dorsal CA1 or dentate gyrus (hpc) and in perirhinal cortex (PRh) are shown on representative sections from the rat brain atlas of Paxinos and Watson (1997). Note that our definition of perirhinal cortex includes ectorhinal cortex as this area corresponds to the perirhinal area 36 (Burwell, et al., 1995; Burwell & Amaral, 1996). Locations for the lowest pole of the bipolar electrode for all experimental animals are represented with dots and for all control animals with squares. The stimulation site is demarked by an asterisk.

Figure 4.4

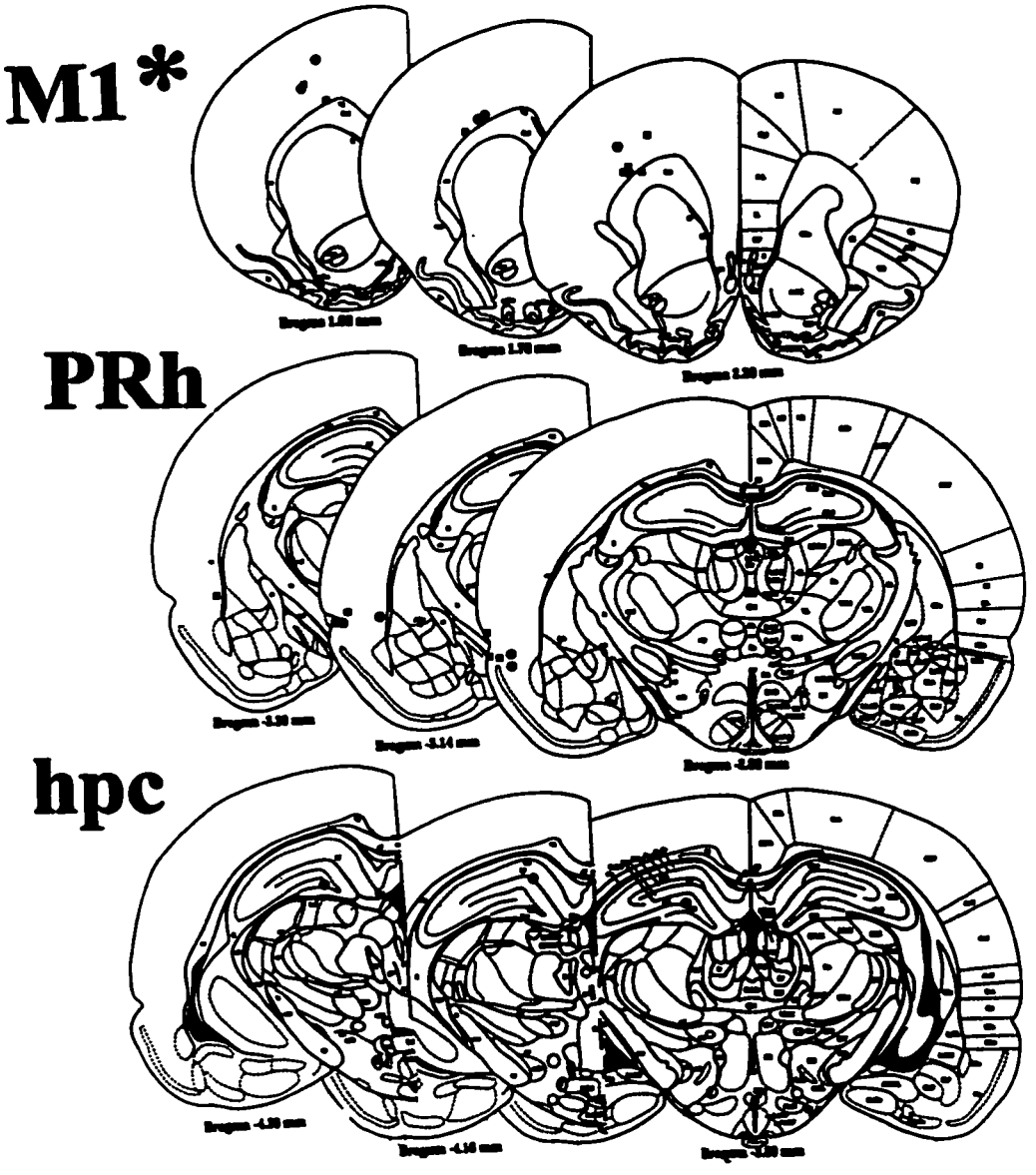


Figure 4.5. Field potentials evoked by primary motor/frontal cortex stimulation in: A) the perirhinal cortex (PRh), and B) the hippocampus (hpc). Note that the solid lines represent the baseline recorded from each site, while the dashed line represents the evoked response recorded 24 hours after the delivery of trains. A thin arrow indicates the monosynaptic peak latency for the field potentials. A thick arrow indicates the polysynaptic peak latency for the field potentials. Horizontal calibration, 10 msec; vertical calibration, 0.5 mV.

Figure 4.5

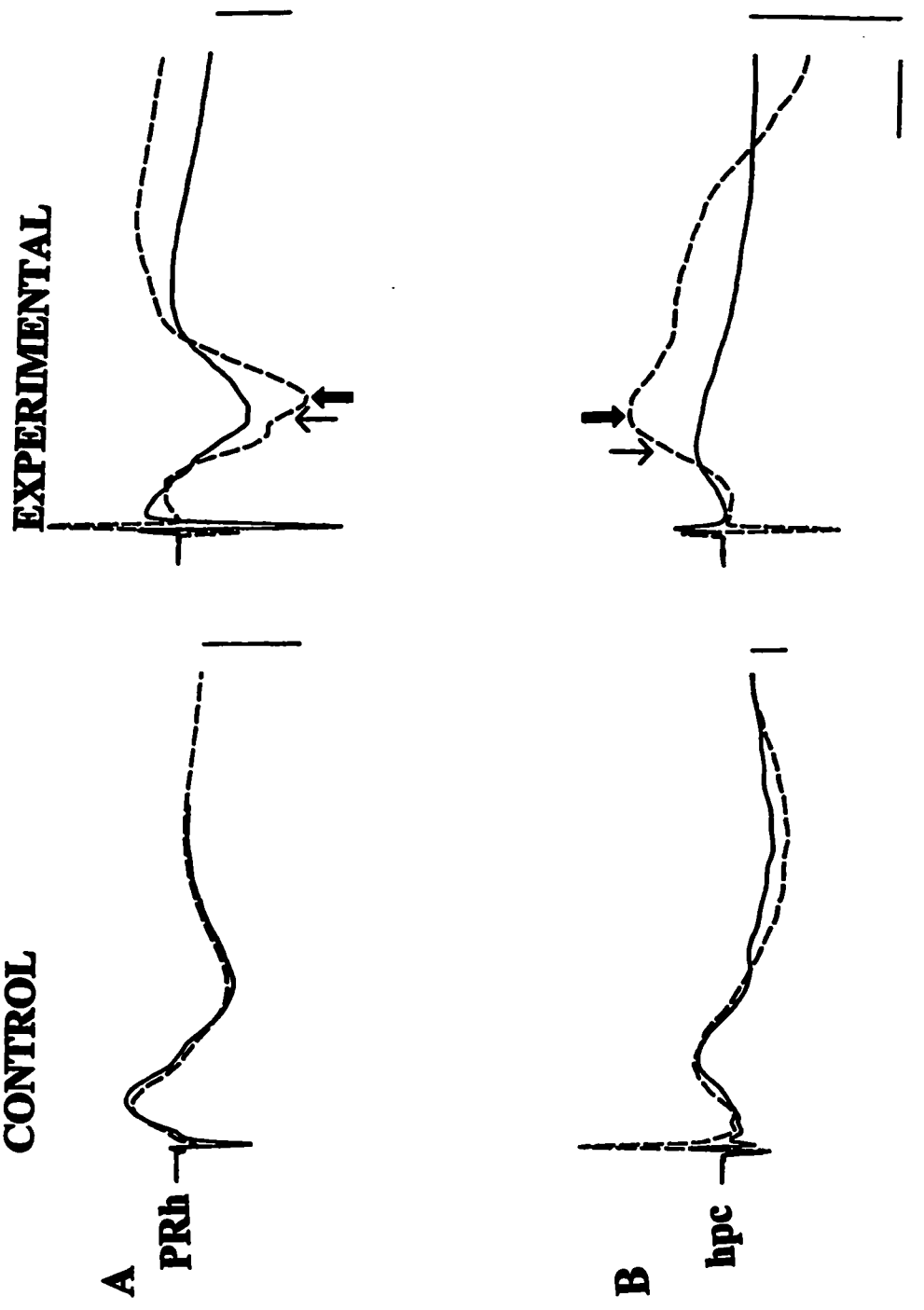
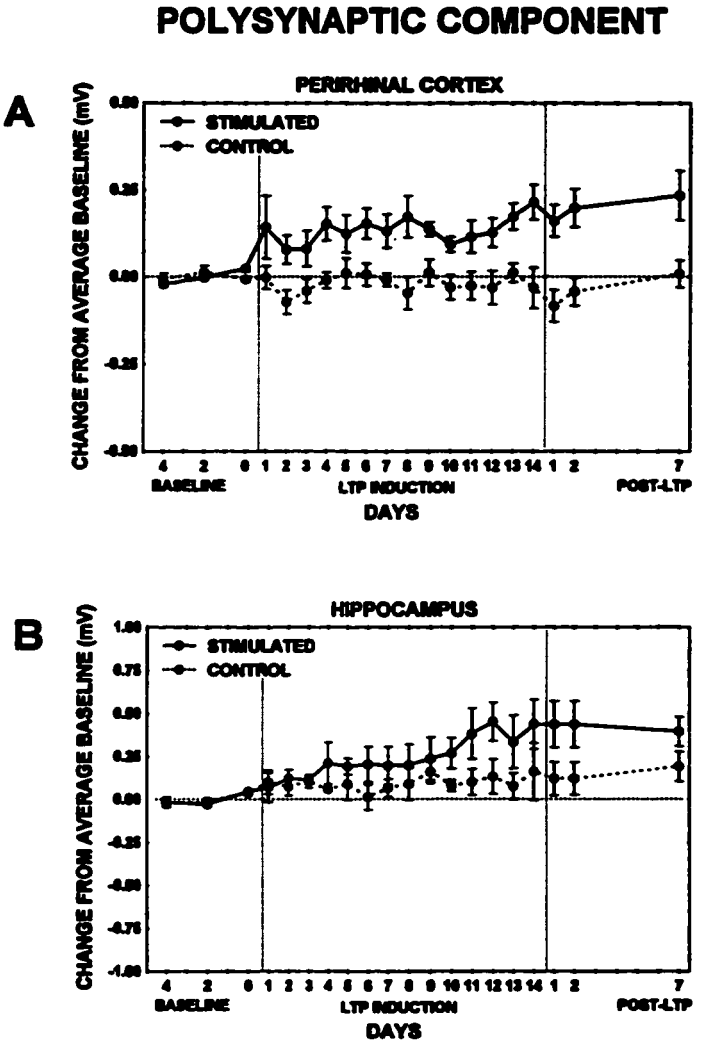


Figure 4.6. Evoked response amplitudes as a function of days following frontal cortex stimulation. Amplitude changes from the average baseline are represented along the ordinate and days along the abscissa. The abscissa shows the pre-induction period (baseline), the induction period, and the follow-up period (post-LTP). Note that the ordinate is labelled differently in A) than in B). A) The amplitude of the polysynaptic component in the perirhinal cortex is shown to increase over days (group x day interaction, $p<0.0001$). B) The amplitude of the polysynaptic component was also found to increase over days in the hippocampus (group x day interaction, $p<0.01$).

Figure 4.6


POLYSYNAPTIC COMPONENT
A
PERIRHINAL CORTEX
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
BASELINE
LTP INDUCTION
POST-LTP
DAYS
B
HIPPOCAMPUS
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
BASELINE
LTP INDUCTION
POST-LTP
DAYS

Chapter 5

Long-term Potentiation in Thalamocortical Projections

Much of the current work on neocortical LTP has been driven by our knowledge of the visual system and visual cortex plasticity. The finding that LTP can be induced in cortico-cortical connections in the developing visual cortex has been confirmed numerous times (i.e. Tsumoto & Suda, 1979, *in vivo*; Kirkwood, Lee & Bear, 1995, *in slice*). LTP can also be induced in the visual cortex slice of an adult animal, but the preparation requires that intracortical inhibition mediated by $GABA_A$ receptors be removed or blocked (Artola & Singer, 1987, 1990) or that an alternate stimulation site be used (Kirkwood & Bear, 1994). Although there is evidence from Crair and Malenka (1995) of LTP in a *geniculo-cortical* pathway in the developing animal, there does not appear to be any evidence for thalamocortical LTP in the adult (see Tyler & DiScenna, 1986).

Several studies by Racine and colleagues (Ivanco, et al., 1996; Racine, et al., 1995; Wilson & Racine, 1983) have reported LTP in cortical areas other than the visual cortex in the adult animal. As described previously, some of these results are in the chronically implanted, behaving animal. In these investigations, LTP induction required the delivery of stimulation over days. The results indicate that the capacity of cortical synapses to undergo LTP is not lost in the adult, but that there are different requirements for the patterning of stimulation for cortical, compared to hippocampal, systems. Such parameters may also be

successful for inducing LTP in thalamocortical inputs to sensory cortex.

Two of the thalamocortical systems chosen to investigate this possibility were sensory and the third was an *association* system thought to be involved in memory. The visual sytem and auditory system were chosen to represent sensory systems, and the mediodorsal nucleus and frontal cortex were chosen to represent associational systems. Also, the latter system has been shown to play a role in memory (i.e. Korsakoffs patients generally have pronounced damage to this area). Additionally, the frontal cortex has previously been shown to sustain LTP following callosal stimulation (Racine, et al., 1995).

GENERAL METHODS

All experiments were performed on male Long-Evans rats from the McMaster Breeding Colony weighing between 300 and 500 grams at the time of surgery. All rats were housed in pairs in plastic cages prior to surgery and individually in hanging wire cages following surgery. Food and water were provided *ad lib*. The colony was maintained on a 12 hr on/12 hr off light/dark cycle.

Bipolar electrodes were constructed by twisting Teflon coated stainless steel wire, 200 μm in diameter, insulated except for the tips. Cortical electrodes had a tip separation of approximately 1.0 mm and subcortical electrodes had a tip separation of approximately 500 μm. Animals were anaesthetized with sodium pentobarbitol (65 mg/kg) and stereotaxically implanted with electrodes under electrophysiological control. Electrodes were connected to gold-plated male amphenol pins that were inserted into a 9-pin connector plug. Four stainless steel jewellers screws were used to anchor and mount the plug to the

skull with dental acrylic. One of the screws had a wire attached with a gold-plated pin that served as a ground electrode. All animals were given at least two weeks of recovery time prior to testing.

Stimulation and Recording. Evoked responses were acquired using ASYST software on a Comptec 486, 33 MHz computer. Electrical stimuli were generated with a Grass S88 stimulator and photoelectric stimulation isolation units (Grass SIU6B). Signals were fed into a Grass Model 12 EEG amplifier and filtered at half amplitude at 0.3 Hz (high pass) and 3 kHz (low pass). The signal was sampled at 10 kHz by a 12 bit A/D converter (Data Translation DT 2821) and stored on the computer hard drive. All analysis was completed off line.

Baseline evoked field potentials were collected three times, once every 48 hours. To determine I/O functions, biphasic test pulses of increasing intensity were delivered to the stimulation electrode at a frequency of 0.1 Hz. Responses were recorded from the cortical sites. Ten responses, 50 ms in duration were evoked, amplified, digitized and averaged at each of 12 logarithmically spaced intensities (32, 40, 63, 100, 126, 159, 251, 398, 501, 794, 1000, and 1259 μA). Following the final baseline measure, animals were matched based on evoked response morphology and separated into control and experimental groups.

To attempt to induce potentiation in the experimental group, high frequency, 50 ms, trains were delivered to the stimulating electrode. Pulse intensity was set at a level that produced an 80% maximum amplitude response in at least one of the sites being recorded from and pulse frequency was set at 300 Hz. Thirty trains were delivered per session at 0.1 Hz. Trains were delivered daily, immediately following an I/O test, for 20 days. Follow-up

I/O tests were conducted 24 hrs and 48 hrs after the last train in experimental animals and after comparable delays in control animals.

Analysis. Potentiation was assessed by comparing the peak amplitudes and rising phase slopes of the initial baseline evoked responses with those of responses evoked after trains had been delivered. An average baseline was calculated by determining the average of the first three I/O tests. All other I/Os were compared against this average to determine millivolt changes in peak amplitude and slope changes over days. Repeated measures ANOVAs were used to test for significant effects of stimulation.

After completion of the electrophysiological experiments, the animals were perfused through the heart with phosphate buffered saline (PBS), followed by 2% paraformaldehyde in PBS in preparation for Methylene Blue-Azure II (MBAII) staining, or with 0.9% saline, followed by a formal-saline solution (4% formalin in 0.9% saline) in preparation for Cresyl Violet staining. Brains were removed and placed in the fixative for at least 24 hours. Brains were then immersed in a 10% sucrose solution and placed in a 4 degree Celcius refrigerator for 24 hours. Each of the brains was sectioned in the coronal plane at 40 μm on a cryostat, mounted on gelatin coated slides, and stained. Slides were examined with a light microscope to confirm stimulating and recording placements.

Experiment 1. Medial Geniculate to Auditory Cortex

To investigate the projection from the medial geniculate to auditory cortex, the MGm was choosen as the stimulation site. This portion of the medial geniculate has been shown to exhibit plasticity in both unit and field potential responses in adult animals (see Edeline

& Weinberger, 1992; Gerren & Weinberger, 1983). The frontal cortex was also implanted with a recording electrode to determine whether this area, which demonstrates cortico-cortical and hippocampo-cortical LTP (see Racine, et al., 1995 and Chapter 4, respectively), would also potentiate following stimulation to the MGm.

Electrode Placement. Animals were implanted with stimulating electrodes in the medial portion of the medial geniculate (MGm) and recording electrodes in primary auditory cortex and frontal cortex (as per Paxinos & Watson, 1997). Electrode placements were as follows: *MGm:* 5.8 mm posterior to bregma, 3.4 mm lateral to the midline, and 5.2 mm ventral from brain surface; *auditory cortex:* 4.8 mm posterior to bregma, 6.5 lateral to the midline with an electrode angle of 10 degrees, and 4.8 mm ventral from bregma; and *frontal cortex (area M1):* 2.0 mm anterior to bregma, 4.0 mm lateral to the midline, and 1.8 mm ventral from brain surface. Depths were adjusted to maximize evoked responses. In total, 13 animals were used to obtain reliable measures from the auditory cortex placements (experimental n=6; control n=7). From these 13 animals, 7 showed responses at the frontal cortex site, as well (experimental n=3; control n=4).

RESULTS

Light microscope analysis of brain sections provided verification of electrode placements in target structures as defined by Paxinos and Watson (1997). Figure 5.1 shows the locations of electrode tips. As the mediodorsal nucleus is small, electrode tips that touched the border of the nucleus and bipolar electrode tips that straddled the nucleus were accepted as being placed appropriately. Paxinos and Watson (1997) had delimited primary

auditory cortex and also labelled dorsal and ventral primary auditory cortex. We defined auditory cortex as including all three of these areas and have removed the lines delimiting the dorsal and ventral areas from our figures.

Test pulses delivered to the MGm generated a surface negative potential in auditory cortex (see Figure 5.2a). High frequency stimulation delivered to the MGm did not result in a potentiation of the evoked response, but did produce a small, significant ($p=0.01$) depression in the evoked response amplitude. The amplitude of the evoked response had decreased 0.26 mV +/- 0.14 mV 24 hours after the last set of trains (see Figure 5.3a). This decrease did not appear immediately and did not reach asymptotic levels until after 9 stimulation sessions.

A surface negative potential was recorded at the frontal cortex site. High frequency stimulation delivered to the MGm did not produce any significant change in this evoked potential ($p=0.3$). Representative examples of a responses evoked in the frontal cortex before and after trains is shown in Figure 5.2b. The change in response amplitude over days is shown in Figure 5.3b.

In the experimental animals, trains produced an interesting behavioural response. Each time a train was delivered, the animals exhibited a behaviour that resembled a startle reflex. They gave a slight jump and froze. With repeated trains the behaviour appeared to grow a little stronger.

DISCUSSION

The results presented here indicate that the pathways between the MGm and auditory

cortex support a long-term depression (LTD) effect, but do not appear to be capable of supporting LTP. This LTD effect was surprising. In a pilot experiment, this pathway did not show any electrophysiological change. In addition, stimulation delivered to the pathways between the two other subdivisions (ventral and dorsal) of the medial geniculate and auditory cortex did not appear to be capable of supporting either LTP or LTD.

These results suggest that the auditory cortex and/or the thalamocortical projection to the auditory cortex does not readily support LTP. As the auditory cortex itself has already been shown to be plastic, it seems likely that the lack of LTP seen in this investigation is due to a reduced level of plasticity in *sensory* thalamocortical projections. This hypothesis can be tested by examining thalamocortical projections to other sensory cortical areas.

The behavioural response that was evoked by MGm stimulation was similar to a startle response. The MGm does have some connections with the amygdala (Edeline & Weinberger, 1992). Stimulating the MGm might be expected to induce an amygdalar response similar to that evoked by a loud noise, which also triggers a startle reflex. When noises are repeatedly presented at consistent intervals, the startle reflex tends to decrease and may disappear altogether. The response to electrical stimulation, on the other hand, actually appeared to grow in magnitude. This increase in the strength of the behavioural response raises the possibility of enhanced transmission in the pathways between the MGm and the amygdala.

Experiment 2. Lateral Geniculate to Visual Cortex.

To extend our analysis of sensory thalamocortical pathways, we tested the projections

leading from the lateral geniculate nucleus to the visual cortex. The auditory and visual geniculocortical pathways show many similarities and might be expected to be similarly 'plastic'.

Electrode Placements. Animals were implanted with stimulating electrodes in the dorsal portions of the lateral geniculate (LGN) and recording electrodes in primary (binocular) visual cortex and frontal cortex (Paxinos & Watson, 1997). Electrode placements were as follows: *LGN:* 3.3 mm posterior to bregma, 2.5 mm lateral to the midline, and 5.0 mm ventral from brain surface; *visual cortex (area V1B):* 5.8 mm posterior to bregma, 4.4 lateral to the midline, and 1.8 mm ventral from brain surface; and *frontal cortex (area M1)* 2.0 mm anterior to bregma, 4.0 mm lateral to the midline, and 1.8 mm ventral from brain surface. Depths were adjusted to maximize evoked reponses. In total, 11 animals were used to obtain reliable measures from the visual cortex placements (experimental n=6; control n=5). From these 11 animals, 10 showed responses at the frontal cortex site, as well (experimental n=5; control n=5).

RESULTS

Examination with a light microscope indicated that electrodes were implanted in target structures as defined by Paxinos and Watson (1997). The distribution of electrode tips is shown in Figure 5.4.

The responses evoked in visual cortex by test pulses delivered to the LGN were surface negative (see Figure 5.5a). High frequency stimulation did not produce any change in the amplitude of the evoked responses recorded from visual cortex (p=0.8) or frontal

cortex (p=0.7). The millivolt changes over days are shown in Figure 5.5.

DISCUSSION

The results presented here suggest that the visual geniculocortical pathway, like the auditory geniculoccortical pathway, does not support LTP with the parameters that were applied. These results suggest that either the thalamocortical pathways or their sensory cortical targets are more resistant to activity dependent change. The visual cortex, however, has been shown to exhibit LTP in the chronically implanted animal following multiple sessions of stimulation. Froc and Racine (1995) found that stimulation delivered to the visual cortex generated a potentiation of the evoked response recorded in the contralateral visual cortex of chronically implanted animals. The auditory cortex also demonstrates plasticity in the form of LTP (i.e. Edeline & Wineberger, 1992), so it seems likely that the lack of plasticity is specific to the thalamocortical synapses. We cannot say, however, whether this lack of plasticity is general to all thalamocortical pathways or only those that project to sensory cortices. Consequently, we selected a non-sensory thalamocortical system for the next experiment.

Experiment 3. Mediodorsal Nucleus to Frontal Cortex

The selection of the mediodorsal nucleus/frontal cortex system was based partly on evidence that this system is involved in memory and on our findings that the frontal cortico-cortical connections are highly plastic. Lesions to either the mediodorsal nucleus or frontal cortex, for example, can produce a decrease in performance on delayed response tasks, as

described in Chapter 2. Korsakoffs' syndrome is characterized by severe memory deficits and by lesions of the mediodorsal nucleus, adjacent thalamic areas, the mammilary bodies, and frontal cortex (Jones, 1985; Kolb & Whishaw, 1990). There are also connections between the mediodorsal nuclei and the hippocampal formation (Jones, 1985), tying the mediodorsal/frontal cortex system to the medial temporal lobe memory system. Connectivity between the frontal cortex and the mediodorsal thalamus has been confirmed in the rat using the axon degeneration method (Jones, 1985).

Electrode Placements. A stimulating electrode was implanted in the mediodorsal nucleus of the thalamus and recording electrodes were implanted in both the lateral frontal cortex and dentate gyrus. Placements were as follows: *mediodorsal nucleus:* 2.4 mm posterior to bregma, 0.8 mm lateral to the midline, and 5.8 mm ventral to brain surface; *frontal cortex (area M1):* 2.0 mm anterior to bregma, 4.0 mm lateral to the midline, and 1.8 mm ventral to brain surface; and *dentate gyrus:* 3.5 mm posterior to bregma, 2.2. mm lateral to the midline, and 3.3 mm ventral to brain surface. In total, 12 animals were used to obtain responses in the frontal cortex and the dentate gyrus (experimental n=6, control n=4).

RESULTS

The distribution of electrode tips is shown in Figure 5.7. All tips were found in target structures as defined by Paxinos and Watson (1997). The mediodorsal nucleus includes many smaller subdivisions. The electrode was considered to be within the appropriate target if it was within any of the subdivisions of the mediodorsal nucleus.

A surface negative potential was recorded in the frontal cortex (see Figure 5.8a).

Following trains, the amplitude of the evoked response remained unchanged. A surface positive potential was recorded in the dentate gyrus (see Figure 5.8b). Following trains, the amplitude of the dentate response showed a significant decrease ($p=0.01$).This depression was not seen until after approximately 6 stimulation sessions (see Figure 5.9b).

DISCUSSION

The results presented here suggest that cortico-cortical inputs are more plastic than the thalamocortical inputs to the same area of frontal cortex. It should be kept in mind, however, that the white matter stimulation of Racine, et al. (1995) presumably activated some thalamocortical fibers, as well as many cortico-cortical projections. It is possible that *combined* stimulation of thalamocortical projections together with cortico-cortical projections might potentiate the thalamocortical inputs. This can be easily tested.

This experiment has provided the first demonstration of an LTD effect in the hippocampus as a result of stimulation delivered to the thalamus. It would be useful to determine if this effect is specific to the activated pathway, and if it could be shifted to a net potentiation effect by pairing with a stronger input.

GENERAL DISCUSSION

The ability of the thalamocortical pathways to exhibit LTP was unresolved (Teyler & DiScenna, 1986) when these studies were conducted. The question is still unresolved, but we did not find potentiation to be inducible in any of the three pathways examined, even though all the cortical target sites have been shown to support LTP induction when other

pathways have been examined. These results indicate that thalamocortical synapses may not support LTP. Possible explanations for this lack of plasticity deserve further exploration.

LTP in sensory neocortex has been primarily investigated in the kitten visual cortex slice, but the rat visual cortex slice has also been used. A number of groups have noted age differences in LTP induction (Artola & Singer, 1987; Kirkwood, et al., 1995; Komatsu, Toyama, Maeda, & Sakaguchi, 1981; Komatsu, Fujii, Maeda, Sakaguchi, & Toyama, 1988). In all cases, LTP seems to be greatest in layer II/III, but it is rarely seen in the adult slice unless inhibition is reduced. Since most inputs into layer II/III come from other cortical sites, it may be the case that cortico-cortical synapses are simply more plastic than geniculo-cortical synapses, which terminate primarily in layer IV (Komatsu, et al., 1981, 1988; Tsumoto, 1992). Frontal cortex, however, has little or no layer IV and there is still no evidence for thalamocortical LTP in this system. It is also possible that the geniculo-cortical inputs, whatever their targets, are plastic only during the critical period.

The differences in the capacity for LTP induction between thalamocortical and cortico-cortical pathways may be related to the activity of the excitatory N-methyl-D-aspartate (NMDA) receptor. The need for bicuculline, a $GABA_A$ receptor antagonist, in slice preparations is consistent with an NMDA-dependent mechanism for LTP (Tsumoto, 1992). NMDA receptors are regulated by inhibition mediated by $GABA_A$ receptors. Artola and Singer (1987, 1990) reported that in the visual cortex slice, LTP was blocked by the administration of NMDA receptor antagonists AP5 (2-amino-5-phosphonovaleric acid). Racine and coworkers have also noted that LTP in their chronically implanted preparation is blocked with CPP, a competitive NMDA receptor antagonist, (Trepel & Racine, in

preparation) and ketamine hydrochloride, a 'blocker' of the NMDA receptor mediated calcium channel (Ivanco, personal observation). As activation of NMDA receptors results in the influx of calcium into neurons, it seems likely that LTP in neocortical areas is due to an activity-dependent increase in calcium.

It is noteworthy that thalamocortical synapses activate primarily *non*-NMDA receptors in the adult, while cortico-cortical synapses activate both NMDA and non-NMDA receptors (Tsumoto, 1990). The relative contributions from NMDA and non-NMDA receptors may mediate the levels of functional plasticity of the two different pathways. The proportion of thalamocortical synapses that are NMDA receptor dependent decreases with age in somatosensory systems, in parallel with decreases in the ability to induce LTP in these same synapses (Crair & Malenka, 1995).

Another contributing factor to the difficulty in inducing LTP in adult animals may be an increase in inhibition directed at thalamocortical inputs, which does not necessarily influence other cortical inputs (Gil & Amitai, 1996a). Agmon and O'Dowd (1992) demonstrated that maturation of inhibition in the cortex occured coincidently with the disappearance of late NMDA receptor-dependent thalamocortical excitatory post-synaptic currents. Gil and Amitai, (1996b) found that the thalamocortical pathways had a lower threshold for the induction of feedforward inhibition than did intracortical inputs. Due to the relatively small number of thalamic cells, they diverge extensively to provide input to a large number of cortical cells, imposing a widespread synchronized activation. Strongly coupled inhibition and excitation could prevent excessive excitation, but bring about a high temporal precision mediated by the short latency responses and low thresholds of thalamocortical cells

(Gil & Amitai, 1996b). Such a coupling could also reduce the number of NMDA receptors participating at thalamocortical synapses.

The coupling of inhibition and excitation may also mediate the pruning of NMDA receptors during development. Two different groups (Fox, Daw, Sato, & Czepita, 1991; Cynader & Mitchell, 1980) have suggested that the loss of plasticity in visual cortex may be accompanied by an activity-dependent pruning of NMDA receptors in different laminae. Using a selective antibody directed against the R1 subunit of the NMDA receptor, it was found that the number of immunoreactive synapses in the middle laminae peaks at an age when the geniculocortical axon arborization into layers III and IV of the visual cortex is near completion (Aoki, Venkatesan, Go, Mong, & Dawson, 1994). It is not clear if the density of immunoreactive synapses decreases with age, but there seems to be a relationship indicating that immunolabelling in the superficial laminae increases with increasing age until adulthood and that these superficial laminae have more immunoreactive synapses than layers III and IV. Aoki, et al. (1994) suggest that the receptors that appear later may mediate a life-long plasticity rather than a developmental plasticity.

We have attempted to induce LTP in primary visual cortex in chronically implanted, behaving young animals (P20) to test whether they would show more plasticity in thalamocortical pathways than older animals. Unfortunately, these preparations are very difficult to maintain for the required multiple session protocol, and we have not been successful in testing the ability of the thalamocortical pathway to support LTP induction. At this time, the question about the ability of the thalamocortical synapses to undergo LTP is still unresolved. It does appear, however, that thalamocortical synapses are less plastic

than cortico-cortical synapses, with the stimulation parameters utilized here.

FIGURES and CAPTIONS

Chapter 5

Figure 5.1. The locations of stimulating electrodes in the medial portion of the medial geniculate (MGm) and recording electrodes in the auditory cortex (AC) and primary motor/frontal cortex (M1) are shown in representative sections from the rat brain atlas of Paxinos and Watson (1997). Stimulating electrode tips that touched the border of the MGm and bipolar electrode tips that straddled the nucleus were accepted. Auditory cortex included the dorsal and ventral distinctions made by Paxinos and Watson (1997). The AuD and AuV have been removed from our figures, as well as the lines delimiting the dorsal and ventral areas. Locations of the lowest pole of the bipolar electrodes for all animals that recieved stimulation are represented with dots and all the control animals with squares. The stimulation site is demarked by an asterisk.

Figure 5.1

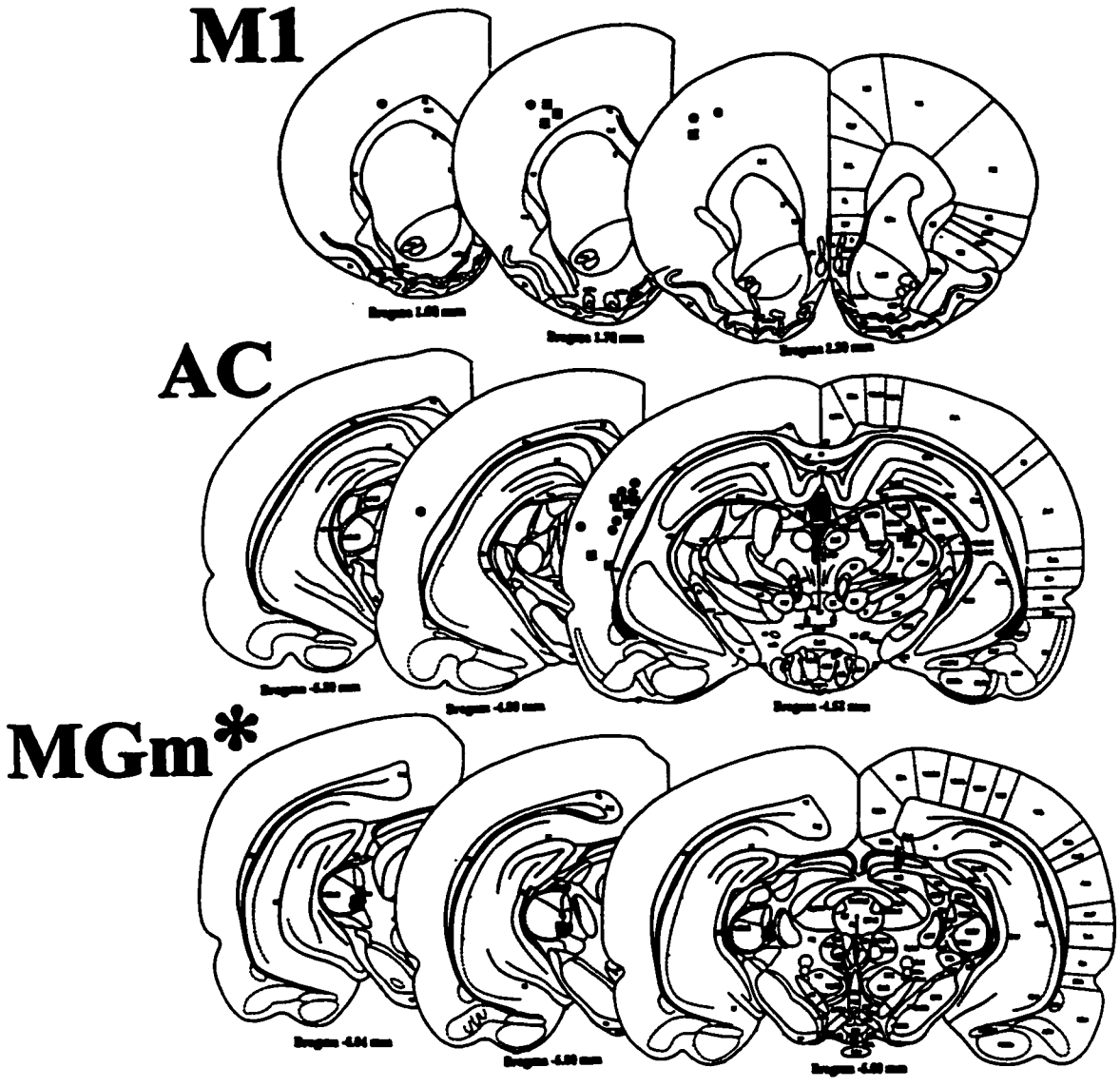


Figure 5.2. Field potentials evoked by medial geniculate (MGm) stimulation in: A) the auditory cortex (AC) and B) the primary motor/frontal cortex (M1). Note that the solid lines represent the baseline recorded from each site, while the dashed line represents the evoked response recorded 24 hours after the delivery of trains. Horizontal calibration, 10 msec; vertical calibration, 1.0 mV, except those labelled. Here * = 0.5mV. The amplitude difference shown in the experimental response is significantly decreased.

Figure 5.2

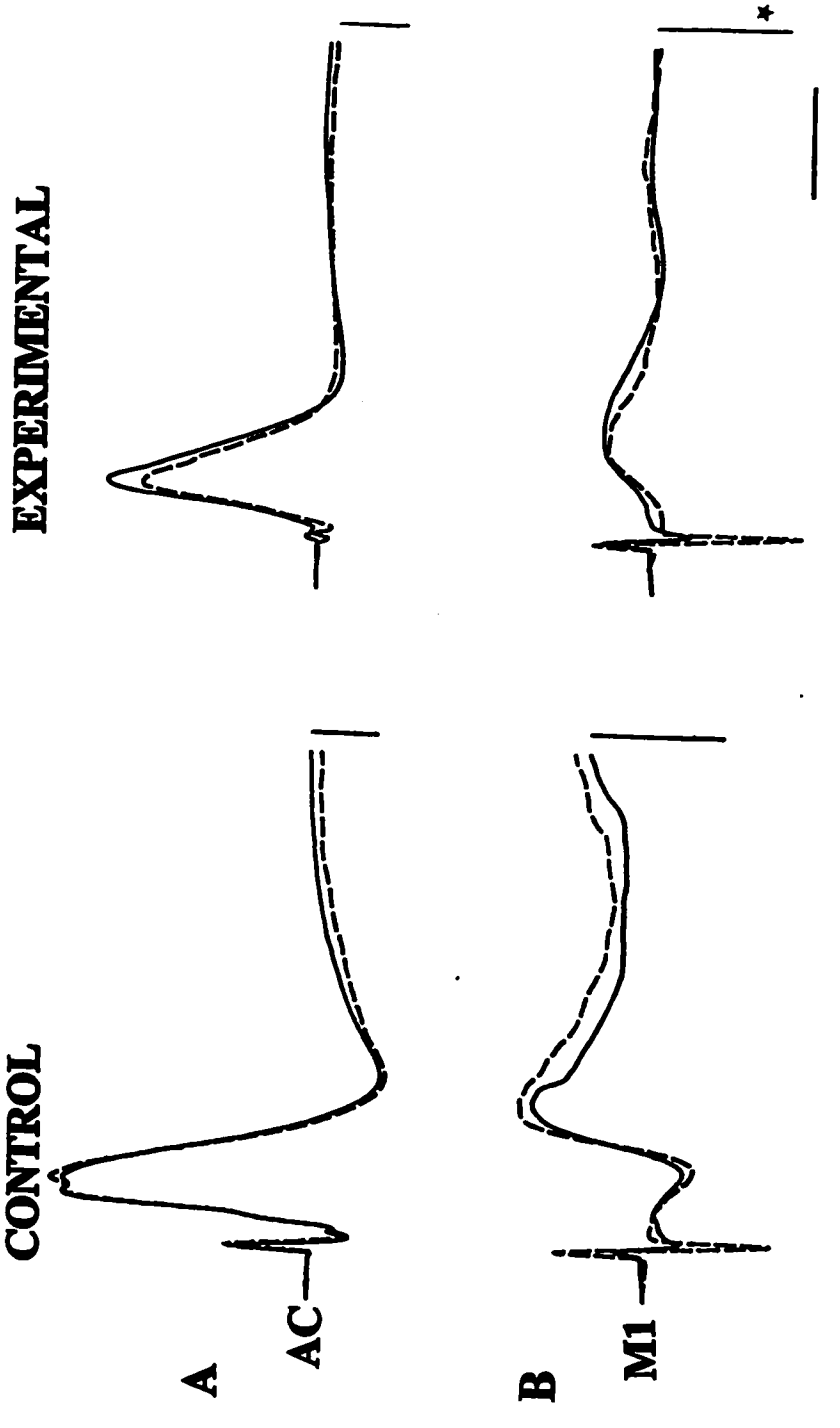


Figure 5.3. Evoked response amplitudes as a function of days following MGm stimulation. Amplitude changes from the average baseline are represented along the ordinate and days along the abscissa. The abscissa shows the pre-induction period (baseline), the induction period, and the follow-up period (post-LTP). Note that the follow-up measures are only collected 24 hrs and 48 hrs after the last stimulation session, but that stimulation is delivered for 20 days. A) The amplitude of the peak amplitude in the auditory cortex is shown to significantly decrease (group x day interaction, $p<0.01$) over days. The amplitude of the response evoked in frontal cortex remained stable over days (B).

Figure 5.3

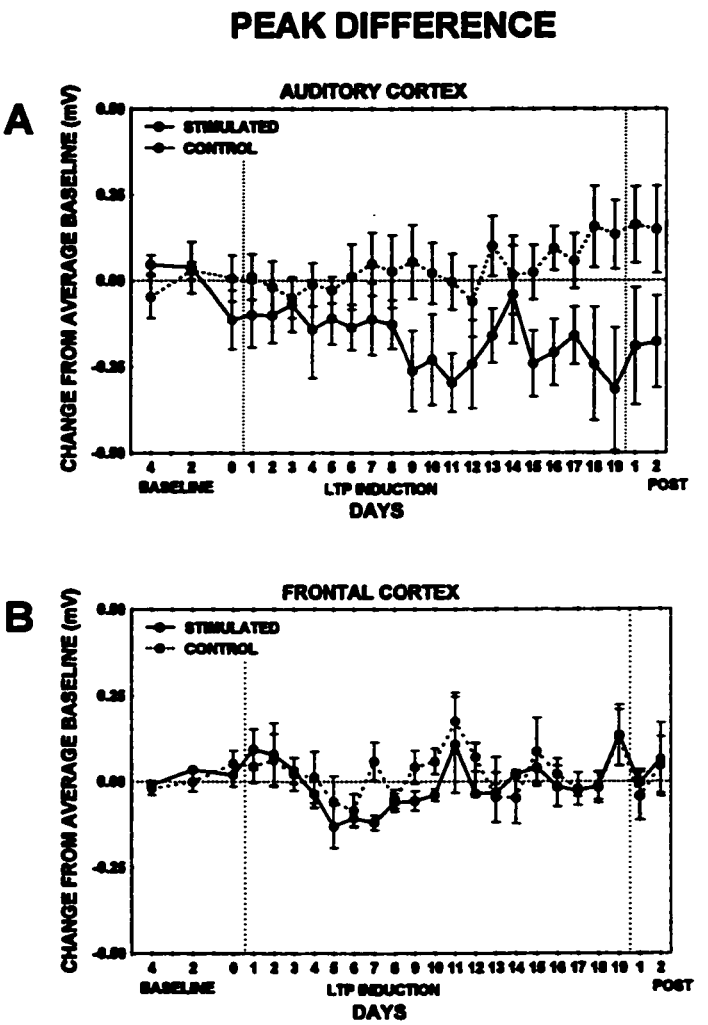

PEAK DIFFERENCE
A
AUDITORY CORTEX
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
BASELINE
LTP INDUCTION
POST
DAYS
B
FRONTAL CORTEX
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
BASELINE
LTP INDUCTION
POST
DAYS

Figure 5.4. The locations of stimulating electrodes in the lateral-dorsal portion of the lateral geniculate (LGN) and recording electrodes in primary binocular visual cortex (V1B) and primary motor/frontal cortex (M1) are shown in representative sections from the rat brain atlas of Paxinos and Watson (1997). The LGN electrodes were considered to be appropriately placed if the tips were in any of the lateral-dorsal nuclei or if the tips of bipolar electrodes straddled the structure. Locations of the lowest pole of the bipolar electrodes for all animals that received stimulation are represented with dots and all the control animals with squares. The stimulation site is demarked by an asterisk.

Figure 5.4

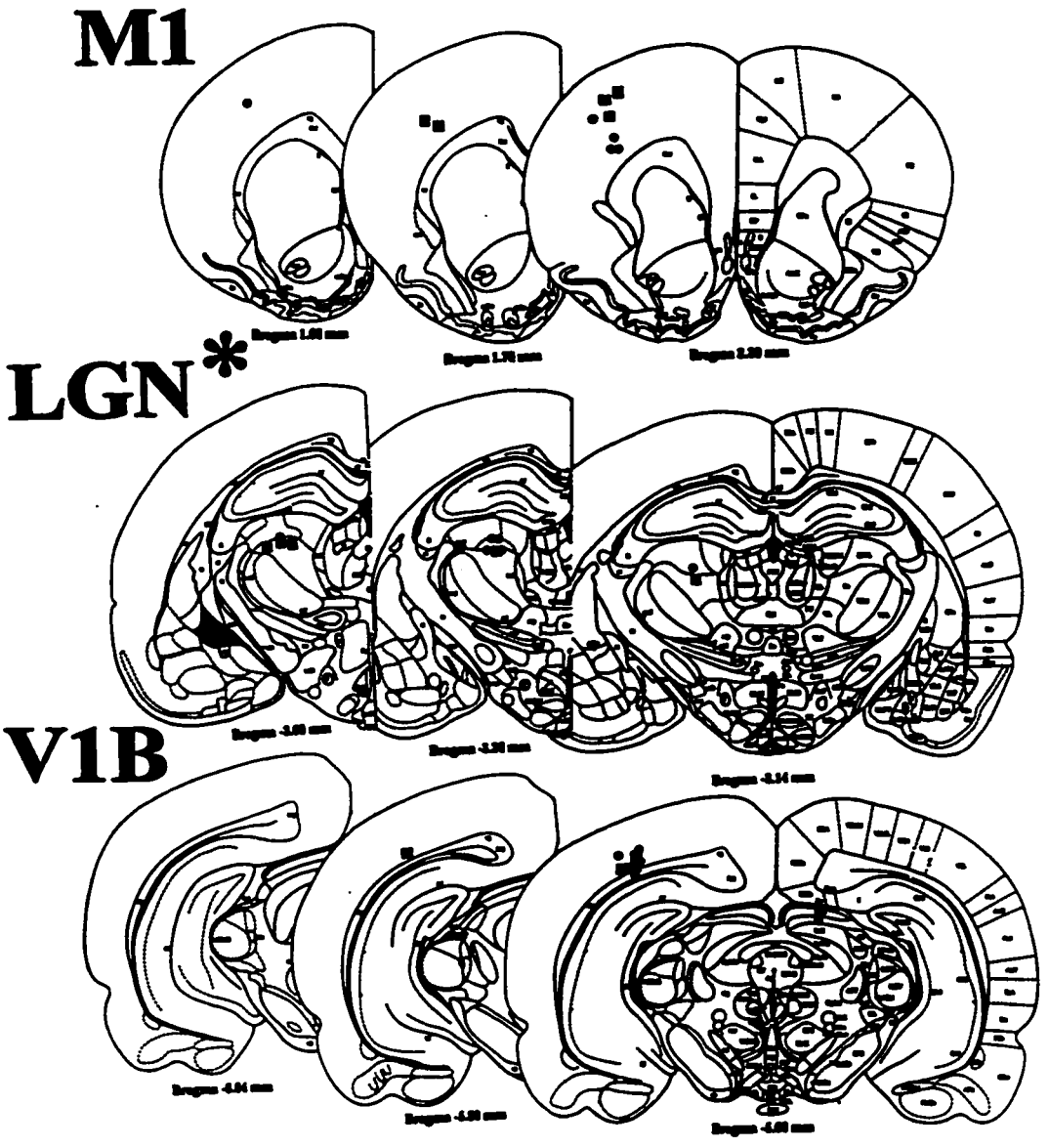


Figure 5.5. Field potentials evoked by lateral geniculate (LGN) stimulation in: A) the binocular visual cortex (V1B) and B) the primary motor/frontal cortex (M1). Note that the solid lines represent the baseline recorded from each site, while the dashed line represents the evoked response recorded 24 hours after the delivery of trains. Horizontal calibration, 10 msec; vertical calibration, 1.0 mV. Note that there is some variability in the evoked responses that are seen here, the differences in amplitude are not significant.

Figure 5.5

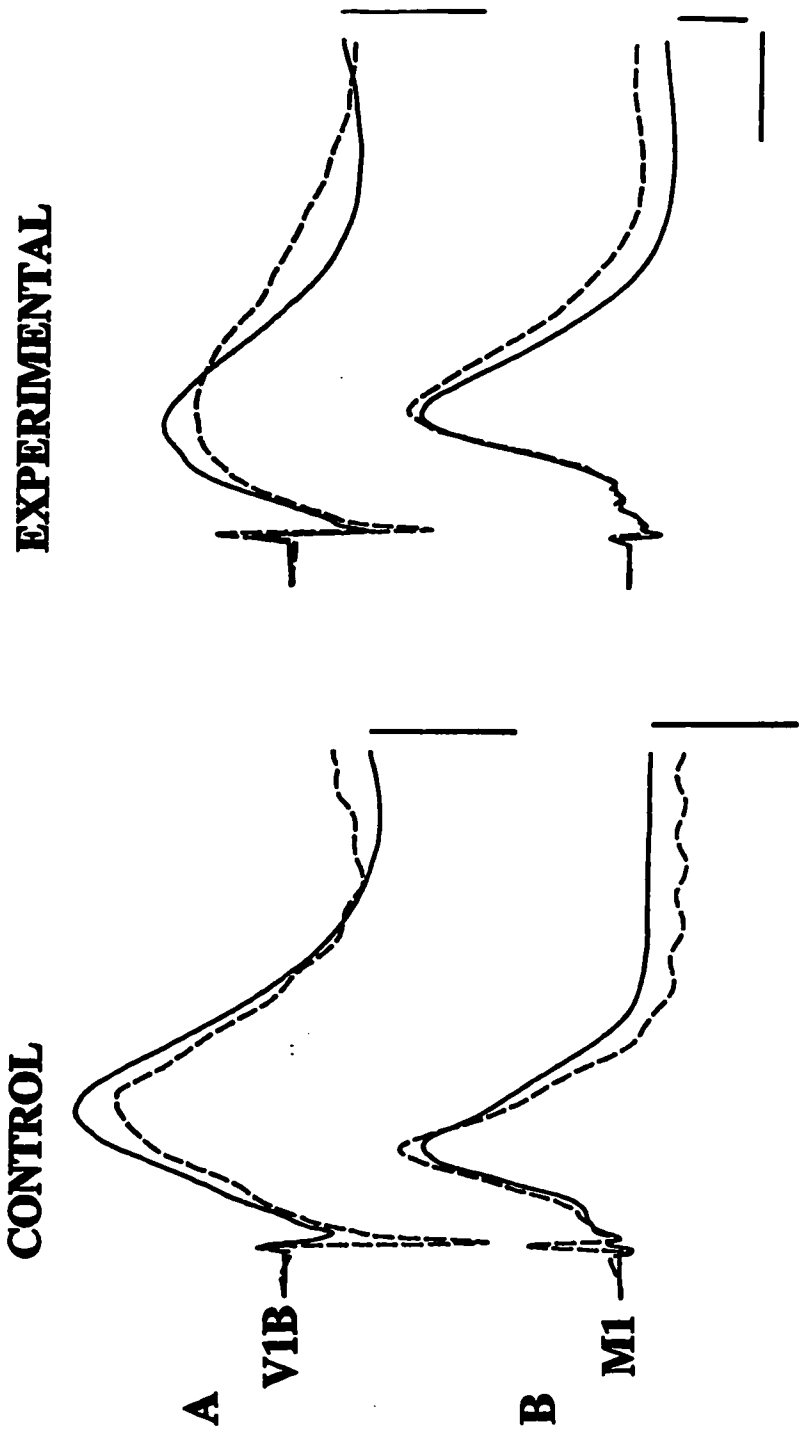


Figure 5.6. Evoked response amplitudes as a function of days following LGN stimulation. Amplitude changes from the average baseline are represented along the ordinate and days along the abscissa. The abscissa shows the pre-induction period (baseline), the induction period, and the follow-up period (post-LTP). Note that the follow-up measures are only collected 24 hrs and 48 hrs after the last stimulation session, but that stimulation is delivered for 20 days. A) The amplitude of the peak amplitude in visual cortex remains stable (group x day interaction, $p=0.8$) over days. The amplitude of the response evoked in frontal cortex also remained stable over days (B).

Figure 5.6

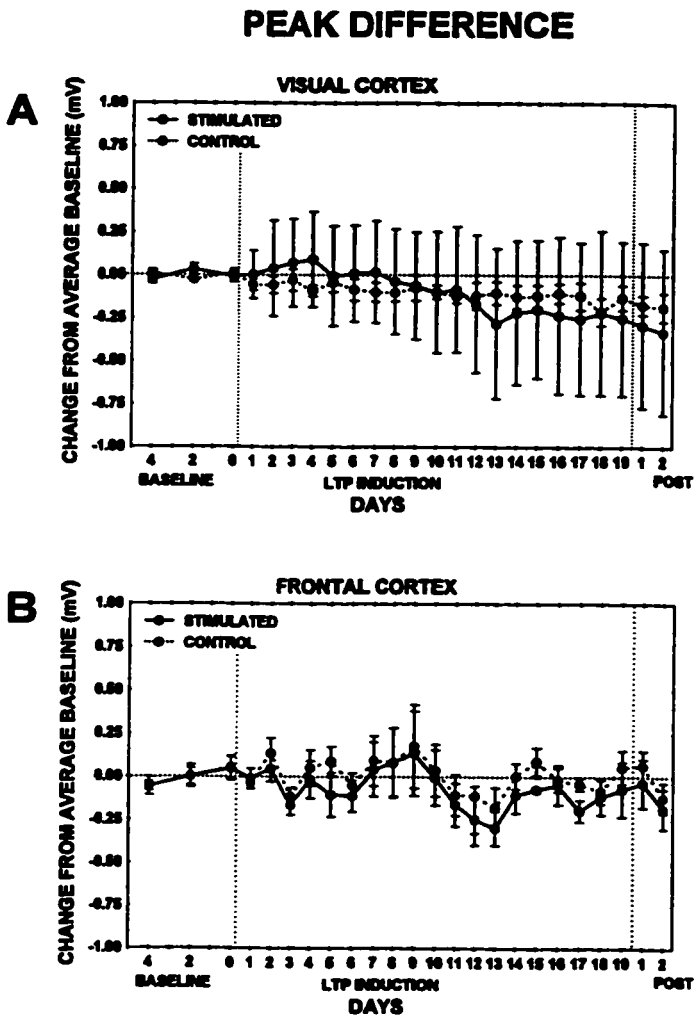

PEAK DIFFERENCE
A
VISUAL CORTEX
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
BASELINE
LTP INDUCTION
POST
DAYS
B
FRONTAL CORTEX
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
BASELINE
LTP INDUCTION
POST
DAYS

Figure 5.7. The locations of stimulating electrodes in the mediodorsal nuclei (MD) and recording electrodes in the dentate gyrus (DG) and primary motor/frontal cortex (M1) are shown in representative sections from the rat brain atlas of Paxinos and Watson (1997). The MD electrodes were considered to be appropriately placed if the tips were in any of the mediodorsal nuclei or if the tips of bipolar electrodes straddled any of the nuclei. Locations of the lowest pole of the bipolar electrodes for all animals that recieved stimulation are represented with dots and all the control animals with squares. The stimulation site is demarked by an asterisk.

Figure 5.7

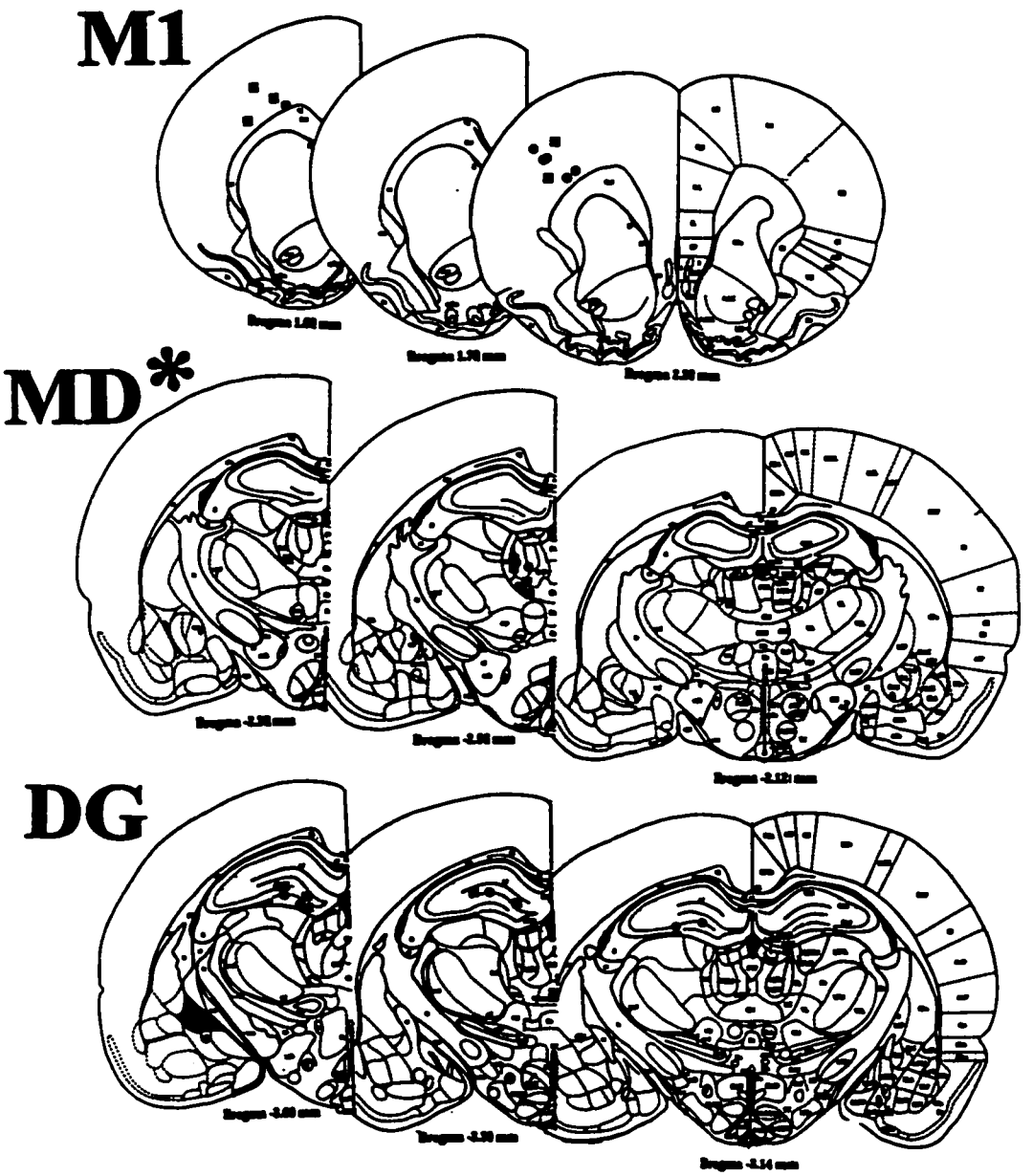


Figure 5.8. Field potentials evoked by mediodorsal (MD) stimulation in: A) the primary motor/frontal cortex (M1) and B) the dentate gyrus (DG). Note that the solid lines represent the baseline recorded from each site, while the dashed line represents the evoked response recorded 24 hours after the delivery of trains. Horizontal calibration, 10 msec; vertical calibration, 1.0 mV, except those labelled. Here * = 0.5mV. Note that there is some variability in the evoked responses that are seen here, the differences in amplitude are not significant.

Figure 5.8

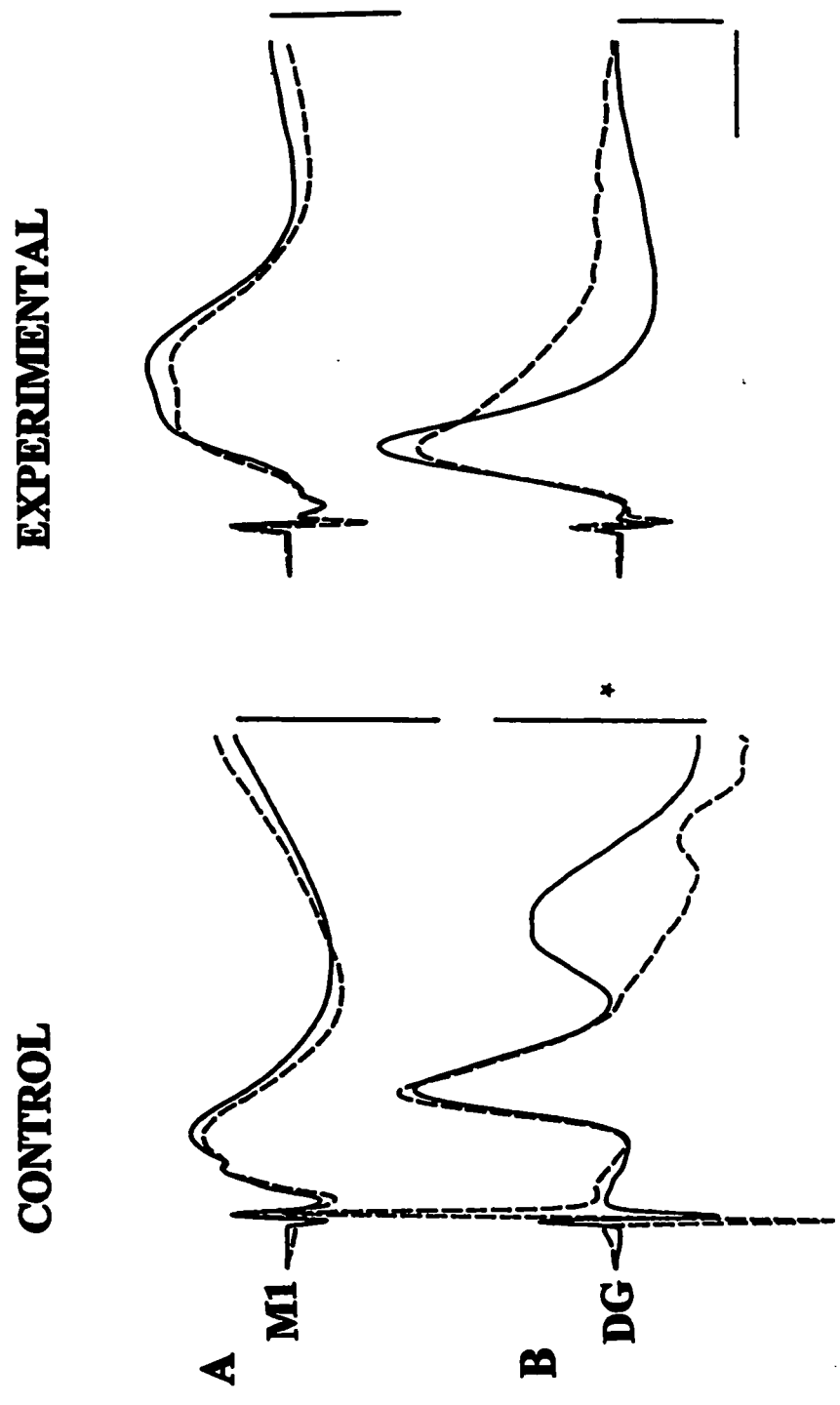
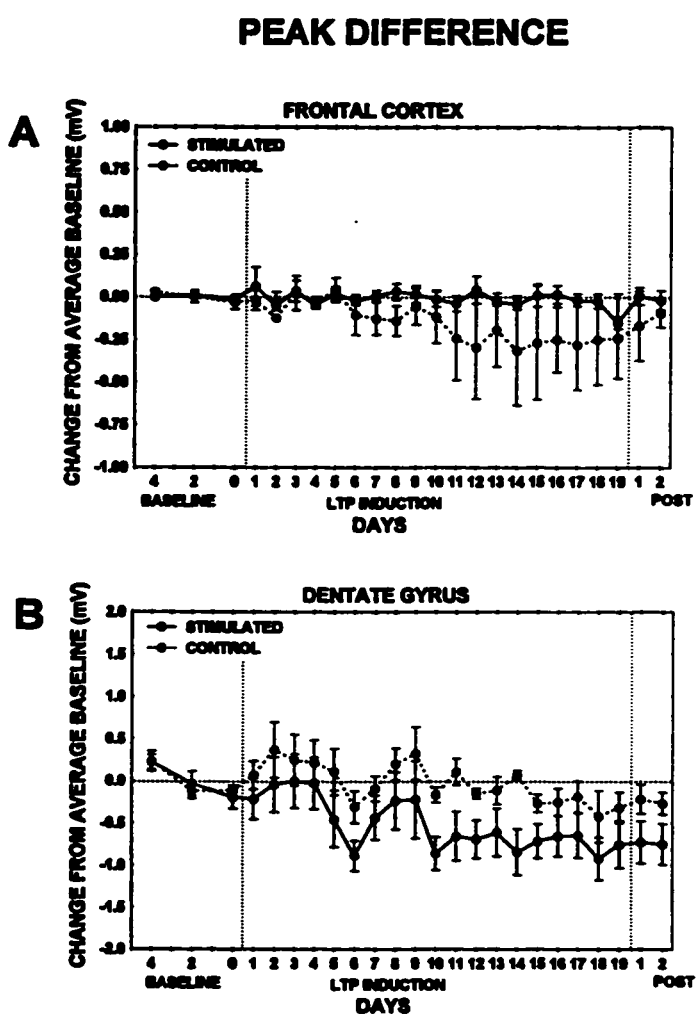


Figure 5.9. Evoked response amplitudes as a function of days following MD stimulation. Amplitude changes from the average baseline are represented along the ordinate and days along the abscissa. The abscissa shows the pre-induction period (baseline), the induction period, and the follow-up period (post-LTP). Note that the follow-up measures are only collected 24 hrs and 48 hrs after the last stimulation session, but that stimulation is delivered for 20 days. A) The amplitude of the peak amplitude in frontal cortex remains stable over days. The amplitude of the response evoked in the dentate gyrus decreased significantly (group x day interaction, $p=0.01$) over days (B).

Figure 5.9


PEAK DIFFERENCE
A
FRONTAL CORTEX
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
BASELINE
LTP INDUCTION
POST
DAYS
B
DENTATE GYRUS
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
BASELINE
LTP INDUCTION
POST
DAYS

Chapter 6

Long-term Potentiation in Corticothalamic Projections

Experimental studies have been unsuccessful in revealing the function of the ubiquitous corticothalamic projections (Jones, 1985; Salt & Eaton, 1996). It has been suggested that they play a role in selective enhancement or gating of sensory information (Miller, 1996; Mumford, 1991). The *ascending* projections in all thalamic sensory areas terminate as large boutons, in an array called a glomerulus, and these are found primarily on proximal dendrites of thalamic cells (Miller, 1996). The *descending* corticothalamic pathways, however, provide the majority of synapses found on distal dendrites. These synapses are in the form of small boutons and, despite their distance from the soma, they still have a considerable influence on the cell. Miller (1996) claims that, on average, corticothalamic influence in somatosensory thalamic areas is virtually equivalent to that of the cuneo-thalamic inputs. One of the unusual characteristics of the thalamus is that thalamic cells have few interconnections, suggesting that neighbouring cells are functionally independent from one another and that most of the input comes from ascending or descending projections (Jones, 1985; Miller, 1996). In electrophysiological recordings, thalamic cells that are in close proximity are not necessarily concordant in their firing. Corticothalamic projections appear to show more divergence than the reciprocal thalamocortical projections (Miller, 1996; Winer & Larue, 1987), but the influence of the

corticothalamic projections on their target cells is greater than that of the reciprocal thalamocortical projections on their target cells.

While there is little known about the mechanisms of action of the corticothalamic input, it may act via NMDA and metabotropic receptors (Salt & Eaton, 1996). The morphology of the corticothalamic synapses and their use of glutamate or aspartate as a neurotransmitter suggest that they are excitatory. The precise physiological conditions to activate such inputs, however, remain to be elucidated. Thalamic cells have a low threshold for firing and corticothalamic fibers impinge on a large number of thalamic cells with, presumably, excitatory connections. As corticothalamic activation could potentially repeatedly fire thalamic cells, we assumed that a synaptic strengthening (Hebbian) may occur at the corticothalamic synapses. Certain types of stimulation protocols, such as those used to induce LTP, may be appropriate for activation of the corticothalamic circuits. In this series of experiments, tetanic stimulation was spaced and repeated to determine the ability of corticothalamic pathways to support LTP induction. The same systems investigated in the thalamocortical study reported in Chapter 5, were targets of interest in this study.

GENERAL METHODS

All experiments were performed on male Long-Evans rats from the McMaster Breeding Colony weighing between 300 and 500 grams at the time of surgery. All rats were housed in pairs in plastic cages prior to surgery and individually in hanging wire cages following surgery. Food and water were provided *ad lib*. The colony was maintained on a 12 hr on/12 hr off light/dark cycle.

Bipolar electrodes were constructed of twisted Teflon coated stainless steel wires, 200 μm in diameter, insulated except for the tips. Cortical electrodes had a tip separation of approximately 1.0 mm and subcortical electrodes had a tip separation of approximately 500 μm. Animals were anaesthetized with sodium pentobarbitol (65 mg/kg) and stereotaxically implanted with electrodes under electrophysiological control. Electrodes were connected to gold-plated male amphenol pins that were inserted into a 9-pin connector plug. Four stainless steel jewellers screws were used to anchor and mount the plug to the skull with dental acrylic. One of the screws had a wire attached with a gold-plated pin that served as a ground electrode. All animals were given at least two weeks of recovery time prior to testing.

Stimulation and Recording. Evoked responses were acquired using ASYST software on a Comptec 486, 33 MHz computer. Electrical stimuli were generated with a Grass S88 stimulator and photoelectric stimulation isolation units (Grass SIU6B). Signals were fed into a Grass Model 12 EEG amplifier and filtered at half amplitude at 0.3 Hz (high pass) and 3 kHz (low pass). The signal was sampled at 10 kHz by a 12 bit A/D converter (Data Translation DT 2821) and stored on the computer hard drive. Analysis was completed off line.

Baseline evoked field potentials were collected three times, once every 48 hours. To construct I/Os, biphasic test pulses of increasing intensity were delivered to the stimulation electrode at a frequency of 0.1 Hz. Responses were recorded from the cortical sites. Ten responses, 50 ms in duration were evoked, amplified, digitized and averaged at each of 12 logarithmically spaced intensities (32, 40, 63, 100, 126, 159, 251, 398, 501, 794, 1000, and

1259 μA). Animals were separated into control and experimental groups.

To attempt to induce potentiation in the experimental group, high frequency, 50 ms, trains were delivered to the stimulating electrode. Pulse intensity was set at a level that produced an 80% maximum amplitude response in at least one of the sites being recorded from and pulse frequency was set at 300 Hz. Thirty trains were delivered per session at 0.1 Hz. Trains were delivered daily, immediately following an I/O test, for 15 days. Follow-up I/O tests were conducted 24 hrs, 48 hrs, and 1 week after the last train in experimental animals and after comparable delays in control animals.

Analysis. Potentiation was assessed by comparing the peak amplitudes and rising phase slopes of the initial baseline evoked responses with those of reponses evoked after trains had been delivered. An average baseline was calculated by determining the average of the first three I/O tests. All other I/Os were compared against this average to determine millivolt changes in peak amplitude and slope changes over days. Repeated measures ANOVAs were used to test for significant effects of stimulation.

After completion of the electrophysiological experiments, the animals were perfused through the heart with phosphate buffered saline (PBS), followed by 2% paraformaldehyde in PBS in preparation for Methylene Blue-Azure II (MBAII) staining, or with 0.9% saline, followed by a formal-saline solution (4% formalin in 0.9% saline) in preparation for Cresyl Violet staining. Brains were removed and placed in the fixative for at least 24 hours. Brains were then immersed in a 10% sucrose solution and placed in a 4 degree Celcius refrigerator for 24 hours. Each of the brains were sectioned in the coronal plane at 40 μm on a cryostat, mounted on gelatin coated slides, and stained. Slides were examined with a light microscope

to confirm stimulating and recording placements.

Experiment 1. Auditory Cortex to Medial Geniculate Nucleus.

Electrode Placement. Animals were implanted with recording electrodes in the medial portion of the medial geniculate (MGm) and stimulating electrodes in primary auditory cortex (as per Paxinos & Watson, 1997). Electrode placements were as follows: *auditory cortex:* 4.8 mm posterior to bregma, 6.5 lateral to the midline with an electrode angle of 10 degrees, and 4.8 mm ventral from bregma and *MGm:* 5.8 mm posterior to bregma, 3.4 mm lateral to the midline, and 5.2 mm ventral from brain surface. Depths were adjusted to maximize evoked responses. In total, 9 animals were used to obtain reliable measures from the MGm placements (experimental n=6; control n=3).

RESULTS

Light microscope analysis of brain sections provided verification of electrode placements in target structures as defined by Paxinos and Watson (1997). Figure 6.1 shows the locations of electrode tips. As the mediodorsal nucleus is small, electrode tips that touched the border of the nucleus and bipolar electrode tips that straddled the nucleus were accepted as being placed appropriately. Paxinos and Watson (1997) had delimited primary auditory cortex and also labelled dorsal and ventral primary auditory cortex. We defined auditory cortex as including all three of these areas and have removed the lines delimiting the dorsal and ventral areas from our figures.

Test pulses delivered to the auditory cortex produced a surface positive evoked

potential in the MGm. High frequency stimulation delivered to the auditory cortex resulted in a long-lasting potentiation effect in the the MGm. Figure 6.2 shows representative responses evoked in the MGm before and after trains were delivered to the auditory cortex. The morphology changes are similar to those seen in neocortical responses following LTP induction in cortico-cortical pathways. There was a reversal in polarity in at least part of the early monosynaptic component and a large potentiation of the polysynaptic component(s). The amplitude shifts in both the early monosynaptic component ($p=0.05$) and polysynaptic component ($p<0.00001$) were significant. The peak had increased an average of 0.13 mV +/- 0.03 mV 24 hours after the last stimulation session. The change in amplitude over days is shown in Figure 6.3 for both the early monosynaptic component and the polysynaptic component. Although it increased slightly with additional stimulation sessions, the increase in evoked response amplitude was apparent after only one stimulation session. Asymptotic levels of potentiation were reached by 4 days of stimulation. The potentiation remained for at least one week after stimulation had ceased.

DISCUSSION

These results indicate that corticothalamic projections may be more plastic than the thalamocortical projections. In a previous series of experiments we observed that LTP could not be induced in thalamocortical pathways using the same stimulation parameters and stimulating for an even longer period. The corticothalamic pathway between auditory cortex and the MGm actually exhibited LTP relatively quickly and reached asymptotic levels quickly. This finding is rather interesting given that other subcortical structures such as the

hippocampus tend to show potentiation with a single stimulation session, but that the cortico-cortical projections require multiple stimulation sessions. Although cortico-cortical potentiation has not been tested in the auditory cortex, other cortical sites appear to require 10 or more days of stimulation to reach asymptotic levels of LTP. If this is also true of the auditory system, it would argue against the cortico-thalamic LTP being due to an increased volley from a potentiated cortical site. The short latency of the corticothalamic response also argues against such a *relayed* LTP effect.

In order to determine if the MGm was the only thalamic area that would show potentiation with cortical stimulation, the visual and the mediodorsal systems were also examined.

Experiment 2. Visual Cortex to Lateral Geniculate

Electrode Placements. Animals were implanted with recording electrodes in the dorsal portions of the lateral geniculate (LGN) and stimulating electrodes in primary (binocular) visual cortex (Paxinos & Watson, 1997). Electrode placements were as follows: *visual cortex (area V1B):* 5.8 mm posterior to bregma, 4.4 lateral to the midline, and 1.8 mm ventral from brain surface and *LGN:* 3.3 mm posterior to bregma, 2.5 mm lateral to the midline, and 5.0 mm ventral from brain surface. Depths were adjusted to maximize evoked reponses. In total, 9 animals were used to obtain reliable measures from the LGN placements (experimental n=6; control n=3).

RESULTS

Electrode placements were confirmed to be located in target structures as defined by Paxinos and Watson (1997) with light microscopy. Electrode tip distributions are shown in Figure 6.4.

Test pulses delivered to the visual cortex produced a surface positive potential in the LGN. While stimulation did not induce a significant potentiation effect, there were morphological changes in the evoked response similar to those seen in the previous experiment. Figure 6.5 shows responses evoked in the LGN before and after trains were delivered to the visual cortex. There was an amplitude shift to a partial reversal that occurred in the early monosynaptic component and an apparent potentiation of the polysynaptic component that did not approach significance ($p=0.8$). The change in amplitude over days is shown in Figure 6.6. The change in morphology occurred after a single stimulation session and was still evident one week after trains had ceased.

DISCUSSION

Although the LGN did not show a significant potentiation effect in this experiment, the amplitude and morphology changes were in the same direction as seen in the auditory corticothalamic responses. There was a small number of animals tested and a relatively large variability in magnitudes of the stimulation-induced changes in the evoked responses. The potentiation effect would likely reach significance with a larger sample.

Experiment 3. Frontal Cortex to Mediodorsal Nucleus and Dentate Gyrus

Electrode Placements. Recording electrodes were implanted in the mediodorsal nucleus of the thalamus and dentate gyrus and a stimulating electrode was implanted in the lateral frontal cortex. Placements were as follows: *frontal cortex (area M1):* 2.0 mm anterior to bregma, 4.0 mm lateral to the midline, and 1.8 mm ventral to brain surface; *mediodorsal nucleus:* 2.4 mm posterior to bregma, 0.8 mm lateral to the midline, and 5.8 mm ventral to brain surface; and *dentate gyrus:* 3.5 mm posterior to bregma, 2.2. mm lateral to the midline, and 3.3 mm ventral to brain surface. In total 8 animals were used to obtain responses in the MD and the dentate gyrus (MD: experimental n=5, control n=3; DG: experimental n=5). As only the experimental condition was examined in the dentate gyrus, statistics were not completed on this group. The frontal to dentate projection was also examined in a previous study reported in this thesis (see Chapter 4, Experiment 2).

RESULTS

Examination with light microscopy indicated that electrode tips were in target structures as defined by Paxinos and Watson (1997). The mediodorsal nucleus includes a series of 4 mediodorsal thalamic nuclei that are situated ventral to the hippocampus. Each of these nuclei are relatively small. Tip locations were considered appropriate if the tip was in one of the 4 mediodorsal nuclei, or the tips of the bipolar electrode spanned a nucleus. Figure 6.7 shows the distribution of tips in the target structures.

Frontal cortex stimulation elicited a potentiation in both the mediodorsal nucleus and dentate gyrus. As the potentiation induced in the dentate gyrus paralleled that seen in

Experiment 2 of Chapter 4, it will not be discussed here. Test pulses delivered to frontal cortex produced a small surface positive potential. Figure 6.8 shows representative examples of evoked responses in the mediodorsal nucleus before and after trains were delivered to frontal cortex. Again, the morphology changes were similar to those seen in the previous experiments. There was an amplitude shift to a partial reversal ($p<0.0004$) that occurred in the early monosynaptic component and an enhancement of the polysynaptic component ($p<0.000001$). The peak had increased an average of 0.60 mV +/- 0.14 mV 24 hours after the last stimulation session. The change in amplitude over days is shown in Figure 6.9a. The polysynaptic component potentiated after only 1 or 2 stimulation sessions and reached asymptote after approximately 9 stimulation sessions. The potentiation of the polysynaptic component and the amplitude reversal of the early monosynaptic component remained for at least one week after stimulation had ceased (see Figure 6.9a and 6.9b, respectively).

DISCUSSION

These results suggest that plasticity in corticothalamic projections is generally greater than in the reciprocal thalamocortical projections, regardless of the system. The rate of onset of potentiation was rapid in all sites. The MGm and the LGN, however, reached asymptotic levels more quickly than those in the MD. These changes were paralleled in the dentate gyrus, which also required more stimulation sessions than the single session required when stimulating the perforant path.

GENERAL DISCUSSION

The mechanisms underlying corticothalamic plasticity have not been elucidated, but experiments in the somatosensory slice preparation indicate that NMDA receptors, which are often associated with a high degree of plasticity, may play a role. Eaton and Salt (1996) have suggested that both NMDA and metabotropic receptors are involved in the mediation of corticothalamic transmission in vivo. Corticothalamic inputs impinge on both NMDA and metabotropic receptors. It is possible that the metabotropic glutamate receptors result in a lowering of membrane conductance, which in turn would enhance NMDA receptor inputs. Responses evoked in cells of the ventrobasal thalamus by cortical stimulation were attenuated by the application of antagonists to NMDA or metabotropic glutamate receptors. Kao and Coulter (1997) reported similar results.

Although the metabotropic gluatamate receptors may enhance the *excitatory* influence of NMDA receptors, they probably also enhance the *inhibitory* influence of the GABAergic afferents. In all systems, corticothalamic fibers form glutamatergic (probably excitatory) terminations on distal segments of the thalamic dendrites (Ray & Price, 1992). In the mediodorsal/frontal cortex system, however, adjacent cortical areas also send GABAergic afferents that target the more proximal segments of thalamic dendrites (Paxinos, 1995). These inhibitory influences may have the ability to regulate the activity of the mediodorsal cells and functionally act to increase the threshold for cell firing and, perhaps, LTP induction. The results reported above are consistent with this hypothesis as more stimulations sessions were required before a potentiation effect was observed, and asymptotic levels were not reached as quickly, in the MD as in the MGm or LGN.

Before too much effort is directed towards explaining the apparently greater plasticity in corticothalamic pathways, an alternative explanation must be excluded. It is possible that the direct cortical stimulation in the corticothalamic LTP experiments potentiated cortico-cortical connections. This, in turn, may have lead to a larger efferent volley triggered by the cortical stimulation. The fact that short latency components were affected in the corticothalamic responses, and that the effects reached asymptotic levels quickly (for sensory cortices) argues against this explanation. Nevertheless, more work needs to be done to confirm that the LTP effects are truly localized to the corticothalamic synapses.

FIGURE CAPTIONS

Chapter 6

Figure 6.1. The locations of stimulating electrodes in in the auditory cortex (AC) and recording electrodes the medial portion of the medial geniculate (MGm) are shown in representative sections from the rat brain atlas of Paxinos and Watson (1997). Stimulating electrode tips that touched the border of the MGm and bipolar electrode tips that straddled the nucleus were accepted. Auditory cortex included the dorsal and ventral distinctions made by Paxinos and Watson (1997). The AuD and AuV have been removed from our figures, as well as the lines delimiting the dorsal and ventral areas. Locations of the lowest pole of the bipolar electrodes for all animals that recieved stimulation are represented with dots and all the control animals with squares. The stimulation site is demarked by an asterisk.

Figure 6.1

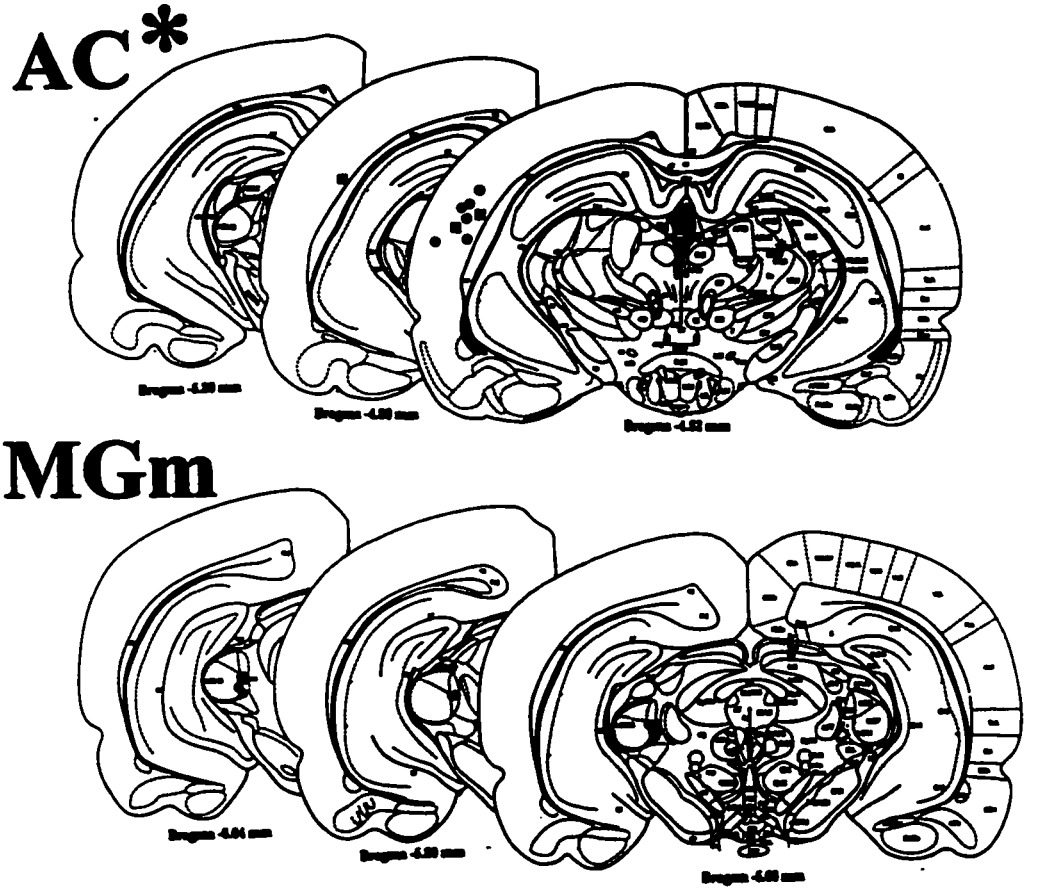


Figure 6.2. Field potentials evoked by auditory cortex stimulation in the medial geniculate nucleus (MGm). Note that the solid lines represent the baseline recorded from each site, while the dashed line represents the evoked response recorded 24 hours after the delivery of trains. Horizontal calibration, 10 msec; vertical calibration, 0.5 mV. The open arrow indicates the portion of the monosynaptic component that shows a reversal in amplitude with multisession stimulation. The thin arrow indicates the monosynaptic peak. The thick arrow indicates the polysynaptic peak that becomes evident with repeated stimulation.

Figure 6.2

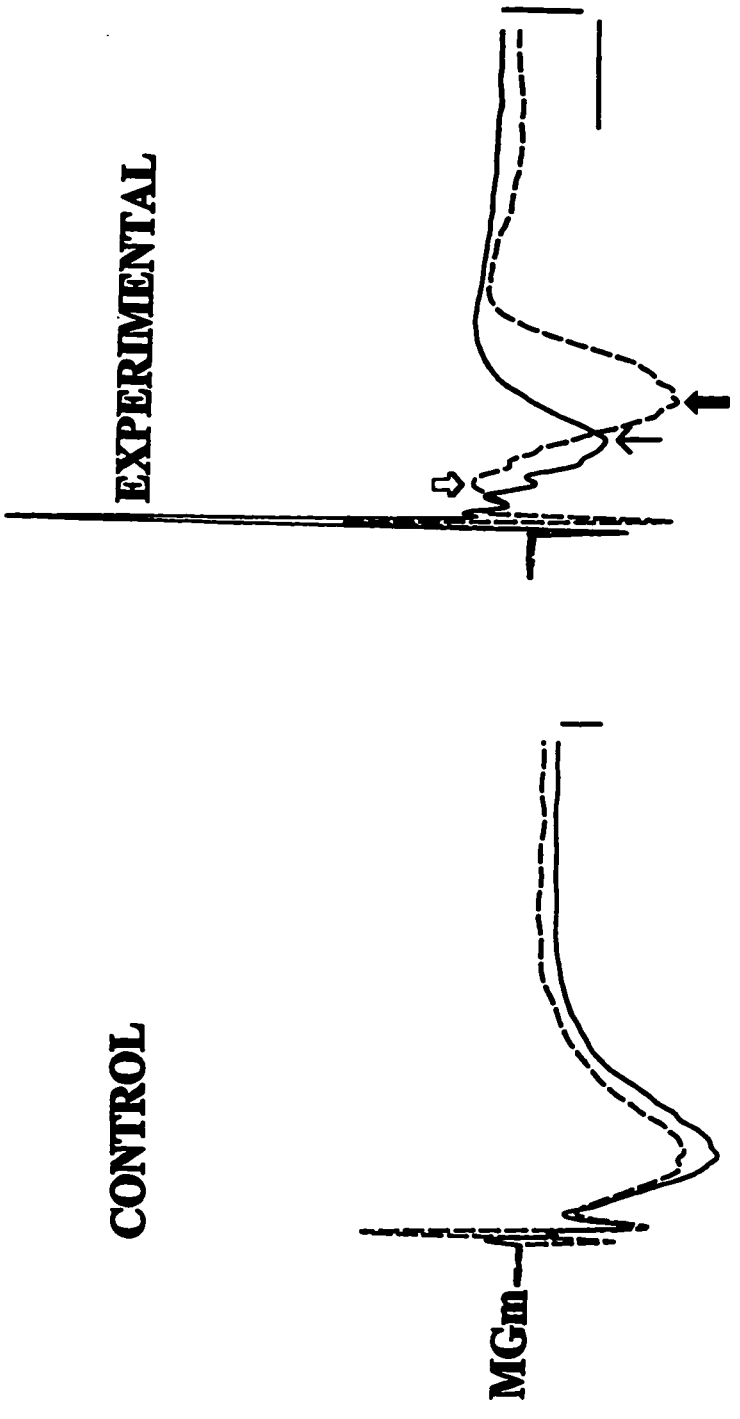


Figure 6.3. Evoked response amplitudes as a function of days following auditory cortex stimulation. Amplitude changes from the average baseline are represented along the ordinate and days along the abscissa. The abscissa shows the pre-induction period (baseline), the induction period, and the follow-up period (post-LTP). Note that the follow-up measures are only collected 24 hrs, 48 hrs, and 1 week after the last stimulation session and that stimulation is delivered for 15 days. A) The amplitude of the early monosynaptic peak shows a significant reversal (group x day interaction, $p=0.05$) over days in the medial geniculate nucleus (MGm). B) The amplitude of the polysynaptic response evoked in MGm shows a significant increase (group x day interaction, $p<0.00001$) with multisession stimulation.

Figure 6.3

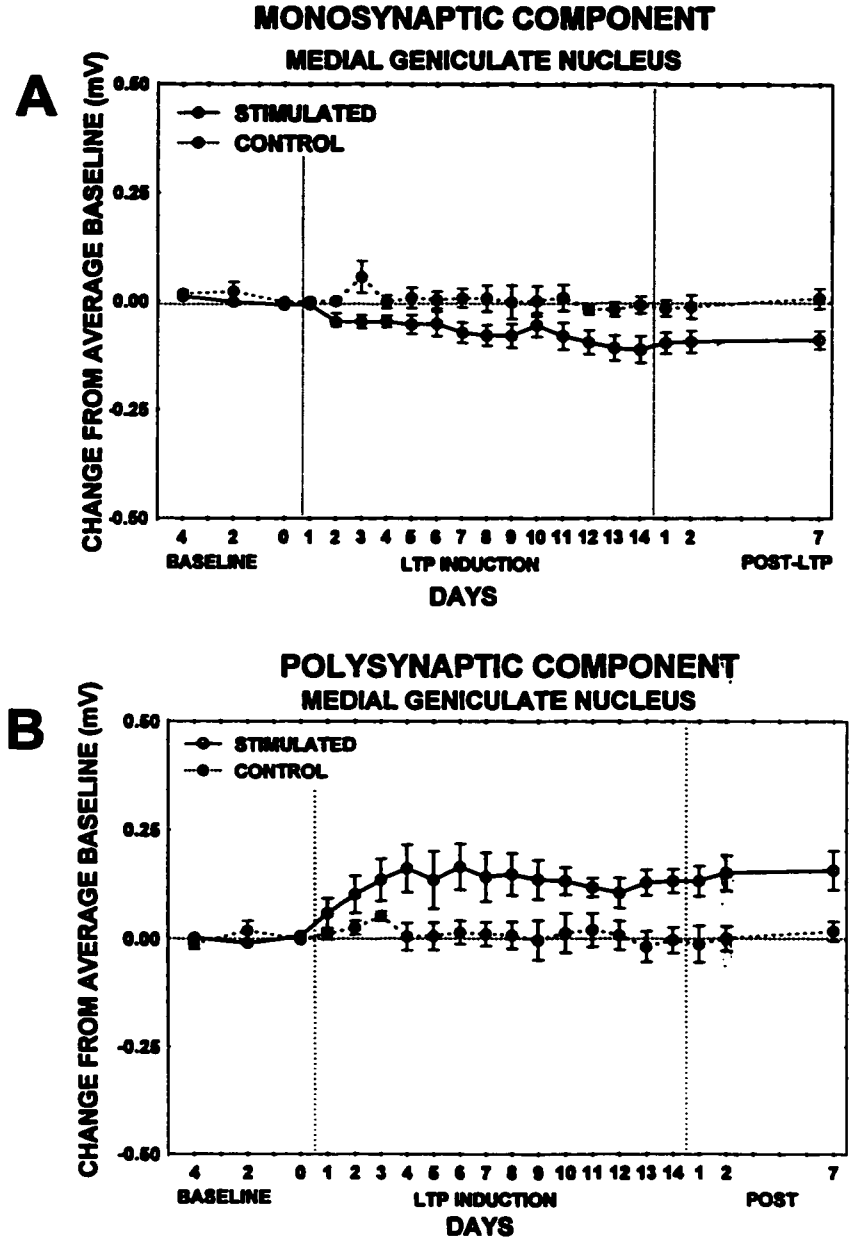

A
MONOSYNAPTIC COMPONENT
MEDIAL GENICULATE NUCLEUS
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
0.50
0.25
0.00
-0.25
-0.50
4 2 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 1 2 7
BASELINE
LTP INDUCTION
POST-LTP
DAYS
B
POLYSYNAPTIC COMPONENT
MEDIAL GENICULATE NUCLEUS
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
0.50
0.25
0.00
-0.25
-0.50
4 2 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 1 2 7
BASELINE
LTP INDUCTION
POST
DAYS

Figure 6.4. The locations of stimulating electrodes in primary binocular visual cortex (V1B) and recording electrodes in the lateral-dorsal portion of the lateral geniculate (LGN) are shown in representative sections from the rat brain atlas of Paxinos and Watson (1997). The LGN electrodes were considered to be appropriately placed if the tips were in any of the lateral-dorsal nuclei or if the tips of bipolar electrodes straddled the structure. Locations of the lowest pole of the bipolar electrodes for all animals that recieved stimulation are represented with dots and all the control animals with squares. The stimulation site is demarked by an asterisk.

Figure 6.4

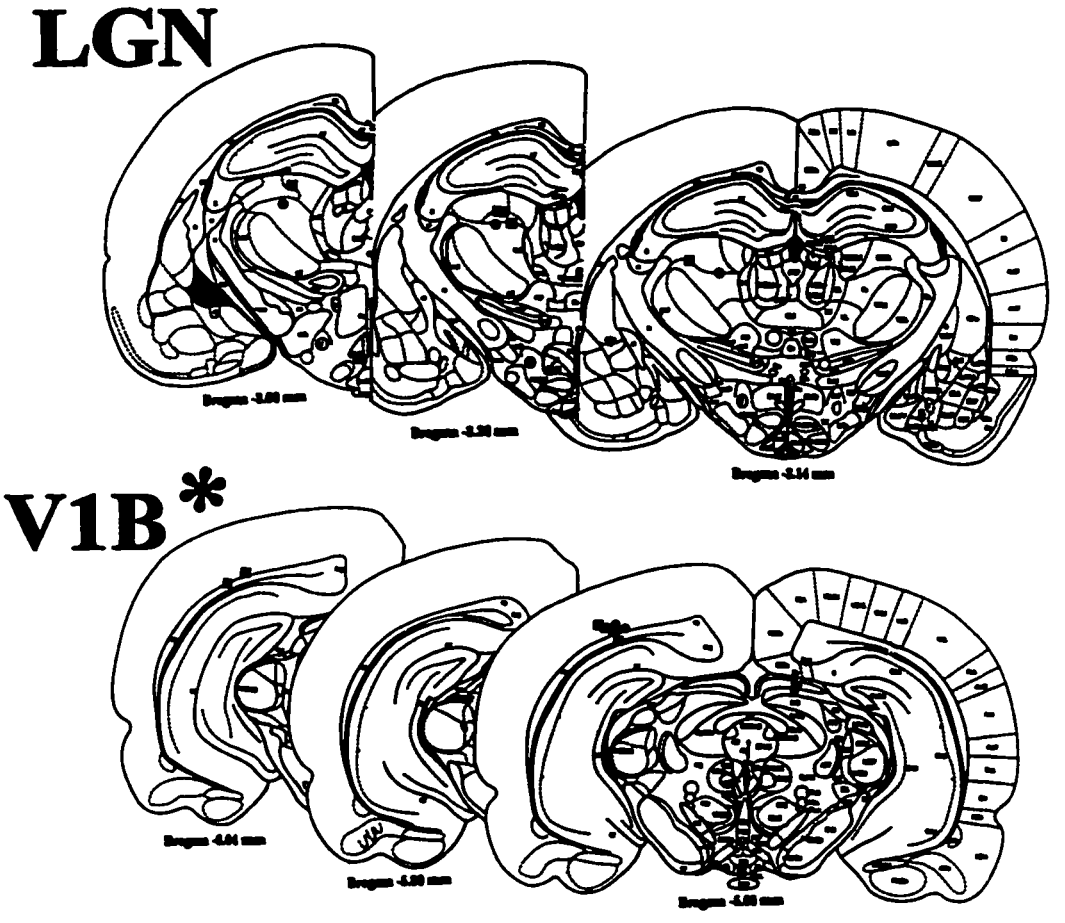


Figure 6.5. Field potentials evoked by visual cortex stimulation in the lateral geniculate nucleus (LGN). Note that the solid lines represent the baseline recorded from each site, while the dashed line represents the evoked response recorded 24 hours after the delivery of trains. Horizontal calibration, 10 msec; vertical calibration, 0.5 mV. The thin arrow indicates the monosynaptic peak. The thick arrow indicates the polysynaptic peak that becomes evident with repeated stimulation.

Figure 6.5

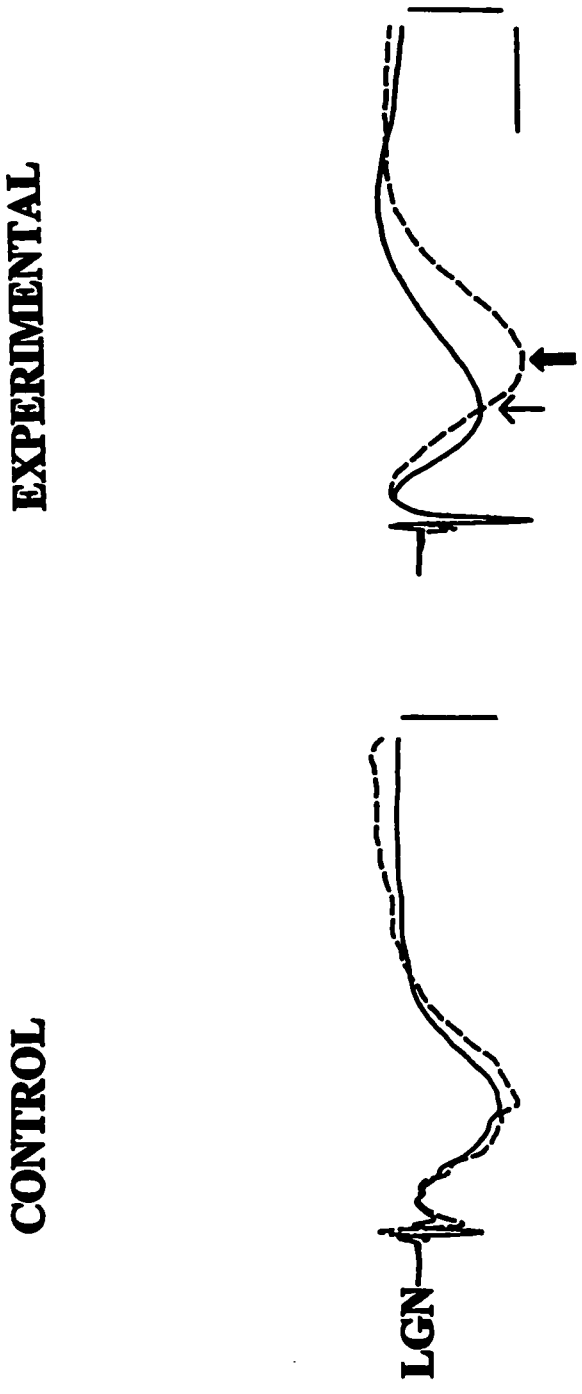


Figure 6.6. Evoked response amplitudes as a function of days following visual cortex stimulation. Amplitude changes from the average baseline are represented along the ordinate and days along the abscissa. The abscissa shows the pre-induction period (baseline), the induction period, and the follow-up period (post-LTP). Note that the follow-up measures are only collected 24 hrs, 48 hrs, and 1 week after the last stimulation session and that stimulation is delivered for 15 days. A) The amplitude of the polysynaptic response evoked in LGN shows a prominent, but non-significant, (group x day interaction, $p=0.8$) increase with multisession stimulation.

Figure 6.6

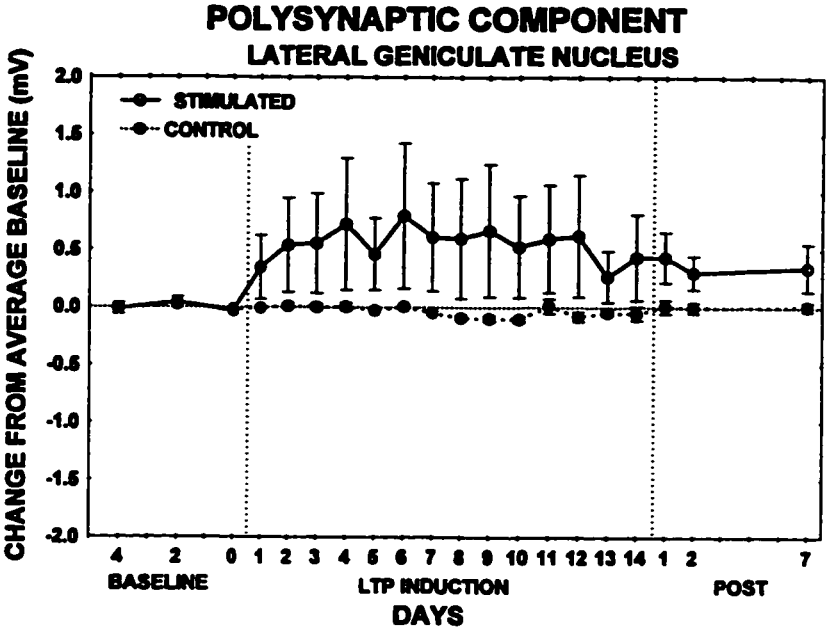

POLYSYNAPTIC COMPONENT
LATERAL GENICULATE NUCLEUS
STIMULATED
CONTROL
CHANGE FROM AVERAGE BASELINE (mV)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
4 2 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 1 2 7
BASELINE
LTP INDUCTION
POST
DAYS

Figure 6.7. The locations of stimulating electrodes in the primary motor/frontal cortex (M1) and recording electrodes in the mediodorsal nuclei (MD) are shown in representative sections from the rat brain atlas of Paxinos and Watson (1997). The MD electrodes were considered to be appropriately placed if the tips were in any of the mediodorsal nuclei or if the tips of bipolar electrodes straddled any of the nuclei. Locations of the lowest pole of the bipolar electrodes for all animals that recieved stimulation are represented with dots and all the control animals with squares. The stimulation site is demarked by an asterisk.

Figure 6.7

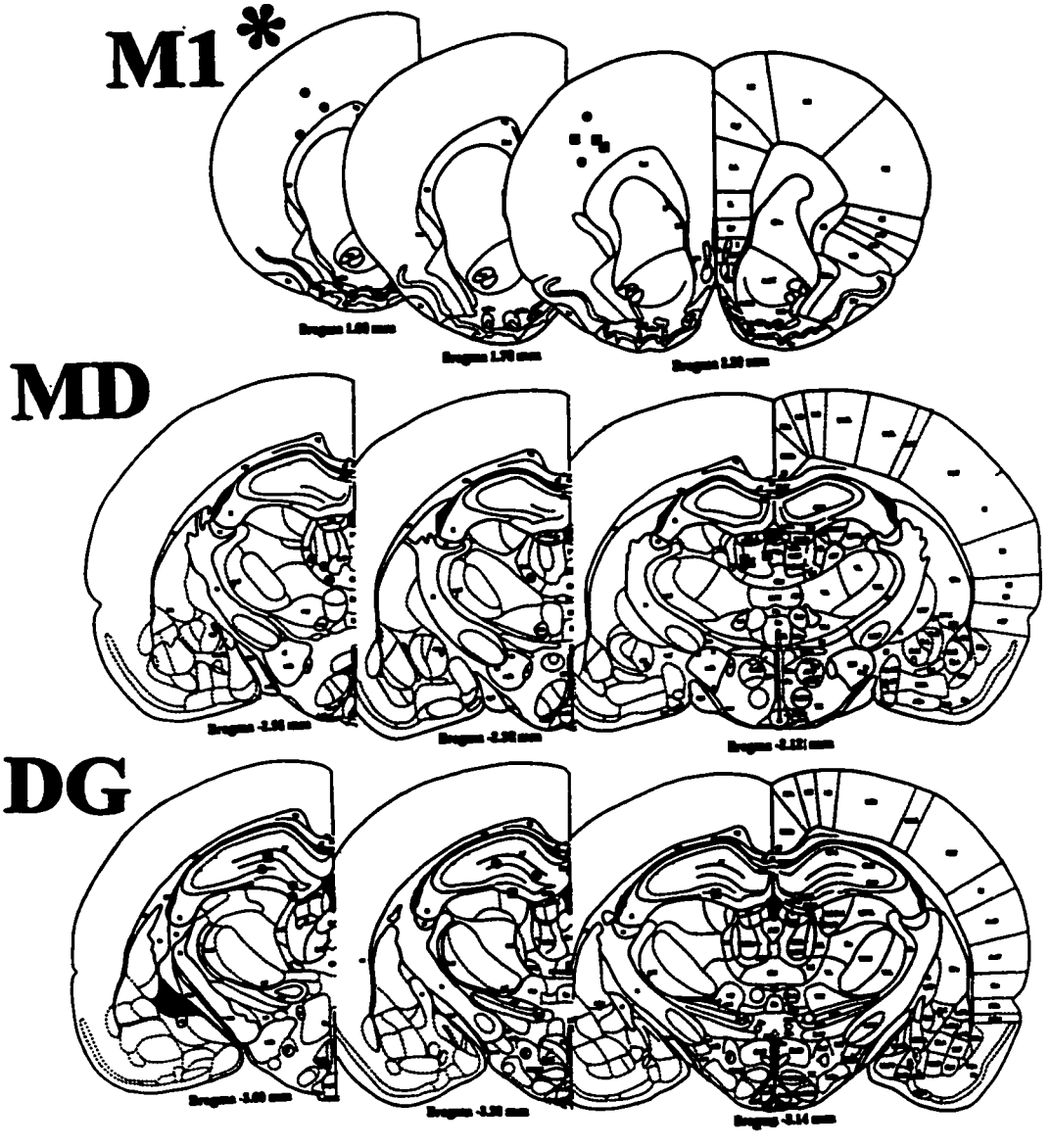


Figure 6.8. Field potentials evoked by frontal cortex stimulation in the mediodorsal nucleus (MD). Note that the solid lines represent the baseline recorded from each site, while the dashed line represents the evoked response recorded 24 hours after the delivery of trains. Horizontal calibration, 10 msec; vertical calibration, 0.5 mV. The open arrow indicates the early monosynaptic component that shows an amplitude reversal with repeated stimulation. The thin arrow indicates the monosynaptic peak. The thick arrow indicates the polysynaptic peak that becomes evident with repeated stimulation.

Figure 6.8

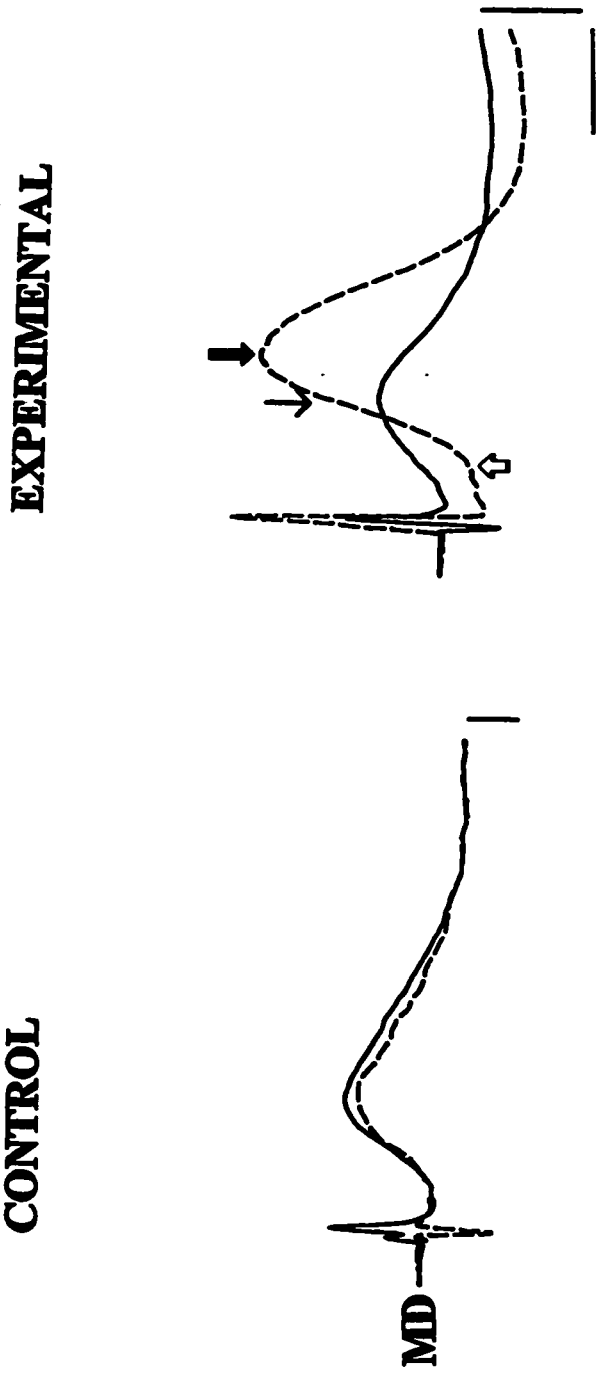
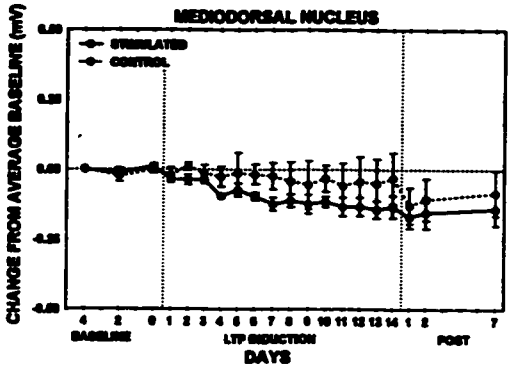


Figure 6.9. Evoked response amplitudes as a function of days following frontal cortex stimulation. Amplitude changes from the average baseline are represented along the ordinate and days along the abscissa. The abscissa shows the pre-induction period (baseline), the induction period, and the follow-up period (post-LTP). Note that the follow-up measures are collected 24 hrs, 48 hrs, and 1 week after the last stimulation session and that stimulation is delivered for 15 days. A) The early monosynaptic component in MD shows a significant shift (group x day interaction, $p<0.0004$) in amplitude with repeated stimulation. B) The amplitude of the polysynaptic response evoked in MD shows a significant increase (group x day interaction, $p<0.000001$) with multisession stimulation.

Figure 6.9

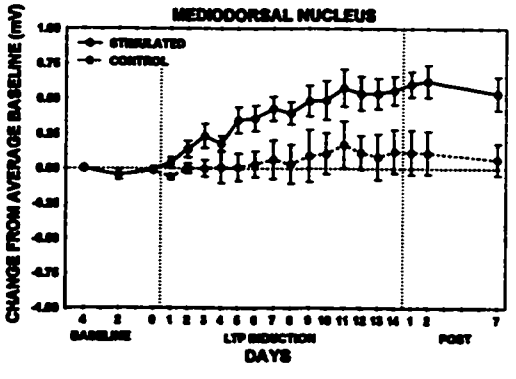
EARLY MONOSYNAPTIC COMPONENT

A



POLYSYNAPTIC COMPONENT

B



Chapter 7

General Discussion

Long-term potentiation is currently the most compelling candidate mechanism for information storage in the mammalian brain. LTP in the hippocampus has received more attention than LTP in any other structure, partially due to the ease with which LTP is induced in the hippocampus and partially due to the role the hippocampus is thought to play in learning and memory. It is clear, however, that many non-hippocampal areas are also critical for learning and memory and that the neocortex is heavily involved in the long-term storage of memories. LTP has recently been demonstrated in the neocortex in the chronically implanted rat (Racine, et al., 1995), but it has been shown to require a different regimen of stimulation than the hippocampus. There had been very little investigation of LTP in the connections between subcortical structures and the neocortex. In this thesis, I have examined two different types of cortical/subcortical systems- the pathways between the hippocampus and neocortex and the pathways between the thalamus and neocortex.

Most theories on the function of neocortical/hippocampal pathways propose that information flows in both directions and that the hippocampus may play a role in the consolidation of information in the neocortex (McClelland, et al., 1995). There have also been several proposals regarding the processing of memory functions by thalamocortical and corticothalamic pathways (i.e. Tyler & DiScenna, 1985). Substrates for two-way

communication have been demonstrated by anatomical investigations for both systems, but the physiology has not been well characterized in either system. In particular, although much is known about the function of projections from the thalamus to the cortex, relatively little is known about the functions of the reciprocal projections. We have proposed that information storage in both of these systems may include the occurrence of synaptic modifications *between* the subcortical and cortical structures.

Whereas the hippocampal-neocortical system is typically viewed as remaining plastic throughout the lifetime of all mammals, the thalamo-neocortical systems (sensory systems) have often been described as plastic only during a specific period of development called the critical period. One of the objectives of this thesis was to test this hypothesis, at least as it applies to LTP induction.

The parameters required for induction of LTP in cortico-cortical pathways in the chronically implanted rat were used here to examine plasticity in the two systems of interest. The results in the hippocampal-neocortical system will be discussed in relation to the presumed memory functions of the medial temporal lobe The results in the thalamo-cortical systems will be discussed in relationship to filtering mechanisms and mechanisms used to boost signals to the cortex.

The hippocampal-neocortical system. The first experiment, which was reported in Chapter 3, indicated that potentiation could be induced in the pathways between the perirhinal cortex and the dentate gyrus, frontal cortex and entorhinal cortex in chronically implanted animals. The results indicate that these pathways may use LTP as a mechanism for information storage. Although the pathways intrinsic to the hippocampus appear to

follow different rules for LTP induction than do the pathways intrinsic to the neocortex, both neocortical and hippocampal inputs from perirhinal cortex show properties that are intermediate to those pathways. As mentioned, current views of the functional roles of the hippocampal-neocortical system require a two-way conversation between the neocortex and hippocampus (Tyler & DiScenna, 1986). In Chapter 4 evidence was provided suggesting that there was a direct influence on the frontal cortex by the hippocampus. There also appeared to be a direct influence on the hippocampus by the neocortex, in addition to the transmission of information via the perirhinal cortex. The plasticity in these projections is relatively long lasting, as the potentiation effects show virtually no decay one week after trains had ceased. Plasticity in the connections between the hippocampus and neocortex could contribute to an indexing function of the hippocampus. It might, in fact, be rather difficult to design a flexible system for reactivation of neocortical traces based on hard-wired hippocampal-neocortical connections.

These LTP effects are consistent with an indexing theory proposed by Miller (1989, 1991). A rule-dependent modification of synapses (i.e. Hebbian and anti-Hebbian learning) is proposed to be the basis for learning and many forms of plasticity. This modification leads to consolidation via a series of recurrent loops that involve the hippocampus. Resonance through the loops at the theta period is suggested to strengthen synaptic connections. Theta activity is generated during exploratory activity when the animal is likely to be learning about the environment. Patterns of resonance are specific to environments and these activate different loops. Thus, phase-locked, self-organizing loops would be sufficient for the hippocampus to engage a consolidation process within the neocortex *and* to keep an index

of what is stored in the there. Data presented in Chapter 3 and 4 are consistent with this proposal.

The thalamo-neocortical systems. The series of experiments reported in Chapter 5 and Chapter 6, indicated that thalamocortical pathways are less plastic in the adult than the reciprocal corticothalamic pathways. While none of the tested thalamocortical pathways exhibited a potentiation effect with a multisession stimulation procedure, the back projections demonstrated a strong potentiation, particularly in the polysynaptic components. Although it tended to reach asymptotic levels more rapidly, the corticothalamic potentiation effects were quite similar to those found in the cortico-cortical connections (Racine, et al., 1995), and it remains to be determined whether the latter contribute in any way to the former.

Mumford (1991) proposed that the thalamus was like an active blackboard that utilized the back projection as a means to update the cortex on the current state of the world. The back projection would act to both write on and read the blackboard. A similar type of metaphor has been proposed for the hippocampus and it has further been suggested that the naturally occurring theta rhythm might be responsible for erasing the blackboards such that sensitivity to new inputs is maximized (McNaughton, personal communication to Rolls, 1987, c.f. Rolls, 1990). The thalamus has its own oscillatory activity (reviewed by Steriade & Llinas, 1988) that might also function to reset, or erase, previously stored information so that it could be updated. How frequently old information would have to be over-written depends, in part, upon the capacity of the corticothalamic circuitry.

If the thalamus can indeed act as a blackboard, then its state should be determined not merely by the ascending inputs, but by processed cortical information (Mumford, 1991).

Mumford argues, for example, that the back projections in the visual system should provide a gradual (approximately 50 ms) sharpening of the image held in the LGN. Such a sharpening effect should be measurable, perhaps in increments that approximate the conduction delays in the geniculocortical loop. The experiments reported in Chapter 7 suggest that this process may also lead to long-lasting changes.

Potential For Change. Since thalamic projection cells do not interact with their neighbours, they are more likely to function in cell assemblies, or circuits that include cortical neurons (Miller, 1996). These cell assemblies could be formed via a Hebbian strengthening of cortico-cortical and cortico-thalamic projections. These would subsequently activate thalamocortical synapses, and so on.

One of the roles of the thalamus is to maintain levels of neural activity in the cortex high enough for normal functioning (Miller, 1996). The thalamocortical and corticothalamic loops may aid in this process. While cortical cells have very high firing thresholds and require multiple excitatory inputs to fire, thalamic cells are normally poised on the verge of firing and fire with only a small amount of corticothalamic activation. Miller proposes that thalamic cells are continually firing at the maximum frequency to simply maintain normal levels of functioning within the cortex. Thus, the firing capacity of thalamic neurons is already near saturation during normal, baseline levels of activation. Therefore, any LTP that might be possible in thalamocortical synapses may itself have already reached saturation. In any case, the dynamic range for inducing change in thalamocortical pathways must be very small. Our stimulation protocol in Chapter 5 may not have produced a change in synaptic efficacy, simply because the system has no additional dynamic range left to sample (Murphy,

personal communication). The reciprocal pathways examined in Chapter 6, however, likely have a huge dynamic range to sample due to the high thresholds for firing of cortical cells.

Associative mechanisms. Cortico-cortical synapses appear to be more susceptible to Hebbian strengthening than thalamocortical synapses. Thalamocortical influences may require the cooperation of cortico-cortical synapses to mediate change. Long-term potentiation of a thalamocortical input to the motor cortex, for example, has been shown following the coactivation of thalamocortical and cortico-cortical afferents in anesthetized cats (Iriki, et al., 1991). One of the potential follow-up experiments to examine thalamocortical pathways would be to stimulate the thalamus with a high frequency tetanus and a cortical area with a phase-locked lower frequency stimulus that would not itself induce potentiation, to activate both thalamocortical pathways and cortico-cortical pathways. The low frequency cortico-cortical stimulus might be sufficient to engage an associative mechanism allowing the high frequency stimulation to influence the thalamocortical synapses. The recording electrode could initially be the site of delivery for low frequency stimulation to determine if activation of local cortico-cortical circuits is sufficient and distal circuits could be examined subsequently.

Oscillations. High frequency stimulation has been found to be highly effective in inducing LTP in the hippocampus and neocortex. It has been found, however, that the most effective pattern of stimulation for inducing LTP in the hippocampus is a series of brief, high-frequency trains delivered at the frequency of a theta rhythm (Larson, Wong, & Lynch, 1986). Optimal LTP induction in the thalamus might also be dependent upon the input

pattern, requiring that the trains be delivered at the dominant cortico-thalamic oscillation frequency. Interestingly, no one appears to have suggested this as a possible requirement for thalamocortical plasticity, not even Miller (1991) who uses the theta rhythm as a fundamental component of his hippocampal indexing theory.

Models. The models proposed by various individuals and groups (McClelland, et al., 1995; Miller, 1996; Mumford, 1991; Tyler & DiScenna, 1985) make a number of predictions. McClelland, et al. (1995) and Tyler and DiScenna suggest that bidirectional communication is necessary in the hippocampal-neocortical memory system. McClelland, et al. (1995) also suggest that a hierarchy of plasticity may exist within the hippocampal-neocortical memory system. The data presented in Chapter 3 and 4 provide some evidence for bidirectional communication as evoked responses could be generated and were modifiable. The data presented in Chapter 3 provide some evidence for a hierarchy of plasticity as stimulation delivered to the perirhinal cortex induced a potentiation in the hippocampus and the neocortex that appeared to have an 'intermediate' induction rate. The variations in induction and decay rates found here are also consistent with the plasticity gradients that are proposed by McClelland, et al. (1995). Miller (1996) and Mumford (1991) both suggest that there is a specific function of the corticothalamic pathway and imply that it should show a high degree of plasticity to influence the thalamus. The evidence presented in Chapter 7 is consistent with this prediction. Although the data does not confirm the ability of the thalamocortical loop to act as a filter, it is congruent with such a proposal.

Conclusion. The evidence presented here suggests that an LTP-like process may be important for information storage within several components of the hippocampal memory

system that are intermediate between the hippocampus and neocortex. The ability to induce LTP in the corticothalamic projections raises a number of interesting questions about the role of plasticity in this system, particularly in the filtering of input and the detection of mismatches between prototypical and novel inputs.

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Appendix 1

Key words and LTP information

1) Stimulation

A basic aspect of brain function is that the living brain is responsive to electrical currents. In general, the goal of experimentation using stimulation in brain regions is to either activate or disrupt a region that is stimulated so that inferences about how that part of the brain participates in behaviour (behaviour in a gross sense of the word).

Although the influence of an externally driven current, which stimulates fibers, inhibitory and excitatory cells and cell bodies, is not the same as the temporal and spatial patterning of a naturally occurring volley of impulses, it is a powerful tool. Current can be delivered to a precise point within the brain by using bipolar electrodes (described below), which allows for stimulation only between its poles (and to tissue in very close proximity).

The dimensions of the stimulus can be precisely controlled and measured (amplitude, shape, frequency, and duration). The onset and termination can also be precisely controlled.

The application of a current through the tips of stereotactically placed electrodes produces a localized excitation of the brain. The properties of current to excite tissue is

important. There are four current parameters of the current that need to be controlled; 1) waveform, 2) intensity, 3) frequency, and 4) duration.

Waveform:

1. Direct current (DC current)

Direct current is characterized by the fact that both the current level and the polarity of the electrical charges remain unchanged over time.

2. Sinusoidal waveform

The current available from a power outlet takes the form of a sine wave, a 60 Hz alternating current (AC) that reverses its polarity from negative to positive 120 times/second.

3. Monophasic square wave

A common waveform is the square wave, which is employed in the laboratory where the LTP demo will take place. The monophasic waveform would be safe at the frequencies being used, but is not as good with higher frequencies and longer durations.

4. Biphasic square wave

Biphasic stimulation is the safest of the stimulating currents as it does not cause any tissue damage with extended stimulation sessions. This is the type of current we use.

Intensity:

The intensity of stimulation corresponds to the amplitude of the wave measured from the lowest peak to the highest peak. With a low intensity, only the neurons in the immediate vicinity of the tips are activated. As intensity increases, the current spreads radially from the tips and affects more neurons. Current is the rate of electron flow in a circuit, and current is measured in units of amperes (A). This is a very large unit with respect to brain tissue and in most cases the current used is measured in milliamperes (mA) or microamperes (uA).

The two factors that effect the rate of current flow are: 1) the voltage that moves the electrons through the circuit and 2) the ease with with the current flows through the material (the resistance, measured in ohms).

In electrically stimulating the brain, or any nervous tissue, we try to use an anatomical circuit that exists and use electrical theory in using the circuit. Output from the stimulatior travels through a wire that is connect to one pole of the bipolar electrode. The current flows through the tissue to the other pole of the electrode and back to the stimulator to complete the circuit.

Frequency:

Frequency of stimulation is measure by the number of pulses, or cycles, per second (PPS) delivered to the stimulating electrode tips. The stimulator will allow for approximately, DC to 1000 PPS. Optimum frequencies for activation of most brain structures is about 30 - 400 Hz. There is some variability on this, but generally, higher

frequencies add the risk of thermal stimulation to the brain structures, which may result in tissue damage. We tend to use a stimulation frequency of 300 Hz.

Duration:

The optimum pulse duration for activating neurons is in the range of about 0.1 to 1.0 milliseconds (msec). A short pulse duration avoids tissue damage by electrolysis or heat. The time delay between pulses also tends to be optimum at 0.1 or greater for similar reasons.

The most convenient way of experimentally stimulating excitable tissue is by using square pulses. Such a pulse is an almost instantaneous change of potential from 0 to some predetermined value, a maintenance of the potential at the value and an almost instantaneous return to 0.

Very often single pulse stimuli are not sufficient to produce a lasting change in the CNS. A barrage of 5-10 pulses, given at a high frequency (called trains), however, will cause a response.

2) Recording

One of the methods that one can use to study brain function is to record and analyze electrical activity. The flow of electrical impulses is thought to be one of the primary methods of transfer of information in the brain. There are a number of things that can be recorded, everything from spontaneous activity from many areas in the brain, as is recorded

with EEG, or individual and discrete events such as quantal release. We record populations of cells.

There are several problems with recording electrical signals from biological tissues. First, the signals are very small, in the order of uV of mV and must be amplified to display and analyse. Second, signals can change very rapidly and must be able to be recorded with good fidelity. Third, sometimes the electrodes used can attenuate or distort the signal. And fourth, noise must be minimized to get good recordings.

Amplification:

Most amplifiers have some standard gain changes that can be used. Our amplifiers have many possible gains on the amplifier banks in the labs. Amplification is usually obtained through the use of operational amplifiers and field effect transistors. Operational amplifiers perform operations (+-*/) on a signal. The amplification requirements can vary depending on what you want to record.

Analog to digital conversion:

The electrical brain signals that we will be recording are analog signals. Consider the difference between an analog watch and a digital watch. One is always moving and changing and the number of points that could be examined is infinite, the other has some discrete time points that you can see. The signal that we will record has to be stored on the computer, but the computer cannot record an analog signal. The number of points that we can record is set

by hardware and software. The resolution of the signal depends upon the number of bits.

We want to amplify our signal enough to use most of the range of the particular A/D converter going to the computer and we want to use filters (low pass) to avoid high frequencies masquerading as low frequencies. Using a computer one must use a filtering that is at least half of the sampling rate (Nyquist Window). We can also use filters to filter (high pass) out low frequency noise that we know could not be signal.

An A/D converter generally utilizes a clock input and logic from negative feedback operational amplifiers. These measure voltage at timepoints and they are then stored.

Measuing voltages.

The measurement of voltage fluctuations in the brain involves the amplification and examination of the potential difference between two points. Neurons are electric dipoles (sort of like a magnet with one positive end and one negative end). The measured potential, with respect to ground or another electrode, will depend upon the angle made with the dipole. A dipole is not necessarily one dimensional, and with layers of cells there is in face a dipole layer. The measured potential could be positive, negative or zero depending upon the position of the electrode. As the geometery of the dipoles becomes more complex, the waveforms become more complex. Monopolar electrodes detect voltage at one tip, but produce some problems if the tip is not exactly in the right position. One of the easiest methods of recording evoked responses involves the use of a bipolar electrode that locates the point between generators of electrical activity.

Bipolar electrodes: These electrodes are constructed by twisting two coated (insulated) wires together and separating the tips such that one is slightly lower than the other. One of the most important characteristics of an electrode is the ability it has to transform the electric field and current circulating in the tissue into voltages available for amplification. So, you want conducting metals that are not reactive with the tissue in the brain. You also want the electrodes to be insulated with a thin material that is also inert and covers all of the electrode but the tip. We use bipolar recording and stimulating electrodes constructed from stainless steel wire that is covered with teflon. The electrodes should also have a tip separation that reflects the kinds of tissue and dipole layers that one is expecting to record from (or that one would like to stimulate directly). The neocortex is about 2mm thick and to record a vertically oriented dipole layer that reflect voltage changes in the cortex, the electrode tips should be separated by about 1mm. The other smaller subcortical structures within the brain require a smaller tip separation of about 0.5mm or less. Horizontal dipoles may also require smaller tip separations with the electrode oriented in a specific direction.

Dipoles will cause you to see positive and negative waves that are normally described as being surface positive or surface negative. The surface portion of the description is describing the surface of the structure (generally top of the structure, unless otherwise stated). The sign of the potential can be predicted from the orientation of the neurons. Structures with dendrites projecting to the top of the structure show a surface negative wave during depolarization of the dendrites (inward current/sink) and a positive wave during an action

potential (outward current/source).

Monosynaptic vs. Polysynaptic responses. The postsynaptic response to stimulation may be direct or indirect. If we recall that mono means one, and that poly means many, the definitions of the number of synapses between two structures of interest becomes a bit more obvious. There are two ways that one can use electrophysiology to determine if connections are monosynaptic or polysynaptic. The first way includes using latencies. Monosynaptic connections pass signals faster than polysynaptic connections. Thus, in a trace, those events that occur early on (within the first 10 msec) are likely to be a result of monosynaptic connections, and those that occur later are more likely to be a result of polysynaptic connections. The second way to determine if connections are monosynaptic or polysynaptic is to use the 'frequency-of-following' test. This test causes a failure of polysynaptic responses at a frequency above about 100Hz. The test requires that connections be examined using a series of trains at increasing frequencies. A direct connection is indicated by high frequency following with a constant delay (Berry & Penreath, 1976). Here following refers to the fact that with a direct connection the evoked response continues to be evident despite the trains. A failure (flattening of the response) indicates that the connection may be polysynaptic because it suggests that there is not enough time for transmitter to be released and taken up before another stimulation pulse arrives. We know that this occurs quicky within a single synapse, but with multiple synapses, it must occur at each synapse. Thus, failure suggests that multiple synapses must be transversed.

LTP

Long-term potentiation refers to a change in synaptic efficacy between neurons that is relatively long lasting, but occurs after a relatively short event. LTP can occur as a result of high frequency stimulation being delivered to an input pathway.

Hebb proposed a rule for such changes in 1949. He proposed that "When an axon of cell A is near enough to excite cell B, and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased" (p.62). There was, however, no firm experimental evidence for such an activity dependent modification for over 20 years. The first demonstrations of a phenomenon that would subsequently be shown to have Hebb-like properties were provided by Bliss and colleagues (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973). They were able to induce long-lasting increases in the strength of synaptic responses in the hippocampal formation of both anaesthetized and freely moving rabbits. Evoked responses in the dentate gyrus were found to be markedly enhanced following an application of brief, high frequency stimulation to the perforant path. The notion is that the first pulse produces a binding of glutamate to the postsynaptic membrane, subsequently calcium channels open and calcium enters the terminal causing a depolarization. This depolarization primes the NMDA receptor by removing the magnesium blockade of the channel. A second pulse activates the NMDA receptor and additional calcium and sodium are able to enter the call causing a cascade of intracellular events that act to increase synaptic efficacy.

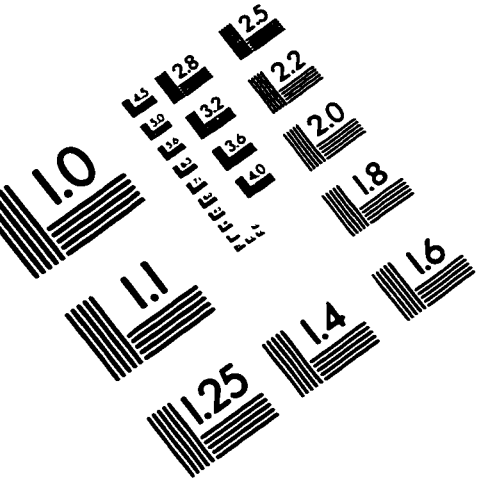
There are a number of features of LTP that make it attractive as a memory mechanism. First, it is long-lasting. LTP has been shown to last up to 5-6 weeks in the non-neocortical areas tested (Racine, Milgram, & Hafner, 1983), and longer in neocortical areas (Racine, Chapman, Trepel, Teskey, & Milgram, 1995). LTP can also be induced rapidly in non-neocortical areas, reaching asymptotic levels within a single session of multiple stimulations. Perhaps the most important features of LTP are that it is specific to the activated pathway and it shows associative properties. Associativity refers to the interaction between co-active pathways. Synapses that are normally unable to elicit LTP on their own, are capable of showing LTP when their activation is paired with the activation of stronger inputs.

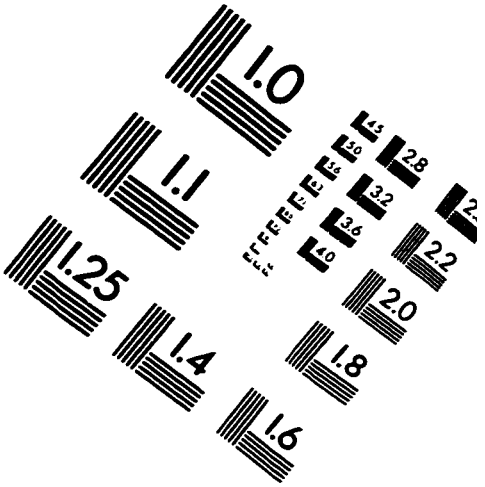
Paired-Pulse Tests

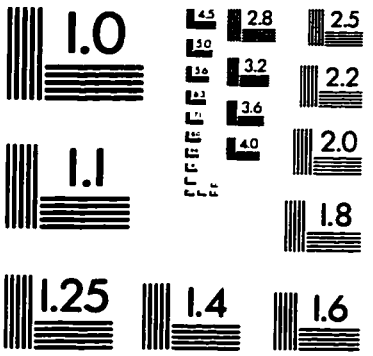
Paired pulse tests can be used to study inhibition or facilitation. Specifically they can be used to measure the strength of inhibitory recurrent circuits (c.f. Robinson & Racine, 1986). Inhibition/facilitation is reflected in the amplitude of the second (test) potential relative to a first (conditioning) potential. The strength of the increase or decrease in amplitude is dependent on the interpulse interval between the two pulses. In each test, a series of pulses are delivered with different interpulse intervals. The effect of the first pulse on the amplitude of the response evoked by the second pulse is assessed by determining the proportion of the response amplitude relative to that of the response evoked by the first pulse. Smaller amplitudes reflect inhibition and larger amplitudes reflect facilitation.

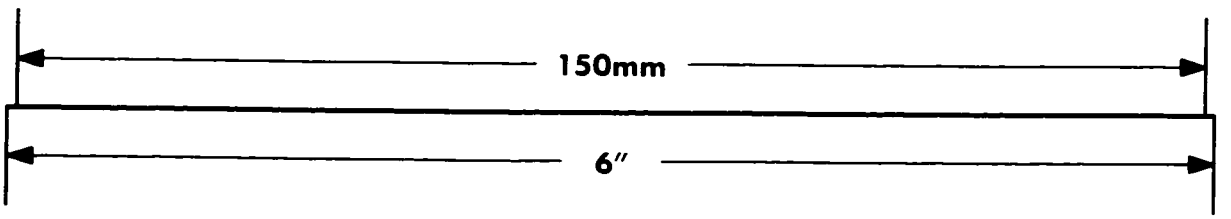
If a first pulse triggers cell discharge, then a subsequent test pulse falling within a period of active inhibition would be depressed (Robinson & Racine, 1986); pulses falling outside of this period may result in facilitation if the postsynaptic terminal is still depolarized.

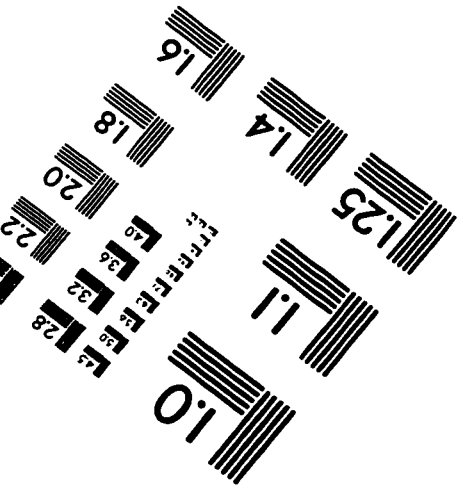
IMAGE EVALUATION TEST TARGET (QA-3)

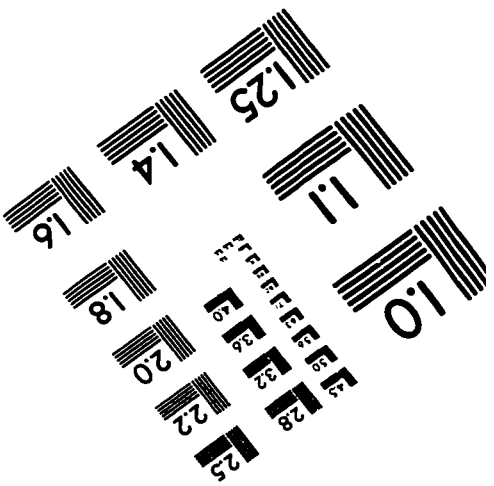












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