UNIVERSITY OF ALBERTA

GENETIC INFLUENCES ON HIPPOCAMPAL AND AMYGDALAR LONG-TERM POTENTIATION AND MEMORY

by

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Abstract

Inbred mouse strains differ in genetic makeup and exhibit diverse learning and memory phenotypes. Studying inbred mouse models of memory impairment can reveal genetic, molecular, and cellular correlates of learning or memory deficits. One candidate cellular mechanism for some forms of learning and memory is long-term potentiation (LTP), an activity-dependent enhancement of synaptic strength. LTP in the hippocampus and amygdala has been correlated with memory function in these brain structures.

Here, genetic variation between inbred mouse strains was utilized to explore the roles of molecules and brain structures in LTP and memory. LTP and short-term plasticity were measured in slice preparations of amygdalar and hippocampal tissue from inbred strains. These measurements were compared with hippocampus- and amygdala-dependent forms of fear memory, and with neural levels of aminergic transmitters.

In a mouse model without an intact hippocampal commissure, deficits in short-term synaptic plasticity and contextual fear extinction were found, although hippocampal LTP and contextual (hippocampus-dependent) fear memory were intact. In other inbred strains, LTP deficits in hippocampal area CA1, but not the medial perforant pathway, correlated with impaired contextual fear memory. LTP deficits in the basolateral amygdala correlated with impairments of amygdala-dependent cued fear memory. Finally, low neural levels of norepinephrine correlated with reduced LTP in hippocampal area CA1, and acute application of a ß-adrenergic receptor agonist during tetanization rescued this LTP.

Thus, the study of LTP and memory in inbred mouse strains has revealed that absence of the hippocampal commissure can lead to subtle changes in hippocampal

information processing, and that LTP deficits in the hippocampus and amygdala correlate with impairments in contextual and cued fear memory, respectively. It has also demonstrated that reduced neural norepinephrine can influence LTP in hippocampal area CA1. Overall, these findings illustrate that inbred mouse strains may be used to investigate the roles of molecules in LTP, and the role of LTP in memory. This thesis provides evidence that some forms of LTP likely underlie specific types of memory, and supplies important information about the phenotypes of inbred strains that should be considered by investigators wishing to use such strains to produce genetically-modified mice.

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5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine (serotonin)
AB	accessory basal amygdaloid nucleus
AC	anterior commissure
ACSF	artificial cerebrospinal fluid
ADHD	attention-deficit hyperactivity disorder
ahl	age related hearing loss 1 mutation
AMPAR	a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AP5	D(-)-2-amino-5-phosphonopentanoic acid
Arc	activity-regulated cytoskeletal protein
B	basal amygdaloid nucleus
ß-AR	ß-adrenergic receptor
BLA	basolateral complex of the amygdala
cAMP	adenosine 3', 5'-cyclic monophosphate
cGMP	guanosine 3', 5'-cyclic monophosphate
Ca^{2+}	calcium
CA	*cornu Ammonis*
CaMKII	Ca^{2+}/calmodulin protein kinase II
CC	corpus callosum
CE	central amygdaloid nucleus
CNS	central nervous system
COMP	catechol-O-methyltransferase
CP	caudate putamen;
CPP	D,L-3[(±)-2-carboxypiperazin-4-yl]- propyl-1-phosphonic acid
CR	conditioned response
CRE	cAMP response element
CREB	cAMP response element binding protein
CREB-P	phosphorylated CREB
CS	conditioned stimulus
DA	dopamine
DCF	dorsal commissure of the fornix
Dbh	dopamine ß-hydroxylase
DG	dentate gyrus

DMSO	dimethylsulfoxide
DOPAC	3,4-dihydroxyphenylacetic acid
E-LTP	early LTP
ERK	extracellular-regulated kinase
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
F	columns of the fornix
fEPSP	field EPSP
FSK	forskolin
GAP-43	growth associated protein 43
GCC	genu of the CC
G-protein	guanine nucleotide-binding regulatory protein
GABA	γ-aminobutyric acid
HbC	habenular commissure
HC	hippocampal commissure
HFS	high-frequency stimulation
HPLC	high-pressure liquid chromatography
HRP	horseradish peroxidase
IBMX	3-isobutyl-1-methylxanthine
I/O	input-output
i.p.	intraperitoneal
ISO	isoproterenol
ITI	inter-train interval
ITM	intermediate-term memory
K^+	potassium
KW	Kruskal-Wallis
L-LTP	late LTP
LA	lateral amygdala
LTD	long-term depression
LTM	long-term memory
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
MAPKK	mitogen-activated protein kinase kinase
MAO	monoamine oxidase
MARCKS	myristoylated alanine-rich C kinase substrate
MECS	maximal electroconvulsive shock
MF	mossy fibre
Mg^{2+}	magnesium

mGluR	metabotropic glutamate receptor
MPP	medial perforant pathway
MRI	magnetic resonance imaging
mRNA	messenger RNA
MTL	medial temporal lobe
MWM	Morris water maze
NE	norepinephrine
NMDAR	N-methyl-D-aspartate receptor
PB	Probst bundle
PBS	phosphate buffered saline
PDE	phosphodiesterase
Pde6brd	phosphodiesterase 6b
PKA	cAMP-dependent protein kinase (protein kinase A)
PKC	protein kinase C
PIR	piriform cortex
PMSF	phenylmethylsulphonyl fluoride
PPD	paired-pulse depression
PPF	paired-pulse facilitation
PTP	post-tetanic potentiation
QTL	quantitative trait loci
RIPA	radio immuno precipitation assay
RNA	ribonucleic acid
SC	Schaeffer collateral
SCC	splenium of the CC
SDS PAGE	sodium dodecyl (lauryl) sulfate polyacrylamide gel electrophoresis
SEM	standard error of the mean
STM	short-term memory
STP	short-term potentiation
TBS	theta-burst stimulation
UR	unconditioned response
US	unconditioned stimulus
VGCC	voltage-gated calcium channel

*CHAPTER 1:

Introduction

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A. Overview

In this thesis, I will describe my investigation of the brain processes that correlate with learning and memory in the mouse. Such study of learning and memory can be challenging – we can't directly observe what's going on in the mind of a mouse, nor ask it whether it remembers something. Thus, we are limited to observing its behaviour in order to deduce what sorts of processes it is capable of carrying out. Here, I chose to measure fear conditioning to gauge the memory capabilities of various types of genetically different mice. In order to investigate the neurobiological mechanisms that are involved in learning and memory in these mice, I focused on synaptic plasticity in the hippocampus and amygdala, two brain regions that are important for fear memory. My general approach in *Chapters 3*, *4*, and *5* was to identify particular types of fear memory impairments in mice, then search for alterations in hippocampal and/or amygdalar synaptic plasticity that correlated with the identified memory deficits. In *Chapter 6,* I focused on the study of hippocampal LTP in genetically different mice, investigating the role of a neuromodulatory transmitter, norepinephrine, in hippocampal LTP. In *Chapter 7*, I will discuss how the conclusions from *Chapters 3-6* can be considered together to shed light on the role of genetic variation in determining neurobiological and behavioural phenotypes.

In this chapter, I will provide a general overview of learning and memory, synaptic plasticity, and neuromodulation. I will describe the inbred mouse models in which I have carried out my studies, and will reveal the aims of my thesis research.

B. Learning and Memory

i. What are learning and memory?

Before examining my thesis work in depth, we should understand clearly what learning and memory are. Learning is the acquisition of knowledge, skill, or the tendency to exhibit a particular behaviour. Memory is the storage of this acquired information. This learned and stored information can modify subsequent behaviours. Here, I will focus on the neurobiology of learning and memory; that is, the biological and physiological means by which information is acquired and stored. First, we must assume that organisms can store information about themselves and the outside world in *internal representations*. These internal representations are structured versions of information that are stored in patterns of neural activity, and they can guide the behaviour of the organism (Dudai, 1989; Milner et al., 1998). Dudai (1989) defines learning as "an experience-dependent generation of enduring internal representations, and/or experience-dependent lasting modification in such representations" (p. 6). This definition illustrates that learning requires an experience that results in either the production of a new representation of information, or an alteration of an already-existing representation of information in the central nervous system (CNS). Dudai explains that memory is the retention of these experience-dependent internal representations over time, and that the use of memory requires a process for retrieval of the previously learned information. Dudai's definitions of learning and memory lead us to a commonly used description of three stages of memory: acquisition, consolidation, and retrieval (e.g., Abel and Lattal, 2001). Acquisition generally refers to the encoding of information into internal representations. Consolidation is the process by which this information becomes stored

in a stable, long-term format in the CNS. Retrieval refers to the extraction of this information from long-term storage, enabling it to enter the stream of consciousness and be used in current mental activities.

There are many types of learning and memory, and they have been studied in a wide range of organisms. Next, I will briefly review the major classifications of learning and memory in order to demonstrate how fear memory fits into this hierarchy.

Learning can be divided into two major types: non-associative and associative. Non-associative learning entails behavioural changes that do not require connections between pieces of information to be realized, and can involve changes in the behavioural response to a particular stimulus. Two prominent examples of non-associative learning are *habituation* and *sensitization*. Habituation is the reduction of a behavioural response to a non-noxious stimulus caused by repetition of that stimulus (Dudai, 1989). An example might be that a person is very much bothered by traffic noise upon moving from a small town to a big city, but gradually reacts less to traffic noise as time passes. Sensitization is the enhancement of a behavioural response to a non-noxious stimulus after experiencing a different, noxious, stimulus (Dudai, 1989). For example, after watching a horror movie, a person might react more strongly to loud noises, or be more "jumpy," for a period afterwards. Habituation and sensitization have been observed in a range of organisms – they seem to be evolutionarily conserved mechanisms (Dudai, 1989; e.g., Davis, 1972; Duerr and Quinn, 1982; Pinsker et al., 1970; Pinsker et al., 1973; Slater et al., 1985). Habituation may serve to prevent organisms from diverting too much attention to innocuous occurrences in their environments. Sensitization may serve as a

defensive mechanism that increases the responsiveness of an organism to a potentially dangerous situation.

In contrast to non-associative learning, in which an organism alters its response to a specific stimulus, associative learning involves making new connections between different items of information. Associative learning processes enable an organism to relate multiple stimuli and/or experiences to one another. Two good examples of associative learning are *instrumental* and *classical* conditioning.

Instrumental (or operant) conditioning uses a reinforcing stimulus to alter the strength or probability of a particular, often spontaneous, behavioural response (Staddon and Cerutti, 2003). For example, a dog might learn that when it barks, it will receive attention, a situation that encourages it to bark more often! The classic example of instrumental conditioning is Thorndike's *puzzle box*, in which cats learned to escape a wooden crate by pulling a lever, releasing a catch, or some other simple action (Chance, 1999; Thorndike, 1898). Skinner (1937) preferred the term "operant conditioning" and developed the *Skinner box*, which contained a lever that when pressed delivered a reinforcer (e.g., a food reward). Animals would learn the association between a lever press and the reinforcer. The reinforcer could be positive or negative; if positive, lever pressing would increase with exposure to the Skinner box (*reward conditioning*), and if negative, lever pressing would decrease (*aversive conditioning*) (Dudai, 1989).

The fear memory testing that I conducted in my thesis research was a form of classical conditioning. Classical conditioning paradigms are based on work done by Ivan Pavlov in the early 1900s. To classically condition an animal, it is presented with a *conditioned stimulus* (CS), which is generally neutral in character. The CS is presented

in association with an *unconditioned stimulus* (US) that yields an *unconditioned response* (UR). Pavlov (1927) studied dogs, and in his case the dog was presented with an auditory click or tone as the CS. The dog was presented with a meat and bread mixture as the US, and Pavlov measured salivation as the UR. After paired presentations of CS and US, the CS alone comes to elicit a *conditioned response* (CR) that is similar to the UR. Thus, after training a dog with CS-US pairings in Pavlov's paradigm, the presentation of the auditory stimulus would stimulate salivation in the dog, regardless of whether food was given. I will discuss the variations of classical conditioning, cued and contextual fear conditioning, that I used in my thesis research in *sections C.i* and *C.ii.*

ii. Multiple memory systems

In higher mammals, more complex types of learning and memory can be studied. This might include the formation of complex internal representations of multiple, interrelated pieces of information or events. These representations can include information from multiple and/or integrated sensory modalities, and can be used in novel situations unrelated to those in which the information was acquired. More intricate tests must be used to investigate these forms of learning and memory in higher mammals (e.g., rodents, monkeys, and humans). For example, mazes were developed for rats that allowed for testing of cognitive mapping of the environment (Small, 1901). Monkeys can carry out tasks that require remembering a particular stimulus for a delay period, then making a choice for reward based on the identity of that stimulus (e.g., Zola-Morgan and Squire, 1990). Humans can be tested for the recall of facts and experiences (e.g., see Squire and Zola, 1998). Specific types of memory have been classified into different memory systems that each rely on particular brain regions. Here I will briefly discuss the

cases of episodic, emotional, and procedural memory to illustrate memory systems operating in parallel.

Dissociation studies are of value in distinguishing multiple memory systems from one another. The premise of a dissociation study is to examine multiple types of memory in an animal with bilateral lesions of a particular brain structure. Within the same study, different groups of animals may receive lesions to different brain regions, then the animals are all tested on the same battery of memory tests to isolate which brain regions have roles in each memory test. McDonald and White (1993) carried out a triple dissociation study that discriminated the brain regions involved in episodic, emotional, and procedural memory in rats. Different groups of rats were given lesions of the fornix (which reduces hippocampal activity), the lateral amygdala, and the dorsal striatum. They were then tested on three separate versions of the radial arm maze. The first was the classic "win-shift" version of the 8-arm radial maze that tests episodic memory. Episodic memory generally refers to memory for events, including their spatial and temporal context (Squire and Zola, 1998). Each arm of the maze was baited with a food reward, and visual cues were placed outside the maze. Upon being placed in the maze, the optimal performance of the rat would be to enter each arm only once to obtain the reward, rather than entering an arm again from which the reward has already been consumed. This task tests episodic memory because the rat must remember which arms it has just visited, within that particular trial, in order to avoid entering the same arm twice. This might be akin to humans remembering which chores they have already completed today, or which stores they have most recently visited. The information that must be remembered in both the "win-shift" version of the maze, and in these examples

of human episodic memory, is associated with a distinct time and context. Rats with fornix lesions committed significantly more errors (entrances to arms in which the reward had already been consumed) than rats with amygdala or striatum lesions. These experiments showed that the fornix, and by extension the hippocampus, are required for episodic memory.

The second memory test conducted by McDonald and White (1993) was the "win-stay" version to test procedural/response learning. Response learning involves the incremental acquisition of a stimulus-response association or a habit (Packard and Knowlton, 2002). In this version of the maze, all eight arms were open, but only four had a light cue at the end that signaled the presence of a food reward. Errors were counted as entrances to unbaited arms. Thus, on this task, rats were required to acquire an entry response to lit arms, but not unlit arms, in order to retrieve a reward. Rats with lesions to the striatum committed significantly more errors than rats with fornix or amygdala lesions. Thus, the striatum was deemed to be necessary for procedural, or response, learning. Knowlton et al. (1996) conducted a test of procedural learning in humans that also found that the striatum is necessary for habit learning. Here, Parkinson's patients, which suffer a major loss of input to the striatum, underwent training on a weather prediction task in which the aim was to make a weather forecast based on combinations of cue cards. In this task, patients were shown two or three such cards at a time, and were asked to predict rain or shine. Each card in fact indicated rain or shine with a particular probability, but this information was not conferred to the participants. Further, the actual probability of rain or shine depended on the conjoint probabilities of all cards presented at once (usually 2-3). After each trial, participants received feedback as to whether their

prediction had been correct or not. In control subjects, their ability to correctly predict the weather from the cue cards improved from about 50% to 70% correct over 50 trials. However, Parkinson's patients with altered striatal function did not exhibit this improvement in prediction ability. Thus, the striatum is necessary in both humans and rodents for response and habit learning, evidenced by impairments of the gradual acquisition of patterns of information in striatal lesioned rats and Parkinson's patients.

McDonald and White's (1993) third radial arm maze test was termed the "conditioned cue preference" task, and examined emotional memory. Emotional memory is memory for emotional experiences (Rodrigues et al., 2004). Here, only two arms of the 8-arm maze were utilized. One arm was brightly lit and the other dimly lit. Rats received several training trials, and in each they were confined to one of the arms of the maze. Half of the trials were spent in the brightly lit, and half in the dimly lit, arm. One of the arms was consistently baited (in relation to the lighting conditions) with an abundance of food, and the other with no food. After the training trials, both arms were opened (and not baited) and rats were allowed to explore the two arms as they wished. Time spent in each arm was measured. Rats with reduced hippocampal activity and striatum lesions spent more time in the arm that was baited during training trials than rats with amygdala lesions. These results indicated that the amygdala is necessary for emotional memory: the rats developed an affective preference for the lighting conditions that yielded food rewards. Another classic example of emotional memory is fear conditioning, in which a neutral stimulus comes to elicit fearful responses by virtue of being paired with an aversive stimulus (Phelps, 2004). For example, Bechara et al. (1995) conducted a fear conditioning experiment in humans in which a visual slide, or a

particular tone, was presented, followed by a very loud, startling, boat horn noise. Following this presentation, Bechara et al. (1995) measured the galvanic skin response of control and amygdala-damaged patients in response to presentation of the visual slide or tone only. This measurement indicates whether patients exhibited increased sweating in response to the stimulus, a response that can occur when patients anticipate (even unconsciously) an unpleasant or fearful occurrence. Control patients exhibited increased sweating in response to the slide or tone, whereas amygdala-damaged patients did not. This result indicates that control patients learned that the visual slide or auditory tone might predict subsequent sounding of the boat horn, whereas amygdala-damaged patients did not. This study provided further evidence that the amygdala has a role in memory for emotionally-charged information, namely, fear of the startling boat horn that was associated with the more innocuous visual slide or auditory tone.

In conclusion, we have evidence that three memory systems exist that rely on processing in different brain regions. In addition to the triple dissociation study conducted by McDonald and White (1993), further double-dissociation experiments have also been conducted that support the notion that these three memory systems rely on different brain regions in the human brain (Bechara et al., 1995; Knowlton et al., 1994, 1996). Finally, the studies of amnesic patient H.M.discussed in *section B.v* were instrumental in initiating the notion that there are multiple memory systems.

iii. Phases of learning and memory

There is variability in the literature in how experimenters describe the phases of learning and memory. Here, I will adopt a description that includes four phases: *sensory* memory, *working* memory, *short-term* memory (STM), and *long-term* memory (LTM).

The first phase of memory, sensory memory, refers to the intial processing of sensory stimuli entering the CNS and generally lasts less than one second (Baddeley, 1996). Examples include *iconic* memory (visual system) and *echoic* memory (auditory system) (Craik, 1979). Information that has been paid attention to while in sensory memory can transfer to working memory (Craik, 1979), which lasts 10-30 seconds. Working memory has a limited storage capacity, and if new pieces of information come along and are attended to, they will replace the information that was already in the working memory store (Baddeley, 1996; Craik, 1979). The prefrontal cortex plays a role in mediating working memory (Goldman-Rakic, 1996).

STM and LTM operate on a longer time-scale. Depending on the organism in question, STM can be considered to last minutes to several hours, and LTM can last hours to years, even a lifetime (McGaugh, 2000). STM may function to provide an interim store of information that can be accessed while processes prepare LTM storage in the CNS, although there is also evidence that STM and LTM are independent processes that operate in parallel, not in series (Izquierdo and McGaugh, 2000). For long-term storage, on the order of days to years in the mammalian brain, a process termed *memory consolidation* occurs (McGaugh, 2000). Memory is initially mediated by the specific structures within a particular memory system (e.g., the hippocampus or amygdala in the above examples). Once a memory trace is stabilized in these brain structures, interactions between memory system brain structures and the neocortex allow information to be transferred to the neocortex and slowly stabilized there. Therefore, the neocortex is the site of long-lasting, or permanent, storage of information. This process of consolidation to the neocortex is very slow – it may take years (Alvarez and Squire,

1994; Lechner et al., 1999; Marr, 1971; McGaugh, 2000). The best evidence for the time scale of this process is the occurrence of temporally-graded retrograde amnesia in patients that have lost the medial temporal lobe (Squire and Alvarez, 1995). The medial temporal lobe includes the hippocampus and surrounding cortical areas that are involved in the episodic memory system (Eichenbauma and Cohen, 2001). These patients exhibit greatest memory loss for events that occurred just prior to brain trauma, and less memory loss for events that occurred long before brain trauma. This indicates that events that occurred long ago have become independent of medial temporal lobe processing, and are stored in the cerebral cortex. However, the idea that hippocampal damage results in temporally-graded (rather than flat) retrograde amnesia has been disputed (Nadel and Moscovitch, 1997; discussed by Knowlton and Fanselow, 1998)

iv. A classification system for LTM

LTM can be classified into two major types: *explicit* (also called declarative) and *implicit* (also called non-declarative) memory (see **Figure 1.1**; Graf and Schacter, 1985; Schacter, 1987; Squire and Zola, 1996). Explicit memory is what people generally think of as "memory," and can be considered *knowing something*. It can involve remembering and consciously recalling facts, experiences, and autobiographical knowledge.

Explicit memory can further be divided into *episodic* and *semantic* memory (Tulving 1972, 1992). Episodic memory was defined in *section B.ii*: an example might be knowing that you ran into your aunt at the store yesterday. In contrast, semantic memory is a store of general knowledge, like a personal encyclopedia. This type of memory does not contain information about how you acquired that knowledge – the episodic qualities of the information have been lost. An example might be knowing how

to get to the store, based on your previous experiences of visiting the store. Particular episodes can contribute to semantic memory – it is thought that commonalities between different episodic memories can be bound together to create a semantic memory that can be generalized to other situations (Eichenbaum and Cohen, 2001).

Implicit memory, on the other hand, is a more indirect form of memory in that it requires no conscious recollection of learning (Schacter et al., 1993). It can be thought of as *knowing how to do something*. Implicit memory might include motor, perceptual, or cognitive skills (e.g., riding a bike, playing the piano, reading). Implicit memory can also include learning and remembering procedures, as well as habit learning and priming (Schacter et al., 1993). Habit learning refers to the acquisition of an association in which a neutral stimulus comes to elicit a particular behaviour via repeated reinforcement (Salmon and Butters, 1995), and can be considered response learning like that described by McDonald and White (1993) and Packard and Knowlton (2002). Repetition priming facilitates the identification of words or pictures from fragments when their intact versions have been observed previously (Schacter et al., 1993). An example might be a word stem completion test in which subjects first study a list of words, and subsequently are asked to complete word stems, which are strings of about three letters that can serve as the beginning of several different words. Priming is evident when subjects preferentially complete the word stems with words form the study list, rather than other words that were not present on the list (e.g., Graf et al., 1984). Classical conditioning, including the fear memory tests I conducted in my thesis research, is also a form of implicit memory.

v. Amnesia and the medial temporal lobe

In 1957, Scoville and Milner published the landmark study of amnesic patient H.M. H.M. suffered from severe epilepsy, and Dr. Scoville performed experimental surgery to remove the epileptic locus. At age 27, H.M. underwent bilateral medial temporal lobe (MTL) resection. This surgery was effective in treating H.M.'s epilepsy, but had an unexpected side effect. H.M. was unable to form long-term memories of events that occurred after his surgery (anterograde amnesia) (Scoville and Milner, 1957). He was unable to remember new people that he met, whether he had eaten meals that day, short stories, or lists of words and numbers (Milner et al., 1968; Corkin et al., 1984). H.M. also exhibited temporally-graded retrograde amnesia: he lost recollection of the events for several years of his life prior to surgery, and memory loss was the most severe for more recent time points prior to surgery (Corkin et al, 1984). Aside from this memory loss, H.M retained intact perceptual, cognitive, and motor functions (Milner et al., 1968). And importantly, H.M. was happy to participate in research on his amnesia. As such, H.M. has been studied in depth for nearly 50 years in an effort to understand the types of memory affected by amnesia, and the roles of particular brain structures in memory function (reviewed by Corkin, 2002).

Some of the most valuable information gained by studying patient H.M. was the identification of types of memory spared by damage to the MTL (reviewed by Corkin, 2002). His working memory was spared – he could remember something as long as he recited it or kept thinking about it, and had a normal digit span (digit span refers to the number of items that may be held in working memory at any one time). The semantic knowledge that H.M. had acquired prior to surgery was unaffected, although he was

impaired in acquiring new knowledge. Priming, skill learning, and classical conditioning were also intact. The patterns of impaired and intact memory in patient H.M. were evidence for different memory systems that likely relied upon different brain regions. The general conclusions from studies of H.M. and other amnesiacs are that MTL damage results in loss of explicit, or declarative memory, although it spares implicit, or non-declarative, memory (Squire and Zola, 1996). **Figure 1.2** illustrates the connectivity of the brain structures thought to contribute to the declarative memory system, including the hippocampus, entorhinal cortex, perirhinal cortex, and parahippocampal cortex (all within the MTL). Another important conclusion from these studies is that the temporally-graded pattern of retrograde amnesia due to MTL damage indicates that memory is consolidated to brain structures outside the MTL over a long period of time after the experience in question.

Because multiple brain structures were bilaterally damaged in H.M., there was still uncertainty as to damage of which structures in particular were responsible for the ensuing amnesia. Scoville and Milner (1957) described the regions of lost brain tissue, estimated from surgery, and MRI analysis has confirmed the locations of bilateral tissue loss in H.M. (Corkin et al. (1997). The rostral regions of the hippocampus and entorhinal cortex were destroyed, and parts of the rostral perirhinal cortex may have been lost. In addition, the amygdala was completely removed. As such, we can only claim that the amnesia suffered by H.M. is due to MTL damage in general, and not due to specific structures like the hippocampus.

Ensuing animal studies attempted to confirm the role of the MTL in memory. In primates, most studies aimed at replicating the brain damage that H.M. suffered, and

devising behavioural tests that revealed anterograde and temporally-graded retrograde amnesia in these primate models. The delayed non-matching-to-sample task revealed anterograde amnesia in MTL lesioned monkeys (Zola-Morgan and Squire, 1985). This task involves presenting a monkey with a stimulus (like an object or a picture). After a delay, the same stimulus was presented along with a different, novel, stimulus. Monkeys were rewarded if they chose the novel, but not the old, stimulus. Intact monkeys could perform this task quite well, but monkeys with MTL lesions were not able to remember the first object for longer delays, and exhibited reduced performance. Zola-Morgan and Squire (1990) also measured temporally-graded retrograde amnesia in monkeys using an object discrimination task. Monkeys were taught to prefer one object over another (by reward) in the months prior to receiving an MTL lesion. The same monkey was taught multiple object pairs at different times prior to brain damage. After recovery from surgery, preference between the old object pairs was tested. Monkeys were generally most impaired in remembering the object pairs they had learned most recently, mimicking the memory patterns observed in human MTL amnesics (see Squire et al., 2001 for review of similar studies in other animals). These studies, however, did not shed light on the roles of particular structures within the MTL in memory.

vi. The hippocampus and memory

Further human case studies since the publication of the case of H.M. have elucidated a specific role for the hippocampus in learning and memory. The case studies of P.B. and R.B. were especially enlightening concerning the role of the hippocampus in memory. Patient P.B. (Penfield and Milner, 1958; Corkin et al., 1997) underwent a unilateral temporal lobe resection for epilepsy, in which the left entorhinal cortex, most

of amygdaloid complex, and the anterior half of hippocampus were lesioned. Upon autopsy, it was found that the right hippocampus was shrunken, although the right entorhinal cortex and amygdala were intact. Thus, the only brain structure that was damaged bilaterally was the hippocampus. Patient P.B. exhibited less severe memory impairments than patient H.M., but still exhibited anterograde and temporally-graded retrograde amnesia. Thus, bilateral damage restricted to just the hippocampus can result in memory loss. Patient R.B. (Zola-Morgan et al., 1986) suffered an ischemic episode that resulted in bilateral damage to hippocampal area CA1, a subregion of the hippocampus. Patient R.B. exhibited clinically significant anterograde amnesia, but little retrograde amnesia. This case study provides further evidence that the hippocampus alone has a role in declarative memory. The overall pattern seen in case studies of amnesia is that hippocampal damage alone can cause memory impairment, but in general the greater the damage to the MTL, the more severe the resultant amnesia (Squire and Zola, 1997).

Rat studies have furthered our knowledge of the role of the hippocampus in memory. From these studies, two major theories about the role of the hippocampus in memory have emerged. The first centers on O'Keefe and Nadel's (1978) landmark book, "*The Hippocampus as a Cognitive Map*," and the second suggests that the hippocampus has a role in encoding relationships between stimuli (*relational memory*). The theory that O'Keefe and Nadel put forth proposes that the role of the hippocampus is to form and use cognitive, spatial maps of the environment. They postulated that the hippocampus could represent places in terms of distances and directions between items in environment, and could encode rough topological maps of multiple environments. Animals could use these

maps for navigation, and could infer locations of objects that are out of sight by using the map.

Two major lines of evidence support O'Keefe and Nadel's theory. Firstly, damage to the hippocampus can cause impairments in spatial learning. The best example of this is the requirement of the hippocampus for the water maze task created by Richard Morris (1982). An overview of the procedures used in the Morris Water Maze (MWM) is illustrated in **Figure 1.3**. A circular pool is filled with opaque water that covers a hidden platform that is anchored to the bottom of the pool. Extramaze visual cues are mounted around the pool at fixed positions. Each rodent is given a series of *training trials* in which it is placed in the pool facing a wall such that its heading is not biased when it starts to swim. It is given about 60 sec to swim around the pool and find the hidden platform. If the rodent does not find the platform within that time it is placed on the platform. The starting position is randomized across trials so that the animal must rely on spatial cues, and not on a static sequence of movement, to find the platform. Over the series of training trials, successful rodents will decrease the time and path length that they swim in order to reach the hidden platform (learning).

A *probe trial* is given after the last training trial. The hidden platform is removed from the pool and the rodent swims freely. The path that the rodent swims is tracked and analyzed for the proportion of swim time and/or path length spent in each quadrant of the pool, swim speed, and the number of times the path crosses the former location of the hidden platform. A rodent that has learned and remembered the location of the hidden platform will spend significantly more time swimming in the quadrant that contained the

hidden platform, and it will cross the former location of the platform significantly more times than it crosses corresponding locations in other quadrants.

Many experimenters also conduct a *visible platform task* in which the motor ability and visual acuity of the rodent (both important in MWM performance) are tested. This task is similar to the training trials of the MWM, but the platform is made visible to the rodent.

Morris et al. (1982) demonstrated that rats without an intact hippocampus were unable to learn the hidden platform version of the task at the same rate as control rats, and could not remember the location of the hidden platform after a delay as measured with the probe trial. This supports the idea that the hippocampus is required for forming cognitive/spatial maps. These rats could perform the visible platform task quite well, however, indicating a selective impairment in spatial learning and memory due to loss of the hippocampus, rather than reduced performance due to motor, sensory, or motivational impairments.

The second line of evidence that supports O'Keefe and Nadel's theory of the hippocampus as a cognitive map is the discovery of *place cells* in the hippocampus (O'Keefe and Dostrovsky, 1971). Place cells are primary neurons in hippocampal areas CA1 and CA3 that increase their firing rate when an animal is in a particular location (Best et al., 2001). The region of an environment in which a single place cell fires selectively is called its *place field.* In rats, place fields are usually 30-50cm in diameter, and fields of multiple cells may overlap (reviewed by Rosenzweig and Barnes, 2003). As few as 50 place cells can encode a 1 m^2 environment (Wilson and McNaughton, 1993). In adult rats, a cell will generally have the same place field every time it enters an

environment (Rosenzweig and Barnes, 2003). With these properties, it is conceivable that place cell activity is a mechanism by which the hippocampus could encode cognitive maps of the animal's environment.

The second prevalent theory of the role of the hippocampus in memory is that it facilitates declarative and relational memory (Eichenbaum and Cohen, 2001). The idea here is that the hippocampus is involved in both the episodic and semantic components of declarative memory. The hippocampus has a role in generalizing across multiple episodes to create semantic memories, and in forming new associations between representations. Eichenbaum and Cohen (2001) have proposed that memories are organized in a "memory space." In this memory space, episodic memories are interleaved into a stable semantic memory structure. A key feature of Eichenbaum and Cohen's proposal is that memories stored in this way can be used inferentially in novel situations, a characteristic they term "representational flexibility." This characteristic allows for comparing memories, flexible and inferential use of memories, the recovery of memories in different contexts, and retrieval of memories via cues.

Two behavioural tests in rats exemplify relational memory. The first is termed the "paired associate task" (Bunsey and Eichenbaum, 1996). In this task, rats were trained to associate odours with each other (**Figure 1.4**). For instance, an odour "A" was presented, and then the rat was given a choice between odours "B" and "Y," with only the choice of odour "B" yielding a food reward. Alternately, if odour "X" were presented first, the rat was then given a choice between odours "B" and "Y," with the choice of odour "Y" yielding a food reward. After learning these initial pairings ("A-B" and "X-Y"), rats went on to learn extensions of these odour-reward pairs. They were presented

initially with odour "B" then given a choice between new odours "C" and "Z" with odour "C" yielding a reward. The analogous new pairing of "Y-Z" was also trained. Bunsey and Eichenbaum (1996) found that rats with intact or lesioned hippocampi could readily learn these odour pairings, but rats with hippocampal lesions could not perform the "transitivity" version of the task. Here, rats were required to form network representations of the two series of odours, "A-B-C" and "X-Y-Z," such that they would choose "C" for reward when presented with "A," and would choose "Z" for reward when presented with "X." Rats were never explicitly trained on the "A-C" and "X-Z" pairings, but had to infer them based on the previously learned pairs. These experiments showed that the hippocampus is required to form network representations by relating different, overlapping associations with one another, and to remember and use this information in new situations.

A second task for rodents that exemplifies relational memory is the social transmission of food preferences task (Bunsey and Eichenbaum, 2005). This task takes advantage of the ethological behaviours of rats by testing their ability to remember the likelihood that a food source is safe to consume. A "demonstrator" rat consumes a scented food, and then interacts with an "observer" rat. After a delay, the observer rat is given a choice between food the demonstrator ate (same scent), or a food with a novel scent. If the observer rat exhibits a preference for the food scented like that the demonstrator ate (by eating more of it than the novel-scented food), this indicates that it learned the association between the scent of the food on the observer rat's breath and its safeness as a food source, and has remembered this association for the delay period. This is an example of relational memory because the observer rat first must infer that the food

scent is safe, and must express this association flexibly in a situation that is different from the learning event. Rats with lesions of the hippocampus express impairments on a 24-hr memory test on this task, reinforcing that the hippocampus is required for relational memory.

vii. Hippocampal anatomy

The hippocampus is a brain structure within the forebrain of the mammal that is continuous with the cerebral cortex and contains several layers. The hippocampus can be divided into several major subregions, including the *dentate gyrus*, the *subiculum*, and areas *cornu Ammonis* 1 (CA1), CA2, and CA3. In the CA regions, the layer *stratum pyramidale* contains the cell bodies of the primary neurons of the hippocampus, the pyramidal cells. Pyramidal cells are multipolar, and extend basal dendrites into *stratum oriens*. The apical dendrites of the pyramidal neurons extend into *stratum lucidum*, *stratum radiatum,* and *stratum lacunosum moleculare* (Johnston and Amaral, 2004). In the dentate gyrus, the primary neurons are granule cells located in the granule cell layer. Granule cells are monopolar. Their dendrites extend into a molecular layer that is nearly devoid of cells, and their axons (called *mossy fibres*) extend into the hilus of the dentate gyrus. For the most part, the cell layers of the CA1 regions and dentate gyrus also contain interneurons, which may be excitatory or inhibitory. These interneurons have a role in synchronizing oscillations at different frequencies in the CA regions, including the theta (~5Hz) and gamma (~40Hz) rhythms (Johnston and Amaral, 2004).

Here, I will focus on the trisynaptic pathway of the hippocampus (see **Figure 1.5**), which is excitatory, and emanates from the primary neurons of the hippocampal subregions. The trisynaptic pathway consists of the *perforant* pathway, which is the

input pathway to the dentate gyrus from neurons in layer II of the the entorhinal cortex (Johnston and Amaral, 2004), the *mossy fibre* pathway, which consists of axonal projections from the dentate gyrus to area CA3, and the *Schaeffer collateral* pathway, which consists of axonal projections from area CA3 to area CA1 (Eichenbaum and Cohen, 2001). Area CA1 pyramidal neurons then send axons out of the hippocampus through the subiculum to the entorhinal cortex (*alvear* pathway) and also to the fornix (Johnston and Amaral, 2004). It is also important to note that there are other influences on area CA1 and CA3 pyramidal cells in addition to the mossy fibre and Schaeffer collateral pathways. First, there are recurrent synapses of CA3 axons back to CA3 pyramidal cells (*association fibres*) (Johnston and Amaral, 2004). Also, *commissural fibres* enter the hippocampus via the hippocampal commissure, and synapse onto primary neurons and interneurons in areas CA3 and CA1 (see *section G.i* for more details). These commissural fibres consist primarily of axonal projections from area CA3 pyramidal cells of the contralateral hippocampus (Johnston and Amaral, 2004). Finally, the *temporoammonic pathway* contains fibres that project directly to area CA1 from neurons in layer III of the entorhinal cortex (Johnston and Amaral, 2004).

The hippocampus connects bidirectionally to many brain structures. The subiculum and area CA1 connect with the entorhinal cortex. The entorhinal cortex sends projections to, and receives projections from, the parahippocampal and perirhinal cortices (within the MTL), which interact with the cerebral cortex (see **Figure 1.2**; Burwell, 2000; Eichenbaum and Cohen, 2001). These connections are responsible for informational inputs and outputs to and from the hippocampus. The hippocampal formation also communicates bidirectionally with the septum and other subcortical areas via the fornix

(Eichenbaum and Cohen, 2001). In contrast with the direct connections to cortical areas, these connections via the fornix are primarily modulatory in nature (e.g., modulation due to attention, arousal, or biological rhythms; reviewed by Eichenbaum and Cohen, 2001). Finally, hippocampal area CA1 sends a direct projection to the prefrontal cortex (Swanson, 1981). In general, the hippocampus processes information and then sends it back to the same brain region that originally supplied that information (Eichenbaum and Cohen, 2001). These connections put the hippocampus in a situation in which it may influence information processing in many brain regions, and across many different modalities.

viii. The amygdala and memory

The amygdala has a role in mediating emotion, and is part of the limbic system (**Figure 1.6**; LeDoux, 2000). Selective loss of the amygdala results in *Kluver-Bucy* syndrome (Kluver and Bucy, 1937), which has been characterized best in monkeys (e.g., Prather et al., 2001), but also occurs rarely in humans. Those with Kluver-Bucy syndrome experience psychic blindness – a general loss of affect and blunting of emotional reactions. Loss of the amygdala can tame normally aggressive monkeys, and may result in social and sexual behavioural abnormalities (reviewed by Eichenbaum and Cohen, 2001; Prather et al., 2001). Patients with Urbach-Wiethe disease, a selective bilateral calcification of the amygdala, show deficiencies in emotional processing and memory (Markowitsch et al., 1994; Siebert et al., 2003). For example, patient S.M. was unable to identify fearful facial expressions (Adolphs et al., 1994, 1995). Also, patient H.M., whose amygdala was removed (Corkin et al, 1997), failed to react to several types of pain or discomfort, such as feeling burning temperatures, or due to medical issues. He

was also unable to gauge hunger very well – he rated his hunger the same before a meal, after a meal, and even after a second meal that he did not finish because he was "finished", but not because he was full (Hebben et al., 1985).

There is evidence that emotional memory depends on the amygdala, and operates independently from hippocampus-dependent, declarative memory. In addition to the radial arm maze experiments discussed in *section B.ii*, several other lines of evidence elucidate the role of the amygdala in memory. First, human case studies suggest that emotional memory remains intact in amnesiacs that have an intact amygdala. A classic example is a patient of Cleparde with Korsakoff's syndrome, which results in amnesia. Cleparde hid a pin in his hand such that when he shook his patient's hand, he pricked her hand. From then on she refused to shake his hand again, although she could not recall the episode (Eichenbaum and Cohen, 2001). Another interesting case is Damasio's patient Boswell, also an amnesic. Boswell developed preferences for new hospital staff based on positive or negative interactions with them, although he could not remember meeting them before (Tranel and Damasio, 1993).

Animal studies have revealed further roles of the amygdala in memory. For instance, experiments have shown that the amygdala has a role in learning stimulus-reward associations (Cahill and McGaugh, 1990). The most prominently studied type of amygdala-dependent memory in the rodent is fear memory. Cued and contextual fear conditioning depend on the amygdala, although contextual fear memory also depends on the hippocampus (Holland and Bouton, 1999; Kim et al., 1993; Phillips and LeDoux, 1992). Cued and contextual fear conditioning will be discussed in depth in *sections C.i* and *C.ii.*

ix. Amygdalar anatomy

The amygdala is located in the MTL, and is surrounded by the parahippocampal region (i.e., parahippocampal, perirhinal, and entorhinal cortices). It is comprised of a number of interconnected nuclei, including the basal, central, lateral, and medial nuclei (see **Figure 1.7**; Pitkanen et al., 2000; reviewed by LeDoux, 2000). Many inputs into the amygdala arrive at the lateral nucleus. For instance, auditory information can arrive at the lateral nucleus from parts of the medial geniculate nucleus of the thalamus (crude auditory information) and also from the auditory cortex (more processed auditory information) (LeDoux, 2000; Maren and Quirk, 2004). The amygdala also receives inputs from the hippocampus – these inputs terminate in the basal nuclei (Pitkanen et al., 2000). The lateral nuclei project directly to the central nuclei, and also send projections to the basal nuclei, which then project to the central nuclei (reviewed by LeDoux, 2000). The central and medial nuclei provide the main outputs of the amygdala (Eichenbaum and Cohen, 2001; LeDoux, 2000; Maren, 2001). They project to the hypothalamus and brain stem, and can influence the autonomic nervous system and the endocrine system (reviewed by Maren, 2001). The basal nuclei can also project back to the hippocampus and other MTL structures (Pitkanen et al., 2000). Its connectivity allows the amygdala to mediate emotional responses, and also modulate memory in other brain structures.

C. Fear Conditioning

In this thesis, cued and contextual fear conditioning were used to measure hippocampus- and amygdala-dependent memory in genetically distinct mice. Cued fear conditioning tasks measure an animal's ability to learn and remember an association between an auditory tone (CS; conditioned stimulus) and footshock (US; unconditioned

stimulus). Contextual fear conditioning tasks require learning and memory for an association between a particular environment (CS) and footshock (US) (see Maren, 2001 for an indepth discussion of cued and contextual fear memory). Animals are trained on this task by presenting them with timed pairings of auditory tone and footshock (cued), and/or environmental context and footshock (contextual). They are then tested for memory by gauging the fear response to the auditory tone alone (cued) or environmental context alone (contextual) after a delay. Fear responses can take many forms – the most commonly analyzed is freezing behaviour, in which a rodent becomes motionless upon presentation of the tone (cued) or upon being placed in the paired environment (contextual). These tests take advantage of the natural tendency of mice to freeze in response to fearful stimuli (Crawley, 2000). Other behaviours may be measured in these fearful situations, such as heart and respiration rate, or auditory startle (Davis et al., 1993; Maren, 2001). Both tasks are sensitive to lesions of the amygdala, but contextual fear conditioning is also disrupted by hippocampal lesions (Holland and Bouton, 1999; Kim et al., 1993; Phillips and LeDoux, 1992). Thus, by testing both cued and contextual fear conditioning in a population of mice, deficits in amygdalar and hippocampal function may be distinguished.

i. Contextual fear conditioning

There is convincing evidence that the hippocampus has a central role in mediating contextual fear memory. Electrolytic lesions of the dorsal hippocampus, which destroy both cell bodies and axon fibres, impair contextual fear conditioning when given either before training or within one month after training (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Selden et al., 1991). There are two possible roles of the hippocampus in

mediating contextual fear conditioning: it could either mediate formation of an association between the context and the footshock, or, it might form an integrated representation of the context that is associated with the footshock in the amygdala (Rudy et al., 2004). There is some evidence that the second idea may be more accurate. Neurotoxic lesions of the dorsal hippocampus, which kill cell bodies and not axons passing through, do not always result in contextual fear memory impairments when given pre-training, although post-training neurotoxic lesions more reliably impair contextual fear memory (Cho et al., 1999; Frankland et al., 1998; Gisquet-Verrier et al., 1999; Maren et al., 1997). These results indicate that there are multiple ways to encode contextual fear memory. The hippocampus is clearly involved in the normal encoding and consolidation of contextual fear memory because loss of the hippocampus after the learning event results in impairments. However, there may be a backup method of encoding contextual fear memory in the absence of the hippocampus because neurotoxic lesions prior to training do not always result in memory impairment.

Maren (2001) and Rudy (2004) have proposed two means of forming context-footshock associations in the rodent brain. The dominant and default method requires the hippocampus, which forms an integrated view of the context: one that combines and relates individual cues within the environment with one another. The hippocampus then communicates this view to the amygdala where it is associated with the footshock. However, if an intact, functioning hippocampus is absent, the second method of relating context and footshock is used. Here, individual cues within the context are related to the footshock in a hippocampus-independent manner, perhaps through interactions between the cerebral cortex and amygdala. This framework is relatively consistent with the

behavioural and lesion data available. Loss of the hippocampus after training results in contextual fear memory impairments because the integrated view of the context supplied by the hippocampus would be lost. Loss of the hippocampus prior to training may or may not result in impairments, depending on the exact training protocol used (discussed by Rudy, 2004). However, why do pre-training electrolytic, but not neurotoxic, lesions of the dorsal hippocampus result in reliable impairments of contextual fear memory? Maren (2001) proposes that electrolytic lesions of the dorsal hippocampus disrupt connections between the ventral subiculum and the nucleus accumbens. Disrupting this connection may interfere with normal exploratory behaviour, and impair the formation of integrated contextual representations of the environment (Fanselow, 2000; Maren, 1999).

In summary, the hippocampus appears to have a crucial role in forming contextual fear memories. However, the amygdala is also involved in mediating contextual fear memory, so examinations of contextual fear memory may benefit from an analysis of amygdalar function as a control.

ii. Cued fear conditioning

Lesions of the amygdala result in impairments of cued fear memory. The lateral amygdala receives information about both the tone and the footshock. Information about the tone can reach the amygdala via nuclei in the medial geniculate nucleus of the thalamus, and via the auditory cortex (Maren, 2001). Either of these pathways is sufficient to provide input about the tone, evidenced by intact cued fear conditioning in animals with lesions of nuclei in the medial geniculate nucleus that project to the amygdala, or lesions of the auditory cortex. When both regions are lesioned, cued fear memory impairments result (Romanski and LeDoux, 1992). Information about the

footshock reaches the lateral amygdala directly via the posterior thalamus and also from the insular cortex (Brunzell and Kim, 2001; Fanselow and Poulos, 2005; Jasmin et al. 2004; Lanuza et al. 2004; Shi and Davis 1999). The lateral amygdala projects directly to the amygdalar central nuclei, and also indirectly via the basal nuclei. The central nuclei provide the bulk of the outputs involved in eliciting fear responses (**Figure 1.7**). The central nuclei project to regions of the brainstem and the hypothalamus to elicit reflexes and autonomic reactions, and the periaqueductal grey to elicit defensive behaviours (Fanselow and Poulos, 2005; Fanselow, 1991; Kapp et al., 1979; LeDoux et al., 1988; Rosen and Davis, 1990; Rosen et al., 1991).

The convergence of tone and footshock information on cells of the lateral amygdala results in changes in synaptic strength at the thalamo-amygdalar and/or cortico-amygdalar synapses that mediate the tone inputs to the amygdala (Maren and Quirk, 2004). It has been proposed that these changes in synaptic strength mediate the formation of an association between tone and footshock, and cued fear memory relies on this synaptic plasticity lasting and becoming stable (Fanselow and Poulos, 2005). Persistent changes in synaptic strength within the basolateral complex (including the lateral and basolateral nuclei) of the amygdala may also have a role in memory storage for cued fear memory (Maren, 2001). The mechanisms of synaptic plasticity in the hippocampus and amygdala will be discussed further in *section E.*

iii. Extinction

Extinction is not merely an erasure of previously learned associations, but a form of new learning (Pavlov, 1927; reviewed by Bouton, 1993, 2004). The extinction of fear memory involves exposing a fear-conditioned animal to the CS (e.g., auditory tone or the

paired environment) after it has already learned a CS-US association. Repeated presentation of the CS without US pairings teaches the animal that it can no longer expect the US to follow the CS. The primary reason that extinction is thought to be new learning rather than destruction of old learned material is that most of the old learned material survives extinction (Bouton, 2002; Bouton, 2004; Myers and Davis, 2002; Rescorla, 2001). Indeed, Pavlov (1927) first witnessed the spontaneous recovery of the old learned behaviour after passage of a period of time following extinction. Here, an animal undergoes extinction, then after a delay (say, a week or two) its response to the CS is tested once again. The response to the CS is greater after this delay than it is at the end of extinction learning, which is evidence that the initial CS-US association remained intact and could be expressed again. Extinction learning may rely on the same brain regions that were involved in learning the initial association (Berman and Dudai, 2001).

D. Synaptic Plasticity and Behavioural Change

Physiological changes in the brain are responsible for changes in behaviour, learning, and memory. Such neurobiological changes likely include synaptic plasticity – the strengthening or weakening of communication between neurons. In this section I will briefly discuss synaptic plasticity (detailed mechanisms will follow in *Section E*), then give evidence in a relatively simple organism (*Aplysia californica*) for a link between synaptic plasticity and observed behavioural change.

i. What is synaptic plasticity?

Synaptic plasticity is a change in the ability of a neuron to excite or inhibit another neuron; such changes in functional connectivity are one way that information may be stored in the brain. Although there are types of synaptic plasticity that are

relatively short-term in duration (i.e., hundreds of milliseconds to seconds in duration; reviewed by Zucker, 1989), longer-term forms may contribute to lasting memory. In particular, long-term potentiation (LTP) and long-term depression (LTD) may be especially important in memory (reviewed by Martin et al., 2000; Milner et al., 1998; Morris et al., 2003). LTP is an activity-dependent strengthening of synaptic transmission (Bliss and Lømo, 1973; Bliss and Collingridge, 1993; Lømo, 1966), whereas LTD is a weakening of synaptic transmission (Dudek and Bear, 1992), and each can last for minutes to weeks. In one striking example, LTP was observed to last a year in the brain of a rat (Abraham et al., 2002). In *section E* I will discuss the cellular and molecular mechanisms of LTP. Here, I will focus on evidence that LTP has a role in learning and memory.

ii. Aplysia californica: a link between synaptic plasticity and behavioural change

Some of the most convincing evidence that synaptic plasticity is directly related to behavioural change was gathered from the marine mollusk *Aplysia californica.* Both non-associative (e.g., habituation and sensitization) and associative (e.g., classical conditioning) learning have been studied using the gill withdrawal reflex in *A. californica* (Carew et al., 1972; Carew et al., 1981; Pinsker et al., 1973; reviewed by Carew and Sahley, 1986). The gill withdrawal reflex is elicited in the laboratory by applying a light tactile stimulus to the siphon or to the mantle, which causes the gill to retract (see **Figure 1.8**). This reflex can be habituated by repeated tactile stimuli applied to the same region to elicit the reflex – the amount of gill retraction lessens with repeated stimuli. The gill withdrawal reflex can also be sensitized by applying a tail shock: subsequent siphon or mantle stimulation elicits a stronger gill contraction (Dudai, 1989).

In *A. californica,* the cells that mediate the gill withdrawal reflex are primarily found in the abdominal ganglion (Kandel, 1976). Sensory neurons respond to tactile stimulation of the mantle or siphon, and excite motor neurons that innervate the gill, which cause it to contract. Sensory neurons also influence interneurons that can affect gill contraction because they synapse onto gill motoneurons (see **Figure 1.8**; Dudai, 1989; Kandel, 2001).

Electrophysiological recordings during habituation revealed a strong correlation between decreased transmitter release from the sensory neuron (termed *presynaptic depression*) and reduced gill contraction during habituation (Carew and Kandel, 1973; Castellucci and Kandel, 1974; Klein et al, 1980; reviewed by Carew and Sahley, 1986). In the case of sensitization, facilitatory interneurons (activated by sensory neurons that sense tail shock) release the transmitter serotonin onto the presynaptic terminal of the siphon or mantle sensory neuron (Brunelli et al., 1976; reviewed by Byrne and Kandel, 1996; Kandel and Schwartz, 1982), resulting in increased transmitter release from the sensory neuron (Castellucci and Kandel, 1976). Serotonin activates two different metabotropic receptors on the presynaptic terminal of the sensory neuron, resulting in the recruitment of the phospholipase C/diacylglycerol/protein kinase C signaling pathway, and the adenylyl cyclase/cAMP/cAMP-dependent protein kinase (PKA) signaling pathway. Recruitment of these pathways ultimately results in prolonged action potential duration (which increases the amount of calcium entering the synaptic terminal) and increased transmitter release from the presynaptic terminal (see Byrne and Kandel, 1996; Siegelbaum et al., 1982 for details). Thus, *presynaptic facilitation* is responsible for the expression of behavioural sensitization.

Long-term forms of habituation and sensitization can be elicited by providing more training sessions (reviewed by Kandel, 2001). In long-term habituation, the number of synapses that connect the sensory and motor neurons is decreased; this effect can last for weeks, and correlates with behavioural changes. In long-term sensitization, the number of synaptic connections between the sensory (siphon/mantle) and motor neurons is increased (Bailey and Chen, 1983). Persistent kinase activity (Greenberg et al., 1987; Sossin et al., 1994; Sweatt and Kandel, 1989) and new protein synthesis (Castellucci et al., 1986, 1989; Montarolo et al., 1986) are also required for long-term sensitization and facilitation. The adenylyl cyclase/cAMP/PKA and mitogen-activated protein kinase (MAPK) signaling pathways are involved in the initiation of protein synthesis in the siphon/mantle sensory neuron in long-term sensitization. PKA and MAPK act to regulate cAMP-response element binding protein (CRE-binding protein; CREB) dependent transcription factors (Martin et al., 1997; Michael et al., 1998; Müller and Carew, 1998; reviewed by Kandel, 2001). CRE-driven gene expression relies on the PKA-dependent phosphorylation of CREB-1, as well as the removal of inhibitory constraints on transcription by MAPK phosphorylation of CREB-2 (reviewed by Abel et al., 1998; Milner et al., 1998). The resultant new protein synthesis has a role in the formation of new synaptic contacts in conjunction with long-term sensitization, potentially through the regulation of actin and cell adhesion molecules (Bailey and Chen, 1989, 1992; Hatada et al., 2000; Kim et al., 2003; Mayford et al., 1992; Michael et al., 1998).

Overall, conjoint studies of behaviour, cellular electrophysiology, and pharmacology in *A. californica* provide compelling evidence that synaptic plasticity underlies changes in behaviour.

E. Long-term Potentiation in Different Brain Regions

Along with his proposed "neuron doctrine," in the 1800s Santiago Ramón y Cajal put forth the notion that experiences elicited changes in neuronal connectivity, and that increased neuronal connectivity was associated with higher intellectual ability (Ramón y Cajal, 1893). Donald Hebb (1949) proposed that synaptic activity might have the ability to elicit lasting changes in the way that neurons interact. This has come to be known as Hebb's postulate:

> "When an axon of cell A is near enough to excite a 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." (pg. 62)

Here, Hebb suggested that synapses might have a mechanism by which past synaptic activity alters future synaptic activity – a form of synaptic memory. Such a change would require concurrent activation of the presynaptic neuron and the postsynaptic neuron. The first comprehensive report of LTP was put forth by Bliss and Lømo (1973), although Terje Lømo (1966) published an earlier account of frequency potentiation in abstract form. This first study of LTP was done in the perforant pathway of the hippocampus of an anesthetized rabbit. A stimulating electrode was inserted in the perforant pathway, and field responses to stimuli were recorded from the dentate gyrus. After application of high-frequency electrical stimulation, the size of the population spike recorded in the dentate gyrus became potentiated, and remained that way for hours. Bliss and Lømo had discovered a way for neurons to "remember" electrical activity long after its cessation.

In mammals, LTP in particular brain regions has been strongly linked to memory processes (reviewed by Martin et al., 2000; Milner et al., 1998; Morris et al., 2003). The

evidence for a role of LTP in memory in mammalian systems will be discussed in *section F*. Here, I will briefly describe the study of short-term plasticity and LTP in the hippocampus and the amygdala *in vitro*, and the mechanisms involved in such LTP.

i. Short-term synaptic plasticity

Short-term plasticity lasts for seconds to minutes, and includes *facilitation* (hundreds of milliseconds), *augmentation* (seconds), and *post-tetanic potentiation* (PTP; up to minutes) (Fisher et al., 1997; Zucker, 1989; Zucker and Regehr, 2002). These phenomena result in an increased postsynaptic response to presynaptic action potentials, and can occur following short trains, or pairs, of high-frequency pulses of electrical stimulation. Mechanistically, they depend on the influx of calcium to the presynaptic terminal due to the arrival of closely temporally-spaced action potentials (reviewed by Zucker and Regehr, 2002). When multiple action potentials arrive at the terminal, calcium concentrations increase and enhance transmitter release for a short time (Delaney et al., 1989; Fisher et al., 1997; Katz and Miledi, 1968). Short-term depression can also occur – this is a decrement in the size of a postsynaptic response to action potentials that can last for seconds to minutes (Zucker, 1989; Zucker and Regehr, 2002). Short-term depression results from a decrease in quantal release (Del Castillo and Katz, 1954), and is likely the consequence of depletion of readily releasable neurotransmitter, and/or a decrease in the efficacy of the exocytosis machinery (Betz, 1970; reviewed by Zucker, 1989).

In the case of PTP, which has a longer duration, calcium influx saturates the calcium buffering capabilities of the terminal during synaptic activity, which may lead to the activation of calcium-dependent enzymes (Zucker and Regehr, 2002). It has been

postulated that Ca^{2+}/calmodulin-dependent protein kinase II can be activated, which phosphorylates the machinery involved in transmitter release (Greengard et al., 1993), although the evidence for this hypothesis remains inconclusive, and other molecules involved in transmitter release are likely involved in PTP (Fisher et al., 1997; Zucker and Regehr, 2002). Although its mechanisms remain uncertain, post-tetanic potentiation enhances transmitter release on the order of minutes, and thus represents a brief form of synaptic memory for prior synaptic activity. Lastly, short-term potentiation (STP), which can last for tens of minutes, is sometimes considered to be a form of short-term synaptic plasticity. Both presynaptic and postsynaptic mechanisms of STP have been identified [including dependence on N-methyl-D-aspartate receptors (NMDARs) and protein kinases], although STP is often spared by blockade of protein kinases (Hanse and Gustaffson, 1992, 1994a, 1994b)

ii. Long-term potentiation

LTP has a much longer time course, and can last for hours to months (Abraham et al., 2002; Andersen et al., 1977; Bliss and Lømo, 1973; Huang et al., 1996). LTP induction depends on concurrent presynaptic activation and postsynaptic depolarization, in concordance with Hebb's postulate. Sufficient amounts and patterns of synaptic activity in the presynaptic neuron result in the release of the excitatory transmitter glutamate, which diffuses across the synaptic cleft and then activates postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPARs), resulting in postsynaptic depolarization that releases the voltage-dependent Mg^{2+} blockade of the NMDAR ionotropic channel (Mayer et al., 1984; Nowak et al., 1984). When activated, NMDARs allow the passage of calcium ions (but also sodium and potassium ions, like

AMPARs) that flow into the postsynaptic dendrites. Both NMDAR activation and postsynaptic calcium influx are required for LTP induction, evidenced by the prevention of LTP induction by NMDAR antagonists and calcium chelators (Collingridge et al., 1983; Lynch et al., 1983; Malenka et al., 1988).

The involvement of NMDARs in LTP induction imparts several unique properties of LTP. Firstly, LTP is specific to the particular synapses that are stimulated (Andersen et al., 1977; Barrionuevo and Brown, 1983; Bliss and Collingridge, 1993). In other words, LTP occurs at the level of the synapse, not the whole neuron. Secondly, LTP requires that a threshold number of presynaptic fibres be active to sufficiently depolarize the postsynaptic neuron and activate NMDARs for induction to be successful, a property called *cooperativity* (Malenka, 1991; McNaughton et al., 1978). Thirdly, LTP can exhibit *associativity*. Within a neuron, weak (sub-threshold) activation of synaptic inputs can interact with strong activation of different inputs that occurs within close temporal proximity to elicit successful LTP induction at the weakly activated inputs (Barrionuevo and Brown, 1983; Levy and Steward, 1979). This property of LTP may confer the ability to associate different inputs within the same neuron – akin to the association of CS and US in classical conditioning within neurons of the lateral amygdala.

The influx of calcium ions through NMDARs can activate enzymes in the postsynaptic neuron such as protein kinase C (PKC) and Ca^{2+}/calmodulin protein kinase II (CaMKII) (Malenka and Bear, 2004). PKC is required for LTP induction (Malinow et al., 1989) and injection of PKC into hippocampal pyramidal cells mimics LTP (Hu et al., 1987). CaMKII is required for LTP induction by different patterns of synaptic activity (Malinow et al., 1989; Otmakhov et al., 1997; reviewed by Lisman et al., 2002). CaMKII

likely has a role in the addition of AMPARs to the postsynaptic membrane, an important component of LTP (Lisman et al., 2002). The cAMP/PKA and the MAPK signaling cascades have a role in LTP induction (English and Sweatt, 1997; Roberson and Sweatt, 1996). Activation of tyrosine kinases, including Src, also occurs with LTP induction (Huang et al., 2001; Lu et al., 1998; O'Dell et al., 1991). In fact, the number of molecules suspected to be involved in LTP is so great (e.g., see Sanes and Lichtman, 1999) that it is beyond the scope of this thesis to identify and discuss them all.

Increases in postsynaptic AMPAR transmission can contribute to lasting LTP. A major mechanism of LTP expression may be activity-dependent changes in AMPAR-trafficking that result in an increased number of AMPARS at synapses (reviewed by Bredt and Nicoll, 2003; Malenka and Bear, 2004; Malinow and Malenka, 2002). Such insertion of new AMPARs can also result in the awakening of "silent synapses" that previously contained NMDARs but not AMPARs (Kullmann, 2003). Modification of AMPARS and their biophysical properties (e.g., conductance) by phosphorylation also contributes to LTP expression (Benke et al., 1998; Lee et al., 2003). In particular, CaMKII-mediated phosphorylation of the AMPAR subunit GluR1 appears to play an important role in these processes, but the specific mechanisms have yet to be worked out (Malenka and Bear, 2004).

Depending on the pattern of synaptic activity experienced at a set of synapses, LTP can have different durations (Huang et al., 1996). Early LTP (E-LTP) can be induced by a single train of high-frequency stimulation (HFS) and decays to baseline levels in an hour or two, whereas late LTP (L-LTP) is induced by multiple trains of HFS (usually three or more) and can last many hours *in vitro* (Huang et al., 1996), and even

months *in vivo* (Abraham et al., 2002). In general, E-LTP relies on the covalent modification of existing proteins (e.g., phosphorylation or dephosphorylation of channels, enzymes, or receptors) whereas L-LTP also relies on new mRNA and protein synthesis (Frey et al., 1988; Huang and Kandel, 1994; Krug et al., 1984; Nguyen et al., 1994). LTP may also be induced by theta-burst stimulation (TBS), in which short bursts of HFS are given at a frequency of about 5Hz. TBS is often considered to be a more physiological induction protocol than longer trains of HFS, as TBS resembles the hippocampal theta rhythm (5-10 Hz) in that it mimics spike discharge patterns in the rodent hippocampus during exploration (Capocchi et al., 1992; Larson et al., 1986; Otto et al., 1991).

Some protein kinases have been identified as having special roles in the maintenance of LTP. For instance, the cAMP/PKA signaling pathway is required for new expression of genes and L-LTP (Abel et al., 1997; Impey et al., 1996; Matthies and Reymann, 1993; Nayak et al., 1998; reviewed by Nguyen and Woo, 2003). PKA can regulate gene transcription by phosphorylation of CREB, which can modulate the transcription of genes with a CRE-response element in their promoter (Bacskai et al., 1993; Gonzalez and Montminy, 1989; Yamamoto et al., 1988). MAPK is required along with PKA to phosphorylate CREB in hippocampal area CA1 (Roberson et al., 1999). MAPK can be activated during LTP either by crosstalk with other signaling cascades (e.g., the cAMP/PKA signaling cascade) or by elevated levels of Ca^{2+} (Iida et al., 2001; Morozov et al., 2003; Vossler et al., 1997). Furthermore, inhibition of the MAPK signaling cascade prevents L-LTP (Rosenblum et al., 2002). Protein kinase M zeta, an

isoform of PKC, can also have a role in the maintenance of LTP (Hrabetova and Sacktor, 1996; Ling et al., 2002).

Synaptic activity can lead to the rapid expression of mRNA from immediate-early genes (e.g., Cole et al., 1989). In particular, activation of the transcription factor CREB leads to the expression of genes involved in LTP maintenance. The expression of brain-derived neurotrophic factor has been shown to be regulated by CREB, and can be important for hippocampal synaptic plasticity (Tao et al., 1998; reviewed by Lu, 2003). Other immediate early genes include *Arc* and *Zif/268* (Cole et al., 1989; Link et al., 1995). It is important to note that a CRE-binding site has not been identified on the *Arc* or *Zif/268* genes, but their transcription has been shown to depend on PKA and MAPK (Ginty et al., 1991; Waltereit et al., 2001). The expression of these genes may mediate new synaptic growth (which may involve an increase in structural proteins such as actin; Fukazawa et al., 2003) that likely supports the long-term maintenance of LTP (reviewed by Abraham and Williams, 2003; Malenka and Bear, 2004).

Just as synaptic strength can be increased during LTP, it can also be decreased during *long-term depression* (LTD). LTD is generally induced by long bouts of low-frequency synaptic activity, and shares many characteristics with LTP, such as NMDAR-dependence (Bear and Dudek, 1992; Bear and Abraham, 1996). Bear (2003) suggests that the overall amount of change in postsynaptic calcium levels, due to NMDAR activation, is what determines whether LTP or LTD will be induced at a particular synapse. In addition to NMDARs and postsynaptic calcium influx, it has been shown that LTD can rely on metabotropic glutamate receptors, protein phosphatase activity, and

the removal of AMPARs from the postsynaptic plasma membrane (Bear, 2003; Kato, 1993; Lee et al., 1998; Malinow and Malenka, 2002; Mulkey and Malenka, 1992).

iii. LTP and memory in hippocampal area CA1

LTP can be elicited in primary neurons in area CA1 of the hippocampus by applying high-frequency stimulation to the Schaeffer collateral pathway; these fibres synapse onto the dendrites of CA1 pyramidal cells. LTP in this synaptic pathway is NMDAR-dependent and relies on the mechanisms described in the previous two sections (reviewed by Malenka and Bear, 2004).

The study of LTP in area CA1 of the hippocampus is quite important because there is strong evidence that this hippocampal subregion is crucial for particular types of learning and memory. Human studies have indicated that selective loss of hippocampal area CA1 result in moderately severe anterograde and mild temporally-graded retrograde amnesia (reviewed by Squire and Zola, 1997). A striking piece of evidence for the role of area CA1 in memory comes from a transgenic mouse in which the NMDAR1 gene was selectively knocked out of CA1 pyramidal neurons (Tsien et al., 1996b). LTP was absent in area CA1, and the mice exhibited severe impairments in spatial memory on the Morris water maze (Tsien et al., 1996b). Raymond Kesner's group has delineated differences in the contributions of hippocampal area CA1 and the dentate gyrus to different types of memory. Using novel behavioural tests, their studies have indicated that area CA1 has a role in memory for the temporal order in which locations were visited, and the dentate gyrus has a role in memory for spatial patterns (Gilbert et al, 2001; also see Huerta et al., 2000, for further evidence of a role for area CA1 in associating events across time). Lee and Kesner (2004) showed that hippocampal areas

CA1 and CA3, and the dentate gyrus, are all important for contextual fear memory in the rat by studying the effects of subregion-restricted neurotoxic lesions. Also, CRE-mediated gene expression is increased in hippocampal areas CA1 and CA3 following contextual fear conditioning (Impey et al., 1998). Thus, hippocampal area CA1 is important for particular types of memory, including contextual fear memory, but other hippocampal subregions are also involved.

iv. LTP and memory in the dentate gyrus

LTP can be induced in the granule cells of the dentate gyrus by stimulation of the perforant pathway, which provides informational inputs to the hippocampus from the entorhinal cortex. The thesis work presented here includes studies of LTP in the medial perforant pathway (MPP), which originates in the medial portion of the entorhinal cortex. LTP in this pathway is NMDAR-dependent, and is thought to rely upon many of the same molecular mechanisms as area CA1 LTP (Bliss and Collingridge, 1993; Colino and Malenka, 1993; Hanse and Gustafsson, 1992, 1994b). Nguyen and Kandel (1996) demonstrated that the MPP exhibits early and late phases of LTP. Like in area CA1, late-LTP in the medial perforant pathway depends on new mRNA and protein synthesis, and on PKA activity (Nguyen and Kandel, 1996).

The role of the dentate gyrus in memory is not well defined, but there are some indications that it has a role in spatial memory (e.g., Gilbert et al., 2001). Place cell activity has been recorded in the dentate gyrus (Jung and McNaughton, 1993), and saturation of LTP in the dentate gyrus can impair subsequent spatial learning (Brun et al., 2001; McNaughton et al., 1986; but see Sutherland et al., 1993). However, LTP saturation in the dentate gyrus may affect other hippocampal subregions, so it is possible

that the spatial learning impairments observed in these studies may at least partly result from changes in other subregions. Other studies suggest that LTP in the dentate gyrus does not correlate with spatial memory (Nosten-Bertrand et al., 1996; Saucier and Cain, 1995). However, considering that the dentate gyrus receives much of the informational input that enters the hippocampus from the entorhinal cortex, it likely has an influential role in hippocampal processing. In terms of memory phases, human fMRI studies have indicated that the dentate gyrus (and areas CA2 and CA3) have a predominant role in the encoding of episodic memory, whereas area CA1 and the subiculum are more involved in memory retrieval (Eldridge et al., 2005; also see Zeineh et al., 2003).

v. LTP and memory in hippocampal area CA3

LTP can be induced at the synapses of the mossy fibres on area CA3 pyramidal cells. This LTP is different from CA1 and MPP LTP in that it is NMDAR-independent (Harris and Cotman, 1986; Zalutsky and Nicoll, 1990). Mossy fibre (MF) LTP is also independent of postsynaptic membrane potential and calcium influx (Mellor and Nicoll, 2001; Zalutsky and Nicoll, 1990). Rather, MF LTP appears to have a presynaptic locus; the first piece of evidence for this was that paired-pulse facilitation is decreased during MF LTP (Zalutsky and Nicoll, 1990). This result indicated that transmitter release is increased with MF LTP, a finding that has been confirmed by Reid et al. (2004). MF LTP depends on PKA activity (Huang et al., 1995; Weisskopf et al., 1994); this PKA activation in the presynaptic terminal may lead to increased transmitter release during LTP. MF LTP exhibits an early phase, and a late phase that depends on protein and RNA synthesis (Huang et al., 1994). Presynaptic kainate receptors are also likely involved in MF LTP (Bortolotto et al., 1999; Lauri et al., 2001).

It is unclear whether MF LTP is necessary for hippocampus-dependent memory. Hensbroek et al. (2003) and Huang et al. (1995) reported that MF LTP does not appear necessary for hippocampus-dependent spatial or contextual learning. However, reduced MF LTP correlates with impaired spatial memory in rats with altered calbindin levels in dentate granule cells (Dumas et al., 2004), and retrieval of contextual memory results in activation of hippocampal area CA3 (Zhang et al., 2005). The presence of place cells in area CA3 (e.g., McNaughton et al., 1989) that may have special roles in spatial pattern completion and separation are a further indication that the mossy fibre pathway and CA3 pyramidal cells have a role in spatial memory (Guzowski et al., 2004).

vi. LTP and memory in the basolateral amygdala

The lateral (LA) and the basolateral (B) amygdaloid nuclei are part of the basolateral complex of the amygdala. The synaptic pathway between the LA and B is part of the circuitry involved in cued fear conditioning in the rodent (LeDoux, 2000; Maren, 2001). LTP in this pathway can be elicited using application of multiple trains of high-frequency stimulation to the LA, and recording from the B in mouse amygdala slices, *in vitro*. This LTP is NMDAR-dependent (Rammes et al., 2000). Other studies have indicated that CaMKII and voltage-gated calcium channels participate in LTP in the basolateral complex (Chapman et al., 2003; Ramsay et al., 2001). Also, PKA and the extracellular-regulated kinase/mitogen activated protein kinases (ERK/MAPK) are required for L-LTP in amygdala (reviewed by Schafe et al., 2001). The Ras/MAPK/CREB signaling pathway may be involved in initiating protein synthesis and LTP maintenance in the amygdala (reviewed by Adams et al., 2000; Chapman et al., 2003). In general, it seems that LTP in the amygdala relies upon many of same

molecules as hippocampal LTP, but these molecules may have slightly different roles and interactions in these different brain structures.

In addition to its role in fear memory, the amygdala participates in mediating stimulus-reward associations (Cahill and McGaugh, 1990; McDonald and White, 2003). The amygdala is also key in the emotional modulation of other memory systems in the brain, including the declarative memory system (McGaugh, 2000; McGaugh, 2004). Much of the evidence that LTP has a role in memory is derived from studies of LTP in the LA during cued fear conditioning in the rat (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997). In the next section I will expand on this idea, and give other convincing evidence that LTP and memory have common mechanisms.

F. Common Mechanisms of LTP and Memory

In their first detailed characterization of LTP in 1973, Bliss and Lømo suggested that they had found a potential cellular correlate of memory. Morris (1989) later detailed five reasons that LTP is a particularly attractive cellular model of memory. These are:

1. LTP occurs in the hippocampus, a brain structure that has a role in memory. The role of the hippocampus in the declarative memory system indicates that it should have a mechanism for memory.

2. LTP is initiated very quickly after synaptic activity – it can be observed within one minute of application a single train of HFS. Quick onset of a synaptic response may be advantageous in encoding new events.

3. LTP can be quite long lasting, like good memory should be. LTP has been observed to last for weeks, and up to a year (Abraham et al., 2002), *in vivo* if repetitive stimulation is given. Such repetition may mimic reminders.

4. LTP is synapse specific, meaning that only the synapses activated by tetanic stimulation are potentiated. This property parallels the nature of episodic memory – different experiences with the same object or same person can be remembered individually. Synapse specificity also enables a large magnitude of storage capacity in the neurons of the brain in that a single neuron might engage in multiple memories, each of which requires a distinct subset of its synapses.

5. LTP is associative – when multiple synaptic inputs on the same cells are activated concurrently, potentiation is promoted. Associations can form between different synaptic inputs, which may provide a mechanism for different experiences to converge on the same neurons and become linked. Synaptic activity can also be integrated over periods of time that have behavioural significance.

Considering that LTP is such an attractive cellular model of memory, much effort has gone into trying to prove that LTP underlies memory, or at the very least, that LTP and memory rely upon the same cellular and molecular mechanisms (reviewed by Martin et al., 2000).

i. Evidence that LTP mediates memory

Two angles of attack have been predominantly utilized in trying to show that LTP plays a role in memory: i) demonstrating changes in synaptic transmission following a learning event; ii) preventing LTP, then measuring subsequent attempts to learn. An example of the first angle is the case of analyzing amygdala LTP during cued fear conditioning. Rogan et al. (1997) conducted experiments in which rats were conditioned using a series of "pips" of acoustic tones that were paired with footshock. This method of conditioning was clever in that each auditory pip elicited a potential that was recorded

in the LA. The slope of the CS-evoked potential was measured throughout training and testing, and was found to change from baseline (pre-training) levels in conditioned, but not unconditioned rats that had received unpaired presentations of CS and US. In a complementary study, McKernan and Shinnick-Gallagher (1997) recorded excitatory postsynaptic currents (EPSCs) in amygdalar slices from conditioned rats. They found that EPSCs were larger in the LA in conditioned vs. unconditioned (unpaired) rats upon stimulation of the pathway entering the LA from the medial geniculate nucleus. LA neurons were more excitable in conditioned animals as measured using the input-output relationship, and paired-pulse facilitation was lessened in conditioned animals, suggesting that transmitter release was enhanced. These two studies suggest that an LTP-like process in the LA underlies fear conditioning in the rat.

Sharp et al. (1985) conducted another study that demonstrated changes in synaptic transmission following a learning experience by measuring excitatory postsynaptic potentials (EPSPs) in the dentate gyrus following environmental enrichment in rats. Rats were placed in a large, spatially complex environment nightly, in groups to provide social interaction. EPSPs were recorded *in vivo* before and after enrichment. Although EPSP amplitude remained the same before and after enrichment, the area of the pop-spike (a measure of action potentials in postsynaptic neurons) was increased after enrichment. This increase declined after enrichment ceased. Thus, it appears that novel experiences may induce a form of potentiation in the dentate gyrus.

One of the pioneering studies that linked LTP to memory was conducted by Morris et al. (1986). Here, the idea was to prevent hippocampal LTP, then measure hippocampus-dependent memory. The NMDAR antagonist D(-)-2-amino-5-

phosphonopentanoic acid (AP5) was infused into the hippocampus of rats via cannulae implanted in the third ventricle. After several days of AP5 infusion, some of the rats were tested on the Morris water maze, and in others *in vivo* LTP was measured in the dentate gyrus. LTP could not be elicited in the dentate gyrus of rats treated with AP5, but LTP was observed in rats treated with the inactive isomer of AP5, L-AP5. Thus, treatment with AP5 prevented LTP induction in the hippocampus. In the Morris water maze, rats treated with AP5 learned the location of the hidden platform more slowly than L-AP5 rats during training trials, and did not exhibit a preference for the correct quadrant of the maze during the probe test. AP5-treated rats could still learn the location of a visible platform in the Morris water maze. These results indicate that blocking NMDARs in the hippocampus prevents both hippocampal LTP and spatial memory, providing a link between the mechanisms of LTP and memory.

Another way to prevent the induction of LTP is to occlude it. The premise of the following occlusion study was that if all synapses are saturated in strength, no further LTP can be induced, and learning and memory will be impaired. Barnes et al. (1994) applied maximal electroconvulsive shock (MECS) to the perforant pathway of rats *in vivo*, which elicited a lasting potentiation of EPSP slope and pop-spike amplitude in the dentate gyrus. Barnes et al. (1994) tested rats on the probe trial of the Morris water maze before MECS, and then conducted reversal trials after MECS in the same rats in which the location of the hidden platform was moved, and rats were required to learn the new location. Rats exhibited a preference for the correct quadrant before MECS, but not for up to 30 days after MECS. This study showed that spatial memory is impaired if

synaptic strength is saturated in the dentate gyrus, and thus, an increase in synaptic strength may be necessary for memory.

A third strategy for interfering with LTP is genetic mutation. Many transgenic and knockout mice have been developed in which genes for proteins thought to be involved in LTP have been modified. These studies have shed light on which proteins are crucial for LTP and memory (for review see Chen and Tonegawa, 1997; Mayford and Kandel, 1999). Several mutant mice have been developed in which decreases in both hippocampal LTP and memory are observed (e.g., Abel et al., 1997; Silva et al., 1992a,b). Silva et al. (1992a,b) developed a mutant mouse in which the a isoform of CaMKII was not expressed in the brain. Hippocampal LTP induced by multiple trains of HFS in area CA1 of hippocampal slices was impaired in these mutant mice (Silva et al., 1992a). These mice also exhibited impairments in both the learning and probe trials on the MWM compared with wildtype controls, indicating that spatial learning and memory were reduced (Silva et al., 1992b). These studies of the a-CaMKII mutant mouse illustrate that a protein crucial for LTP is also important for a form of hippocampus-dependent memory. These findings provide support for the hypothesis that hippocampal LTP has a role in specific types of memory.

I have discussed just a few examples of experiments that support the notion that LTP is important for memory; these, along with many others (Brun et al., 2001; Doyere and Laroche, 1992; Ishihara et al., 1997; Maren, 1999; Moser et al., 1998; Roman et al., 1987; Skelton et al., 1987) make a convincing case for a strong relationship between LTP and memory in the mammalian brain.

ii. Overlapping molecular mechanisms for short- and long-term memory and LTP

Another persuasive angle of research that supports the hypothesis that LTP underlies memory has shown, through pharmacological manipulation, that the molecules important for LTP are also important for memory. In general, the molecular mechanisms involved in STM parallel those for early LTP, and the molecular mechanisms of LTM parallel those for late LTP. In mammals, both early LTP and STM have a duration of a few hours or less, and rely on the covalent modification of already-existing proteins, but not transcription or protein synthesis. Late LTP and LTM can last much longer, and both processes rely on translation and transcription (Agranoff *et al.*, 1965; Castellucci et al., 1989; Izquierdo and McGaugh, 2000; Rose, 1995a,b; Tully et al., 1994; Wustenberg *et al.*, 1998; reviewed by Davis and Squire, 1984). The dependence of LTM on protein synthesis was first studied by the systemic injection or intracerebral infusion of antibiotics, and these experiments revealed that protein synthesis is required in the first few hours after a learning event for successful memory consolidation (Agranoff *et al.*, 1965; Cherkin, 1969; Flexner *et al.*, 1967; Matthies, 1989). Further experiments showed that new mRNA and proteins peaked at two times following a learning event in the rat: around one hour later, and about 3-6 hours later (Matthies, 1989). Behavioural pharmacology experiments, in which drugs are infused to brain regions involved in particular types of memory at various time points before, during, or after a learning event (e.g., Morris, 1986), have since been utilized to investigate the roles of various receptors, other proteins, and transmitters in learning and memory.

Such behavioural pharmacology experiments have provided insight into which molecules are required for STM versus LTM. Izquierdo and colleagues conducted many

experiments in which different inhibitors of molecules involved in LTP were infused into hippocampal area CA1 of rats during the course of inhibitory avoidance learning. By infusing such drugs to the hippocampus during or after training, then testing memory at different intervals after training (STM: 1.5 hrs after training; LTM: 24 hrs after training), they identified molecules required for both STM and LTM, and also for STM or LTM alone. Izquierdo and McGaugh (2000) reviewed these experiments; see **Table 1.1** for a summary of some molecules involved in the hippocampus for STM and/or LTM of inhibitory avoidance. Notably, glutamate receptors (NMDARs, AMPARs, and mGluRs) and many components of the cAMP/PKA signaling pathway (adenylyl cyclase, PKA, D1/D5 receptors, and $5HT_{1A}$ receptors) are required for both STM and LTM. The synthesis of nitric oxide and carbon monoxide were found to be necessary for LTM but not STM, as were CaMKII, CREB-P, and ß-adrenergic receptor activity.

A very interesting finding from this behavioural pharmacological investigation of inhibitory avoidance learning is that some molecules are necessary at particular time points for STM alone. This means that blocking D1/D5 receptors, adenylyl cyclase, or MAPK during training, for instance, prevents the expression of STM but not LTM (Izquierdo et al., 1998; Izquierdo and McGaugh, 2000). The observation that an animal that did not remember the learning event at a short interval can yet express memory at longer intervals is strong evidence for parallel systems for STM and LTM (Izquierdo et al., 1998). Indeed, other lines of research have suggested that there are parallel systems for STM, LTM, and *intermediate-term memory* (ITM), each of which have different durations in different organisms. In *A. californica*, these phases of memory have been studied using sensitization; STM generally lasts minutes, ITM lasts 1-3hrs, and LTM

lasts days to weeks (Castellucci et al., 1989; Cleary et al., 1998; Frost et al., 1985; Goldsmith and Byrne, 1993; Levenson et al., 2000; Scholz and Byrne, 1987; Sutton et al., 2001). STM does not require protein or RNA synthesis, ITM requires protein but not RNA synthesis, and LTM requires both (Castellucci et al., 1989; Levenson et al., 2000; Sutton et al., 2001a). STM, ITM, and LTM for sensitization in *A. californica* rely on different mechanistic processes, and are not continuous with one another because ITM decays long before LTM emerges.

Multiple phases of memory have also been identified in *Drosophila melanogaster* by studying olfactory associative learning. This learning task involves applying electric shock to flies when in an environment scented with a particular odour. Subsequently, flies are given a choice between two environments, one scented with the same, shock-associated odour, and one with a different odour. Flies that avoided the shock-associated odour exhibited successful learning. Examination of single-gene learning mutants (flies in which a single gene has been mutated, and which do not learn to avoid the shock-associated odour) has uncovered four distinct phases of memory: short-term, middle-term, long-term, and anesthesia-resistant memory. Dubnau and Tully (1998) suggest that memory processing is sequential from short-term to middle-term memory, but then long-term and anesthesia-resistant memory operate in parallel (**Figure 1.9**). Long-term memory for olfactory associations begins about 24 hr after conditioning, and lasts over a week in *D. melanogaster*. Like in *A. californica*, LTM appears to depend on protein synthesis and transcription in *D. melanogaster* (Yin et al., 1994; Yin and Tully, 1996). Anesthesia-resistant memory begins not long after learning, and decays within four days. It is distinguished by being resistant to cold-shock, unlike the other memory phases in *D.*

melanogaster, and does not require protein synthesis (Tully et al., 1994) or CREB (Yin et al., 1994). There is also evidence for parallel memory systems in honeybees, with the cAMP/PKA signaling cascade having a selective role in a late, transcription-dependent form of memory (Friedrich et al., 2004).

Overall, we can conclude that organisms express different phases of memory that have different time courses and rely on distinct mechanisms, and many of these mechanisms overlap with those involved in LTP. Some of these phases may operate in parallel, such that loss of one phase will not influence other phases.

G. The Hippocampal Commissure, and its Role in LTP and Memory

The hippocampal commissure is a fibre tract that bridges the two hemispheres of the brain to allow communication between the two hippocampi. Because of its role in mediating hippocampal information transfer between hemispheres, the hippocampal commissure has the potential to greatly influence hippocampal LTP, learning, and memory. Here I will describe the anatomy of the hippocampal commissure, and what we know about its influence on synaptic transmission in the hippocampus and its role in memory.

i. Anatomy and physiology of the hippocampal commissure

The (ventral) hippocampal commissure crosses the midline of the brain, and consists of fibres that run alongside the fimbria (O'Keefe and Nadel, 1978; Raisman et al., 1965). The fibres that comprise the hippocampal commissure are fairly small in diameter, and conduct rather slowly (7.5m/s in the rat; Andersen 1960).

In the rat, the hippocampal commissure is comprised primarily of axons of CA3 neurons that project to areas CA3, CA2, and CA1 of the contralateral hippocampus,

although a small number of CA1 pyramidal cells and mossy cells (excitatory interneurons in the hilus of the dentate gyrus) send projections to the contralateral hippocampus as well (Buchhalter et al., 1990; Johnston and Amaral, 2004; Laurberg and Sorenson, 1981; Swanson et al., 1978; Van Groen and Wyss, 1990). CA3 commissural fibres terminate both on contralateral CA1 and CA3 pyramidal neurons, and on interneurons, and dentate mossy cell fibres terminate primarily on the dendrites of ipsilaleral and contralateral dentate granule cells (Frotscher and Zimmer, 1983; Johnston and Amaral, 2004; Gottlieb and Cowan, 1972; Kishi et al., 1980; Voneida et al., 1981). Hippocampal regions that give rise to commissural efferents tend to send equivalent projections to the ipsilateral hippocampus, although the ipsilateral projections are usually more extensive (Swanson et al., 1978; Swanson et al., 1980).

The various types of commissural projections are all glutamatergic (Johnston and Amaral, 2004; although see Ribak et al., 1986, for evidence of a γ-aminobutyric acid (GABA) -ergic component of the hippocampal commissural pathway). Electrical stimulation of hippocampal area CA3 *in vivo* elicits field EPSPs in areas CA3 and CA1 of the contralateral hippocampus (Andersen, 1959; Finnerty and Jefferys, 1993; Thomson, 1979). High-frequency stimulation of commissural fibres *in vivo* can result in LTP in hippocampal area CA1 (Markevich et al., 1997; Young et al., 1998). Hernandez et al. (1994) elicited LTP in area CA3 by high-frequency stimulation of pyramidal cells in the contralateral area CA3, and found that this LTP was reduced by the NMDAR antagonist D,L-3[(±)-2-carboxypiperazin-4-yl]- propyl-1-phosphonic acid (CPP), indicating that LTP is NMDAR-dependent at commissural synapses. Kaibara and Leung (1993) found that tetanization of stratum oriens in hippocampal area CA1 or CA3 was

effective in eliciting LTP in the basal dendrites of pyramidal neurons in contralateral area CA1, but not the apical dendrites. Tetanization of stratum radiatum was ineffective in eliciting LTP in both the apical and basal contralateral dendrites, suggesting that the basal and apical dendrites in CA1 differ in their receptiveness to commissural LTP (Kaibara and Leung, 1993).

Low-frequency stimulation of commissural fibres can elicit LTD in area CA1 that is NMDAR-dependent (Thiels et al., 1996). An *in vitro* study in which the commissural pathway received low-frequency stimulation in guinea pig hippocampal slices determined that such stimulation resulted in the secretion of GABA (Skrede and Malthe-Sorenssen, 1981). The authors concluded that commissural fibres stimulated pyramidal neurons, which activated interneurons that released GABA as a transmitter. Similarly, Douglas et al. (1983) found that stimulation of the hippocampal hilar region resulted in inhibition of the perforant path evoked potential in the contralateral hippocampus, presumably by commissural stimulation of local interneurons. Commissural/associational synapses in area CA3 can also undergo associative LTP and LTD depending on activity at mossy fibre-CA3 synapses (Chattarji et al., 1989), indicating that the commissural input to the CA3 pyramidal neurons can interact with other synaptic influences on these neurons to elicit different types of synaptic plasticity. Overall, activity in commissural fibres has the potential to influence information processing in multiple hippocampal subregions, and likely has a key role in the transfer of information between the two hippocampi in the brain.

ii. Memory in split brain patients and animals lacking the hippocampal commissure

Commissurotomy, or transection of the cerebral commissures, was first used in the 1940s as a treatment to reduce the spread of epileptic seizures in the brain (Springer and Deutsch, 1998). Such "split-brain" patients have become the focus of much study into the lateralization of function between the cerebral hemispheres. They are an interesting model in which to study the influence of the commissures on memory because these patients don't exhibit any gross behavioural impairments in day to day activities. Speech, conversation, reasoning, motor coordination, personality, social interaction, and intelligence all appear intact after recovery from surgery (Zaidel and Sperry, 1974). Specialized tests that restrict performance to the use of a single brain hemisphere are often needed to detect any behavioural change (Bogen et al., 1965; Zaidel and Sperry, 1974). Indeed, defining changes in memory function in split-brain patients has been challenging. There are no obvious memory impairments observed in the population of split-brain patients as a whole (Clark and Geffen, 1989), but if we closely examine exactly which commissures have been transected in which patients, some patterns emerge.

In general, if the commissurotomy included the posterior regions of the corpus callosum, patients were more likely to exhibit memory deficits than if only the anterior regions are transected (Phelps et al., 1991; Springer and Deutsch, 1998). This may be because the hippocampal commissure is more likely to be damaged with posterior than with anterior transections. The memory deficits in these commissurotomy patients tended to encompass verbal and visual recall memory more than recognition memory (Phelps et al., 1991). Zaidel (1995) argues that short-term memory is impaired in split-

brain patients, although long-term memory remains intact (Zaidel and Sperry, 1974), but this notion has been disputed (see Clark and Geffen, 1989, for review).

It is generally accepted that an individual brain hemisphere is capable of carrying out most types of tasks without communicating with the other hemisphere (although, because of lateralization of certain functions, the left or right brain may offer better performance on particular tasks). Animal studies have provided convincing evidence of this in learning and memory tasks. For instance, Majkowski (1967) transected the hippocampal commissure, corpus callosum, and optic chiasma in cats, and then trained them on a cued conditioning task. Cats were given co-terminating pairings of light flashes with electrical shock; shock could be avoided if the cats pulled a string during the light presentation to shut off the switch controlling the shock. Majkowski's cats were trained in only one hemisphere of the brain by restricting sensory input to the other hemisphere. The cats learned this task over multiple trials and became proficient in avoiding shock even in the absence of a hippocampal commissure, indicating that communication between brain hemispheres is not required for this type of learning and that one hemisphere is sufficient. There is also evidence that place learning is preserved in rats with a lesion of one hippocampus (Czeh et al., 1998; Kolb et al., 1984; but see Astur et al., 2002; Port et al., 2000). As such, animal models have not yet reflected the subtle, yet detectable, consequences of loss of communication between the hippocampi in the human.

Aside from lesion studies, the role of the cerebral commissures in cognition has also been inferred from the study of humans and animals with callosal agenesis. Callosal agenesis is a deformity of the midline brain structures due to defective axonal migration

during early development (Lassonde and Sauerwein, 2003). Saul and Sperry (1968) reported that patients with callosal agenesis did not display the same cognitive deficits as split-brain patients. This is not surprising because callosal agenesis is congenital, whereas split-brain patients experience their brain damage later in life. Perhaps brain development is altered in a compensatory fashion in callosal agenesis patients. In addition, in humans, the hippocampal commissure often remains intact in callosal agenesis patients (Loeser and Alvord, 1968).

Some animal models of callosal and hippocampal commissure agenesis have been identified. A number of inbred mouse strains exhibit reductions in the size of, or complete absence of, particular commissures (Wahlsten, 1989). Strain BALB/c, for instance, can exhibit a small, or completely absent, corpus callosum (Wahlsten, 1974; Wimer et al., 1969). Genetic factors may influence the development of the brain structures that axons must migrate along during development, potentially interfering with proper formation of the commissures (Wahlsten and Bulman-Fleming, 1994). Reflecting what is seen in human patients, lack of development of a corpus callosum in mouse strains seems to have little effect on a variety of behaviours (Bulman-Fleming et al., 1992; Schmidt et al., 1991; Wahlsten et al., 2001). However, it is uncertain whether absence of the hippocampal commissure, in particular, in these mice might result in memory impairment as seen in humans (Phelps et al., 1991).

Via recombinant breeding and inbreeding, mouse strains 9XCA/Wah and BALB/cWah1 have been generated that express agenesis of different combinations of forebrain commissures (Wahlsten, 1989; Wahlsten and Sparks, 1995). 9XCA/Wah exhibits complete absence of the corpus callosum, and a hippocampal commissure that is

greatly reduced in size (Wahlsten and Sparks, 1995; D. Wahlsten and K.M. Bishop, unpublished observations). Only 4% of BALB/cWah1 mice, on the other hand, express a reduced hippocampal commissure size, but 44% show a reduction or absence of the corpus callosum. These mice present an interesting opportunity to investigate the consequences of hippocampal commissure agenesis on memory and hippocampal synaptic physiology, especially because by comparing the phenotypes of the two strains, the potential influence of absence of the corpus callosum can be controlled for. An important note is that the effect of absence of commissural input to the hippocampus on synaptic plasticity has not been previously studied, and remains an important question.

H. Inbred Mouse Strains

i. Genetic approaches to the study of synaptic plasticity, learning, and memory

The advent of murine transgenic and gene-targeting techniques has provided behavioural geneticists and neurophysiologists with invaluable experimental technology for clarifying the functions of particular genes, molecules, and signalling pathways in certain forms of learning and memory (Chen and Tonegawa, 1997; Martin et al., 2000; Micheau and Riedel, 1999; Picciotto and Wickman, 1998; Wehner et al., 1996). Within specific brain regions, such as the hippocampus, single genes may be artificially overexpressed (Mayford et al., 1996; see also Costantini and Lacy, 1981; Jaenisch and Mintz, 1974; Palmiter et al., 1982, for the seminal reports of the development of transgenic techniques), or their expression may be reduced or eliminated by targeted mutagenesis (e.g., Bradley, 1993; Capecchi, 1989; Smithies et al., 1985; Soriano, 1995; Thomas and Capecchi, 1986).

Genetically modified mice have provided great insight into the specific signaling molecules responsible for the expression of LTP, learning, and memory (Mayford et al., 1995; Picciotto and Wickman, 1998). A three-pronged approach to the mechanistic analysis of learning and memory, involving molecular biological, electrophysiological, and behavioural techniques, has commonly been used to investigate the cellular and molecular mechanisms of mammalian learning and memory (see **Figure 1.10**) (Chen and Tonegawa, 1997; Grant et al., 1992; Picciotto and Wickman, 1998; Tsien et al., 1996a). However, genetic manipulation comes with a few caveats and challenges (see Gerlai, 1996; Gerlai, 2001; Wehner and Silva, 1996; Wolfer et al., 2002). For example, deletion or overexpression of a gene can lead to compensatory up- or down-regulation of other genes that can mask the effect of the genetic manipulation. Furthermore, these compensatory mechanisms can vary according to the animal's genetic background. Mutant mice are frequently generated using inbred strains or F1 hybrids (first generation offspring) of two inbred strains as backgrounds. Inbred strains are created by at least 20 consecutive generations of matings between siblings, resulting in genetically identical mice within a strain. Inbred strains differ from one another in genetic makeup, and they can display marked variations in neurochemistry (Ingram and Corfman, 1980), synaptic plasticity, learning, and memory (e.g., Nguyen et al., 2000a; Nguyen et al. 2000b; reviewed by Nguyen and Gerlai, 2002). Thus, directed mutation of one gene might elicit distinct compensatory mechanisms in different inbred strains, depending on the presence of other genes and signalling molecules that modify the function of the mutated gene and its encoded protein. In short, there is always potential for specific interactions between a certain gene and a particular genetic background.

Because inbred strains vary in performance on behavioural tests and display differing levels of hippocampal synaptic plasticity, the inbred strains used for genetic manipulation will influence the phenotypes of mutant mice. If an inbred strain that performs poorly on the MWM were used to generate mutant mice to test the hypothesis that knockout of a protein will impair spatial memory performance, subsequent testing of mutant mice on the MWM will produce ambiguous results. Similarly, enhancement of memory by genetic manipulation of inbred strains that exhibit strong memory function may lead to falsely negative findings. Thus, it is important to choose inbred strains with known behavioural and physiological phenotypes that are suited to an experiment's goal. Crawley (2000) has suggested that most studies requiring production of genetically altered mice would benefit from selecting an inbred mouse strain in which bidirectional changes in behavioural performance can be readily detected. For example, if a mutation is expected to increase memory performance, a strain with modest behavioural memory performance might be selected to provide a good chance of detecting improvements of memory performance.

The complications that arise in choosing an inbred strain background for generating mutant mice can also be viewed as an opportunity to shed new light on the neural mechanisms of learning and memory. The inherent genetic variability of different inbred strains can be studied to reveal novel mechanisms of learning and memory. Using this *forward genetics* approach, inbred strains may be phenotyped for particular types of learning and memory deficits, and then the molecular, cellular, and genetic correlates of these deficits can be identified. This contrasts with the *reverse genetics* used in the study

of genetically modified mice, in which a gene of interest is mutated, and alterations in physiology and behaviour arising from the mutation are assayed (Crawley, 2000).

An early use of forward genetics to study learning was developed by Tolman and Tryon in the 1920s and 1930s (reviewed by Innis, 1992). Here, rats were trained to run a maze for reward, and individual performance differences between the rats were observed. A selective breeding program followed in which the best-performing rats (those that made the fewest errors on the maze) were mated with one another, and poorly performing rats were mated with one another. Through successive generations, maze-bright and maze-dull rat strains were developed; the maze-bright strain consistently outperformed the maze-dull strain on the maze (Tryon 1940, 1942). These experiments illustrated that the ability to learn has aspects that can be inherited. Another very productive forward genetics approach has contributed greatly to the study of genes in learning and memory. The single-gene learning mutants of *D. melanogaster* are an example of forward genetics study because their mutations were induced randomly (usually by exposing flies to mutagenic chemicals; Dudai 1989). Different single-gene mutants were phenotyped for learning and memory, as well as other characteristics, and the gene loci of mutation corresponding to such phenotypes were mapped. The discovery of learning mutants, subsequent gene mapping and cloning, and biochemical measurements led to the identification of several signaling molecules that have a role in learning and memory, such as cAMP-phosphodiesterase (*dunce* mutant; Chen et al., 1986, 1987; Davis and Davidson, 1986) and adenylyl cyclase (*rutabaga* mutant; Dudai et al., 1983; Livingstone et al., 1984).

In particular, forward genetics using inbred strains offers several advantages. First, the genetic variability between strains is the result of breeding, and thus reflects genetic differences that might occur in nature. The allelic differences between strains reflect those that occur via breeding within a population, and they result in detectable learning and memory phenotypes across strains. Although targeted mutagenesis can create alleles that are identical to disease alleles, directed mutagenesis is an artificial genetic manipulation that can give rise to memory impairments that might not fully replicate the molecular characteristics and etiologies of memory deficits that exist in nature. Alternatively, the more complex phenotypes found in inbred strains may more closely mimic naturally occurring memory impairments. A second advantage is that mouse models of specific memory impairments may be discovered by isolating particular types of memory deficits in individual strains. These mouse models can then be examined for alterations in neurophysiology and/or synaptic plasticity as compared to a mouse strain with intact memory. Finally, inbred mouse models of memory impairment can be used to identify ways to enhance learning and memory through experiential or pharmacological intervention.

The use of inbred strains to identify cellular and molecular contributions to memory impairment has some disadvantages. Because the genetic differences between strains are not artificially imposed and controlled by the experimenter, it can be difficult to discern exactly which genes are different from strain to strain. Furthermore, because multiple genes are different between strains and many cognitive traits are polygenic, it can be difficult to determine which genes are responsible for altered cognitive phenotypes. These difficulties can be partially resolved by incorporating sophisticated,

sometimes complex, genetic analyses (e.g., quantitative trait loci, or QTL, analysis; microarray techniques).

A number of studies have gauged behavioural performance of inbred mice on tests of hippocampal learning and memory. In contrast, much less information is available concerning the synaptic and molecular mechanisms underlying strain-dependent differences in hippocampus-dependent learning and memory. In the following sections I will review studies of learning and memory, focussing on fear memory and MWM testing, and hippocampal synaptic plasticity, in inbred mouse strains

ii. Survey of fear conditioning in inbred mouse strains

Table 1.2 summarizes cued and contextual fear conditioning results in a number of inbred strains. Because very few studies examined sex differences within or between strains, this variable was not included in the table. A strain was rated to have 'good' performance if it performed comparably to another strain that displayed good freezing behaviour (i.e., C57BL/6). A strain was rated as 'intermediate' if some memory was evident but was not expressed as strongly as in a 'good' performing strain, or if memory was only evident under restricted conditions. 'Poor' ratings were given to those strains that failed to show any evidence of having fearful memories.

About half of inbred strains tested exhibited good memory for both contextual and cued fear conditioning at 24 hrs after training (see **Table 1.2** for references). All "129" substrains that were tested, and strains C57BL/6, C57BL/10, LP, and SJL performed well. These strains can be postulated to have intact hippocampal and amygdalar function underlying contextual and cued fear memory.

Several strains exhibited poorer contextual than cued fear memory. These strains, including AKR, BALB/cByJ, C3H/He, DBA, and FVB/N probably have some impairment in hippocampal function. Because some cued memory is still intact in these strains, amygdala function is likely intact. Thus, the hippocampal contribution to contextual fear memory, but not the amygdalar contribution, is probably diminished in these strains. These five strains are potential models for the study of hippocampal memory deficits, and further work is needed to pinpoint the physiological mechanisms that underlie these behavioural memory deficits.

A number of inbred strains are homozygous for the age-related hearing loss mutation *ahl*. Hearing loss might affect the ability of these strains to perform in the cued fear conditioning paradigm. Strains BALB/cByJ, BuB/Bn, and C57BL/6 exhibit hearing loss beginning at about 10 months of age (The Jackson Laboratory, 2003). However, BALB/cByJ and C57BL/6 exhibited intact cued memory, and most groups have used mice younger than 10 months. Thus, hearing loss probably did not influence the outcomes of cued conditioning experiments for these three strains. A/J and DBA/2J exhibit earlier onset hearing loss (around three months) as a result of *ahl* (The Jackson Laboratory, 2003), and both were rated 'intermediate' on the cued fear conditioning test. Thus, expression of cued memory may be compromised in these two strains because of hearing loss.

Some of the strains tested for contextual and cued fear conditioning also carry the retinal degeneration allele, *Pde6b*^{rd1}, and could have vision deficits (The Jackson Laboratory, 2002). However, Bolivar et al. (2001) determined that this mutation does not result in fear conditioning deficits in C3H/He mice. Vision is likely not essential for

strains to perform well on the fear conditioning tests. It should also be noted that strain A/J has been described to exhibit generalized freezing (Owen et al., 1997), and this must be taken into consideration when conducting fear conditioning in this strain.

iii. Survey of MWM data in inbred mice

Table 1.3 summarizes MWM learning and probe trial results for a variety of inbred strains. Ratings were assigned to inbred strains using the same criteria as for fear conditioning.

In most cases, within a strain, mice performed similarly on the learning and probe trials – that is, if a strain was capable of learning during the training trials, it also performed well on the probe trial. Many strains performed well on the MWM learning and probe trials (see **Table 1.3** for references). C57BL/6 performed well on both, justifying its use as a strain commonly used to generate mutant mice, and as a "standard" performance strain for multi-strain comparisons of spatial learning and memory ability. C57BL/10J also performed well, as did most of the "129" substrains, with the exception of 129X1/SvJ.

Many of the strains that showed poor performance on the MWM also performed poorly on the visible platform task. This indicates that they are likely impaired in visual perception of spatial cues. Thus, the poor performance of strains A, BALB/cByJ, BuB/BnJ, C3H, CBA/J, FVB/N, and SJL on the MWM does not reflect deficits in learning abilities, because these strains did not successfully complete the visible platform task. Several of these strains are albino (A, BALB/cByJ, BuB/BnJ, FVB/N, SJL) and as a result may have poor vision. In addition, BuB/BnJ, C3H, CBA/J, FVB/N, and SJL are homozygous for the retinal degeneration allele *Pde6b*^{rd1} (The Jackson Laboratory, 2002),

which compromises vision. 129X1/SvJ and BALB/c have not been tested on the visible platform task, but they are also albino, which may contribute to their poor and intermediate performance, respectively, on the MWM. The strains DBA/2, DBA/2J-I, and LP have no known visual impairments, and yet they still performed poorly on at least one aspect of MWM testing (see also Experiment 2B in Paylor et al., 1993). These three strains most likely have impaired spatial learning and memory as a result of altered hippocampal function. However, an important note is that some groups have shown that DBA/2 mice can display good probe trial performance (see **Table 1.3** for references). This disagreement between groups may be explained by small differences in methodology, genetic drift between different mouse suppliers, or variations in rearing and in housing environments. Crabbe et al. (1999) have shown that even when separate groups go to great lengths to replicate methods, behavioural tests done in different locations can yield significantly distinct results.

In summary, several strains have been tested on both the MWM and contextual fear conditioning. Inbred strains that performed well on both the MWM and contextual fear conditioning include 129S6/SvEv, 129T2/SvEmsJ, C57BL/6, and C57BL/10. Of the strains considered in this section, these strains are the most likely to have intact hippocampal function. In contrast, BALB/c showed intermediate performance on both tests. CBA, DBA/2, and FVB/N performed poorly on both tests and most likely have impaired hippocampal function. However, CBA and FVB/N may have performed poorly on the MWM due to deficits in visual acuity.

Other strains showed divergence in performance on these two tests of hippocampus-dependent learning and memory (i.e., 129X1/SvJ, A, BALB/cByJ, C3H,

LP, and SJL). Although the MWM task and contextual fear conditioning have both been shown to depend on intact hippocampal function, they may require distinct hippocampal processes that engage different hippocampal circuits or recruit distinct types of synaptic plasticity (e.g., Bannerman et al., 1999; Ferbinteanu and McDonald., 2001; Hock Jr and Bunsey, 1998; Maruki et al., 2001; Moser et al., 1993). Both tasks also depend on brain regions outside of the hippocampus. If extra-hippocampal brain structures are dysfunctional in a particular strain, it may show a memory impairment when compared to another strain that has equivalent hippocampal but normal extra-hippocampal function. Thus, it is advisable to conduct several behavioural tests of hippocampal function that vary in their documented dependence on extra-hippocampal brain structures.

iv. Survey of hippocampal synaptic transmission in inbred mouse strains

Hippocampal synaptic plasticity in mice has often been examined along with behavioural performance (see reviews Chen and Tonegawa, 1997; Martin et al., 2000). The common goal of these studies is to demonstrate a causal relationship between certain types of hippocampal synaptic modification (e.g., LTP or LTD) and specific forms of hippocampus-dependent learning and memory (e.g., spatial or contextual learning). The issue of whether LTP, or other forms of synaptic plasticity, is causally linked to particular types of learning and memory is hotly debated (for discussion, see Martin et al., 2000). There is evidence that LTP can critically influence the expression of some types of learning and memory (e.g., spatial learning and memory: Moser et al., 1998; Brun et al., 2001).

There is little detailed information on the comparative electrophysiology of hippocampal neurons in inbred strains of mice. The few studies that have started to

investigate this important aspect of the comparative physiology of inbred strains are reviewed below.

Table 1.4 summarizes studies that have examined hippocampal synaptic transmission in inbred mice. Fewer inbred strains have been studied for hippocampal synaptic transmission than for expression of hippocampus-dependent learning and memory. Several strains exhibit impairments in particular forms of synaptic plasticity, including 129/SvEv, 129T2/SvEmsJ, CBA/J, and DBA/2. Deficits in LTP were detectable using both *ex vivo* (hippocampal slice) and *in vivo* preparations (see ***Notes*, Table 1.4**).

The most interesting finding in **Table 1.4** is that LTP deficits can vary greatly between inbred strains. It does not appear that an inbred strain has 'good' LTP or 'poor' LTP *per se*, with the exception of C57BL/6, which has been chosen to represent a "standard" for robust LTP. Rather, most strains show selective deficits in a subset of LTP variants. Furthermore, the composition of the deficient subset is different between strains.

LTP induced by different types of tetanic stimulation can rely on different cellular and molecular mechanisms for induction and maintenance (as discussed in *section E*). For example, in area CA1 of hippocampal slices, using different stimulation protocols either E-LTP or L-LTP can be induced, each reliant upon different molecular mechanisms (Abel et al., 1997; Andersen et al., 1977; Bourtchouladze et al., 1994; Collingridge et al., 1983; Frey et al., 1988; Frey et al., 1993; Huang and Kandel, 1994; Huang et al., 1996; Impey et al., 1996; Malinow et al., 1989; Matthies and Reymann, 1993; Nguyen et al., 1994; Silva et al., 1992a; also see Matsushita et al., 2001; Schulz et

al., 1999). Thus, it is plausible that if any of the signaling pathways involved in E-LTP or L-LTP are expressed differentially in particular inbred strains, then specific forms of LTP may be altered in these strains. Indeed, the finding that LTP deficits can vary across inbred strains indicates that the different genetic makeup of the strains affects the availability and/or function of the cellular and molecular mechanisms, or the neural anatomy and structure, that underlie different types of hippocampal LTP. For example, Nguyen et al. (2000b) demonstrated that induction and maintenance of CA1 LTP were increased in 129T2/SvEmsJ hippocampal slices following stimulation by 4 trains of HFS at 3 sec intervals compared with stimulation by 4 trains of HFS at 20 sec intervals. Thus, the same total number of stimuli applied on a slightly different time scale yielded LTP with differing magnitudes in this strain. In contrast, in the other three strains tested in that study (C57BL/6J, CBA/J, and DBA/2J), changing the inter-train interval did not affect the magnitude of LTP. The authors concluded that the hippocampal neurons of some mouse strains might be optimally tuned to particular temporal patterns of synaptic activity due to variations in genetic background. It is unclear which specific properties of hippocampal neurons in strain 129T2/SvEmsJ differ from those of the other strains examined by Nguyen et al. (2000b). The authors examined several biophysical and cellular electrophysiological properties of CA1 pyramidal neurons (including spike-frequency accommodation, membrane input resistance, and number of spikes elicited by current injection) in these strains and found no differences between strains. Thus, the frequency-dependent deficit in LTP observed in this study cannot be readily explained by inter-strain variations in the biophysical and spike-firing properties of hippocampal

neurons. Rather, this L-LTP deficit is potentially the result of biochemical alterations in 129T2/SvEmsJ (e.g., modified spatial localization of kinases such as PKA).

v. Correlates of memory impairments in inbred strains

Cellular and molecular differences between inbred strains likely contribute to the modifications of learning and memory that have been observed. For instance, several neurochemical differences between DBA/2 and C57BL/6 have been reported in the literature. Compared with C57BL/6 mice, DBA/2 mice have reduced hippocampal PKC activity (**see Table 1.5**) (Bowers et al., 1995; Paylor et al., 1996; Wehner et al., 1990). PKC is required for induction of LTP (Malinow et al., 1989), and it has a role in hippocampal learning and memory (Abeliovich et al., 1993; Olds and Alkon, 1991; Van der Zee et al., 1992). For a more in-depth discussion of cellular and molecular alterations that are suspected of having a role in impaired hippocampal LTP and memory in strain DBA, see the *Appendix.*

The studies of inbred mice described in this section indicate overall that inbred mouse strains exhibit widely varying phenotypes. Different types of memory impairment in different strains are abundant, and provide us with the opportunity to investigate the cellular and molecular mechanisms of specific memory functions. Already, some studies have attempted to correlate hippocampal LTP with memory function, but no studies have examined synaptic plasticity in the amygdala, another brain structure that is important for memory (cued fear memory, in particular). Further studies have the potential to reveal the roles of hippocampal and amygdalar synaptic plasticity in contextual and cued fear memory, and identify specific molecules and forms of synaptic plasticity that are important in particular types of memory processes in these mice.

I. Neuromodulation in Inbred Mice

The neuromodulatory systems in the brain can facilitate or suppress different types of synaptic plasticity, as well as learning and memory processes. Alterations in neuromodulation might result from the genetic differences between inbred mouse strains, and might influence synaptic plasticity, learning, and memory in these mice. Although neuromodulation has not been the focus of much study in inbred mice, some aspects have been examined. In this section I will briefly introduce the concept of neuromodulation and its role in synaptic plasticity, learning, and memory. I will then focus on the special role of the noradrenergic neuromodulatory system in these processes, and will consider this interaction in inbred mouse strains.

i. The neuromodulatory systems of the brain

Neuromodulation is not generally involved with directly processing bits of information; rather, it influences the circuitry that is responsible for such processing by altering its responsiveness to stimuli (Saper, 2000; Nestler et al., 2001). Functionally, neuromodulation can influence sensory awareness, motor responses, and levels of arousal (Saper, 2000). Neuromodulators usually take longer to act than a conventional transmitter, and their effects often last longer. The same neurochemicals may act as both transmitters and neuromodulators; in this case, the chemical might have a quick, transmitter-like effect followed by a slow-onset, long-lasting modulatory effect (Hasselmo, 1995). The slow, long-lasting quality of neuromodulation often results from the activation of metabotropic (G protein-coupled) receptors by the neuromodulator. Because metabotropic receptors exert their influence by activating other proteins (e.g.,

enzymes, second messengers, ion channels), they take longer than ionotropic receptors to affect neurons.

The major factor that seems to define the neuromodulatory systems of the brain is that each originates with a relatively small number of neurons, usually in the brainstem, that project to and influence wide areas of the brain by secreting a particular transmitter (Hasselmo, 1995; Nestler et al., 2001; Saper, 2000). Some major neuromodulatory systems that originate in the brainstem are the dopaminergic, noradrenergic, adrenergic, serotonergic, cholinergic, and histaminergic systems. Although all of these systems can influence cognitive function, in my thesis research I examined the dopaminergic, noradrenergic, and serotonergic influences in the hippocampi of inbred mice, and thus I will discuss these particular modulatory systems in greater depth here.

Neurons in the substantia nigra pars compacta, ventral tegmental area, and arcuate nucleus secrete dopamine (DA; Nestler et al, 2001). The neurons of the ventral tegmental area, located in the ventral midbrain, project to limbic structures, including the hippocampus, and release DA (called the mesolimbocortical dopamine system). This dopaminergic mesolimbocortical system has a role in cognitive and emotional function, learning and memory, and includes projections to the nucleus accumbens involved in reward (Nestler et al, 2001; Saper, 2000). Norepinephrine (NE) is produced by neurons in the locus ceruleus, located in the rostral pons (Nestler et al., 2001; Saper, 2000). The locus ceruleus projects to every major region of the brain, including the hippocampus (Moore and Bloom, 1979; Saper, 2000). Three sets of fibres provide NE innervation of the hippocampus (the ansa peduncularis-ventral amygdaloid bundle system, the fornix, and the cingulum), and together these fibres innervate all major hippocampal subregions

(Moore and Bloom, 1979). The noradrenergic modulatory system has been proposed to impart vigilance and responsiveness to unanticipated environmental stimuli (Saper, 2000), and has a role in sleep/arousal, attention, and learning and memory (Nestler et al, 2001). DA and NE are referred to as catecholamines, and share a synthetic pathway (along with epinephrine; see **Figure 1.11**). Via the thorough innervation of the brain by the dopaminergic and noradrenergic neuromodulatory systems, these catecholamines can facilitate or inhibit communication between neurons, and can regulate synaptic plasticity (Nestler et al., 2001).

The hydroxylation of tyrosine is the rate-limiting step in the synthesis of DA and NE (Nestler et al., 2001; Schwartz, 2000), and *tyrosine hydroxylase*, the required enzyme, can be regulated rapidly by phosphorylation and dephosphorylation. PKA, CaMKII, PKC, and MAPK are some of the protein kinases that can act on tyrosine hydroxylase to increase its catalytic activity (Nestler et al., 2001). This increased activity can enhance DA and NE levels in dopaminergic and noradrenergic neurons, respectively. Long-term changes in expression of tyrosine hydroxylase can be accomplished via the activation of CRE-mediated transcription (Nestler et al., 2001).

After the synthesis and release of DA or NE into the synaptic cleft, several things may happen to the molecules of transmitter. DA and NE may activate presynaptic or postsynaptic receptors, may be degraded by the enzymes catechol-O-methyltransferase (COMP) and/or monoamine oxidase (MAO) into metabolites, may diffuse from the synaptic cleft, or may undergo uptake into the presynaptic terminal by specific active transporters (Nestler et al., 2001). DA and NE can activate G protein-coupled receptors on postsynaptic neurons. For DA, D_1 and D_5 receptors are G_s-coupled, whereas D_{2-4}

receptors are G_i-coupled. For NE, α_1-adrenergic receptors are G_q-coupled, α_2-adrenergic receptors are generally G_i-coupled, and ß-adrenergic receptors are G_s-coupled (Nestler et al, 2001). α_2-ARs and D_2 receptors are often found on the presynaptic neuron, and inhibit further release of their respective transmitters (Nestler et al., 2001). Activation of G_s-coupled receptors can lead to activation of adenylyl cyclase and PKA; phosphorylation of ß-adrenergic receptors (ß-ARs) by PKA and PKC can lead to receptor desensitization (Nestler et al., 2001). In the hippocampus and cortex, ß-AR activation can promote pyramidal cell excitation by blocking a Ca^{2+}-activated K^+ channel (Madison and Nicoll, 1982, 1986).

Neurons in the raphe nuclei of the brainstem synthesize and release serotonin, or 5-hydroxytryptamine (5-HT). Like the DA and NE systems, these serotonergic neurons project axons and can secrete 5-HT to many brain structures, including the limbic system and the cerebral cortex (Nestler et al., 2001). 5-HT is thought to participate in many cognitive processes (e.g., mood and sleep; Nestler et al., 2001). Two enzymes are required in the synthesis of 5-HT from tryptophan (see **Figure 1.12**). The first, tryptophan hydroxylase, is very similar to tyrosine hydroxylase; the genes that encode these enzymes are even located near each other on the same chromosome (Schwartz, 2000). 5-HT is degraded by MAO's and aldehyde dehydrogenase into 5-hydroxyindoleacetic acid (5-HIAA). Like DA and NE, serotonin can also influence both presynaptic and postsynaptic receptors. Fourteen types of 5-HT receptors have been identified; 13 are G protein-coupled, and one is ionotropic (Nestler et al., 2001). The 13 types of G protein-coupled receptors utilize G_s, G_i, and G_q proteins, like the dopaminergic and adrenergic receptors.

ii. Norepinephrine and hippocampus-dependent memory

The noradrenergic modulatory system has a role in regulating memory storage for events that occur when emotionally aroused (Cahill et al., 1994; McGaugh, 2000). Stressful events can activate the sympathetic nervous system and the pituitary-adrenal axis, leading to the release of epinephrine and glucocorticoids by the adrenal glands. Epinephrine and glucocorticoids have the same eventual influence on the amygdala in that they increase levels of norepinephrine within the amygdala, resulting in norepinephrine release by the amygdala to many different brain regions that are involved in learning and memory (Izquierdo and Medina, 1997; McGaugh, 2000). ß-ARs in the amygdala are necessary for this norepinephrine-mediated enhancement of learning and memory because blockade of amygdalar ß-ARs after training prevents the enhancement of learning and memory in different memory systems, and infusions of ß-AR agonists enhance memory (Gallagher et al., 1977; Hatfield and McGaugh, 1999; Ji et al., 2003; Ji et al., 2003a; Liang et al., 1986; Packard and Teather, 1998).

Several groups have studied the role of NE in memory by testing memory in mutant mice that are unable to synthesize NE via a targeted disruption of the dopamine ß-hydroxylase gene (*Dbh*$^{-/-}$ mice; Thomas et al., 1995). Marino et al. (2005) found that social memory is impaired in these mutant mice. Thomas and Palmiter (1997) found that these mutant mice are able to learn the location of the hidden platform in the Morris water maze, and exhibit a preference for the correct quadrant on a probe trial conducted immediately after training. However, the mutant mice were impaired, compared to controls, on a probe trial conducted two days after training ended; the authors suggested that memory consolidation was impaired in the mutant mice (Thomas and Palmiter,

1997). Murchison et al. (2004) then investigated whether retrieval processes are impaired in these *Dbh*$^{-/-}$ mice using a single-trial fear conditioning paradigm. By testing contextual fear at various time points after training, Murchison et al. determined that the mutant mice exhibited a contextual fear memory deficit beginning 2hrs after training. However, contextual fear memory recovered to control levels in the days following training, leading the authors to hypothesize that retrieval, rather than consolidation, is impaired in these mutant mice. Indeed, pharmacological restoration of NE levels in the mutant mice before memory testing, but not during training, rescued contextual fear memory. Murchison et al.'s (2004) experiments indicate that β_1-AR activation is required for the retrieval, but not the formation or consolidation, of hippocampus-dependent memory for a period of several days after training (see also Ouyang and Thomas, 2005). Zhang et al. (2005) studied contextual fear memory and transcriptional imaging in *Dbh*$^{-/-}$ mice, and suggested that adrenergic signaling is important for the transfer of retrieved information from area CA3 to CA1, where this information may be compared to sensory input received from the cortex.

Overall, there is strong evidence that the noradrenergic neuromodulatory system can influence multiple memory systems. It follows that norepinephrine, and activation of ß-ARs, are likely to influence hippocampal LTP.

iii. Norepinephrine and hippocampal LTP

Activation of different subtypes of adrenergic receptors in different hippocampal subregions can influence LTP in distinct ways. Here I will focus on the effects of ß-AR activity on LTP in hippocampal area CA1, although substantial work has also focused on the role of NE and ß-AR activity in LTP in hippocampal area CA3 and the dentate gyrus

(Frey et al., 2001; Hopkins and Johnston, 1984,1988; Munro et al., 2001; Stanton and Sarvey, 1985; Swanson-Park et al., 1999).

Some studies have suggested that ß-AR activity is not required for LTP in hippocampal area CA1 (e.g., Swanson-Park et al., 1999). Murchison et al. (2004) found that E-LTP induced in area CA1 of transverse hippocampal slices by one train of 100Hz stimulation, and L-LTP induced by 4 trains of 100Hz stimulation (4-min intertrain interval) were intact in NE-deficient *Dbh*$^{-/-}$ mice. Also, Katsuki et al. (1997) found that application of NE to area CA1 of hippocampal slices had no effect on LTP induced by theta-burst stimulation. However, rats in which the endogenous adrenergic system was eliminated at birth exhibited deficits in CA1 LTP induced by theta-burst stimulation (Yang et al., 2002).

Rather than directly mediating LTP, it may be that adrenergic receptor activation has a role in modulating, or enhancing, LTP in area CA1. Moody et al. (1999) found that ß-AR activation could enable additional LTP induction at already-potentiated synapses when low-frequency stimulation was used for tetanus. Also, ß-AR activity can modulate the synaptic response to activity that is subthreshold for inducing LTP. Thomas et al. (1996) showed that long trains of 5Hz stimulation don't result in lasting potentiation in area CA1, but applying isoproterenol to activate ß-ARs during this low-frequency stimulation elicits LTP that lasts at least one hour. Katsuki et al. (1997), Winder et al. (1999), Brown et al. (2000), Giovannini et al. (2001), and Gelinas and Nguyen (2005) have replicated Thomas et al.'s (1996) finding that LTP in area CA1 can be elicited by activation of ß-ARs during stimuli sub-threshold for inducing LTP. Winder et al. (1999) determined that is it likely the $ß_1$-AR subtype that is required for this facilitatory effect on

LTP. There is some indication that ß-AR activation in this situation recruits the cAMP/PKA signaling pathway, which inhibits the activity of protein phosphatases (Brown et al., 2000; Thomas et al., 1996), enabling LTP. CaMKII may also be required for this form of LTP (Brown et al., 2000; Giovannini et al., 2001). Other studies have suggested that the MAPK signaling pathway is recruited by conjoint ß-AR activity and sub-threshold stimulation, and is required for LTP to occur (Gelinas and Nguyen, 2005; Giovannini et al., 2001; Winder et al., 1999). Interestingly, Gelinas and Nguyen (2005) found that ß-AR activation during both prolonged 5Hz stimulation, and during one train of 100Hz stimulation elicited L-LTP in area CA1, and this form of LTP required dendritic translation, but not transcription, and was ERK-dependent. In summary, ß-AR activation during sub-threshold stimulation can promote LTP in area CA1. This finding supports the notion that NE can facilitate hippocampus-dependent memory.

iv. Neuromodulatory transmitters in inbred mouse strains

A number of studies have examined catecholamine concentrations in brain tissue, or in particular brain structures, of inbred mouse strains. NE, DA, and 5-HT levels in the brain of strain B6 have been measured in several studies (Dolfini et al., 1970; Kempf, 1974; Scudder et al., 1966; Yoshimoto and Komura, 1987). Few studies directly compared brain levels of these catecholamines between inbred mouse strains. For example, Yoshimoto and Komura (1987) found that strain C3H exhibited less brain NE than B6.

Future studies that measure levels of catecholamines in brain and/or hippocampal tissue from inbred mouse strains, and relate this data to hippocampal LTP may shed further light on the relationship between neuromodulatory transmitters and LTP. These

studies should also take into consideration the expression of receptors for these neuromodulatory transmitters, because the potential action of the transmitter may be defined by the subtype(s) of receptors available in the brain regions/circuitry of interest.

J. Aims of the Present Thesis

The many factors that contribute to learning, memory, and synaptic plasticity discussed here can be influenced by the genetic background of the organism that is being studied. In this thesis, I have taken advantage of the varying memory and synaptic plasticity phenotypes exhibited by inbred mouse strains in order to shed light on a few important questions. My objectives were as follows:

1. Examine the role of the hippocampal commissure in hippocampus-dependent memory, and to identify potential alterations in hippocampal synaptic plasticity that result from absence of the hippocampal commissure that may alter information processing and memory function (*Chapter 3*). In concordance with the rest of my thesis work, I used an inbred mouse strain model for this investigation.
2. Investigate the role of genetic background in memory and synaptic plasticity by identifying specific memory and LTP phenotypes in inbred mouse strains. Here I focused on hippocampus- and amygdala-dependent memory, and LTP in the hippocampus and the amygdala.
3. Investigate the link between LTP and memory by correlating hippocampal LTP with hippocampus-dependent memory, and amygdalar LTP with amygdala-dependent memory (*Chapters 4* and *5*).
4. Determine whether the genetic differences between inbred mouse strains led to altered expression of neuromodulatory transmitters in the brain, and if so, to discern the effects of this altered expression on hippocampal LTP (*Chapter 6*).

The overall theme of this thesis is that genetic differences in inbred mouse strains can influence learning, memory, and synaptic plasticity. I have used inbred mouse strains as models in which to investigate the objectives outlined above. In this thesis I will present data that support the notion that synaptic plasticity in particular brain regions can influence the types of memory supported by these brain regions. I will also examine LTP and memory in genetic models in which the hippocampal commissure does not develop properly, and will relate genetic differences in neuromodulatory transmitter expression to hippocampal LTP. This body of work contributes valuable insights toward one of the central questions of current neuroscience research: does LTP = memory?

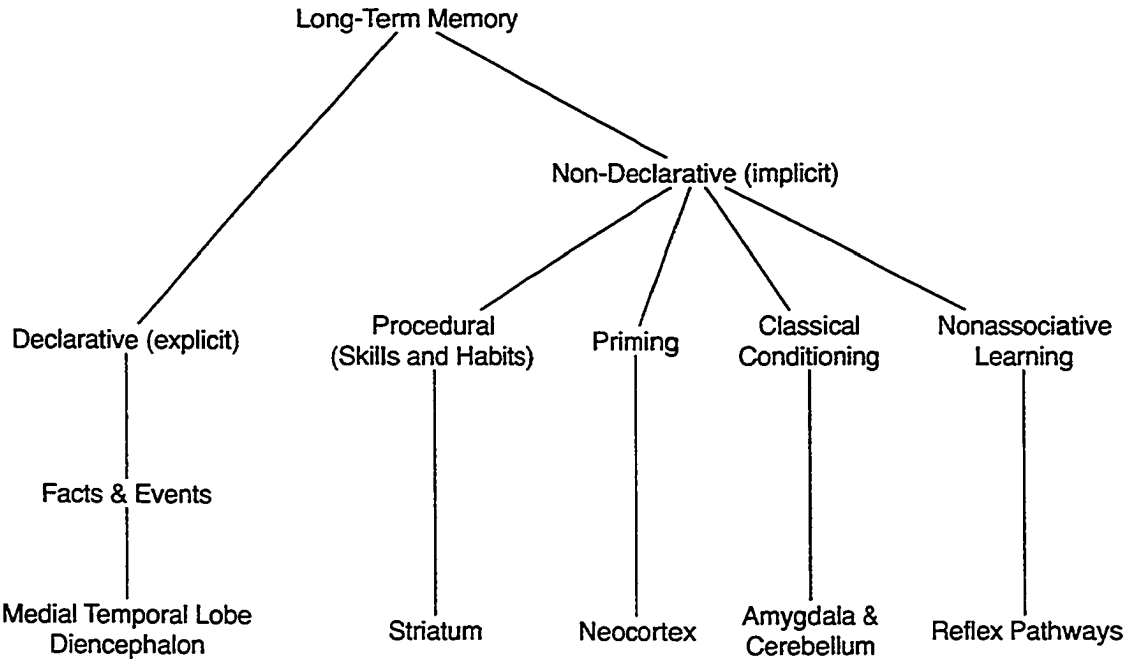

Figure 1.1: Classification system for long-term memory illustrating the taxonomy and anatomy of different forms of memory (adapted from Squire and Zola, 1996).

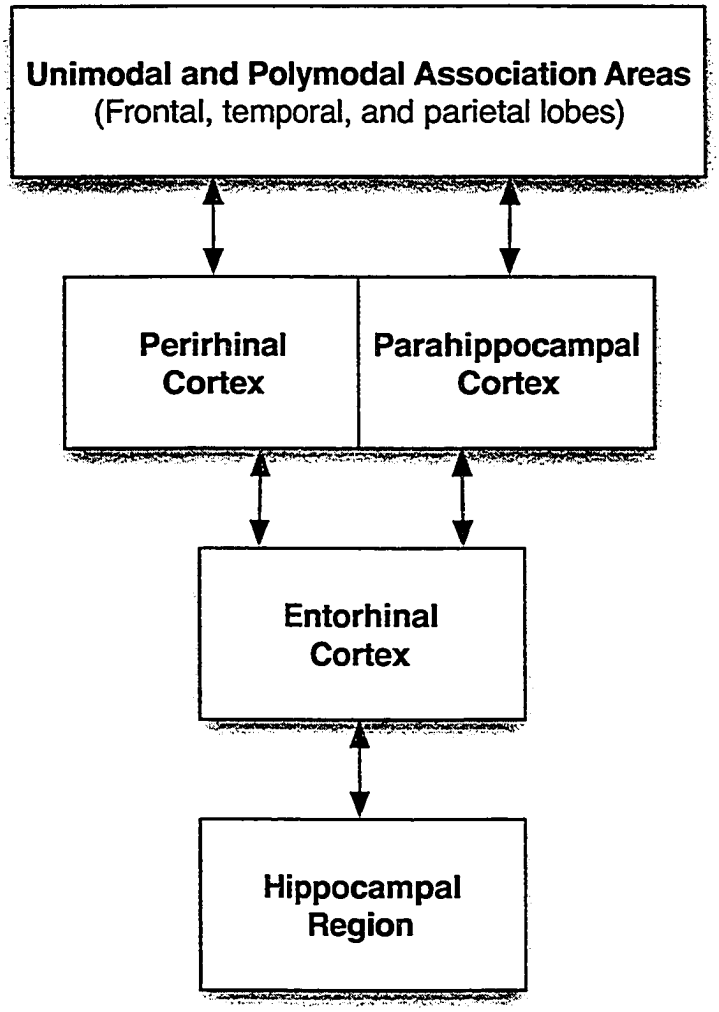

Figure 1.2: The anatomy of the declarative memory system (adapted from Squire and Zola, 1997). The entorhinal cortex receives about two thirds of its inputs from the perirhinal and parahippocampal cortices, and provides the majority of informational input to the hippocampus. All connections are bidirectional.

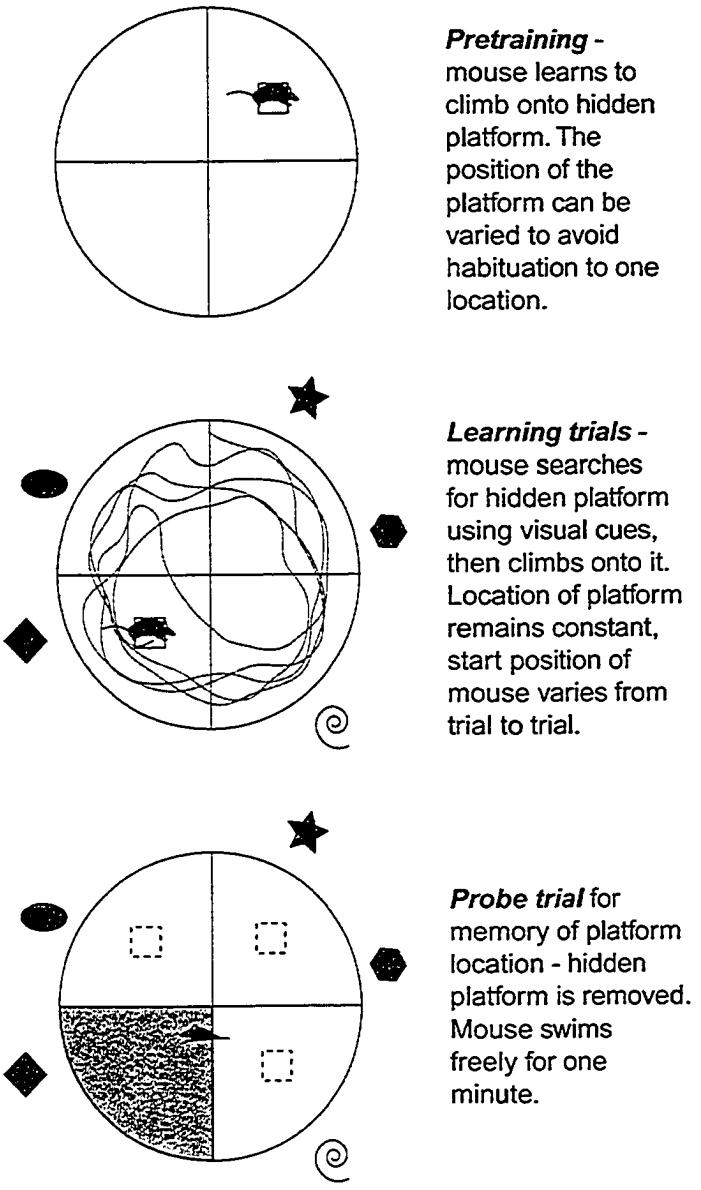

Figure 1.3: General methodology for the Morris Water Maze (from Schimanski and Nguyen, 2004).

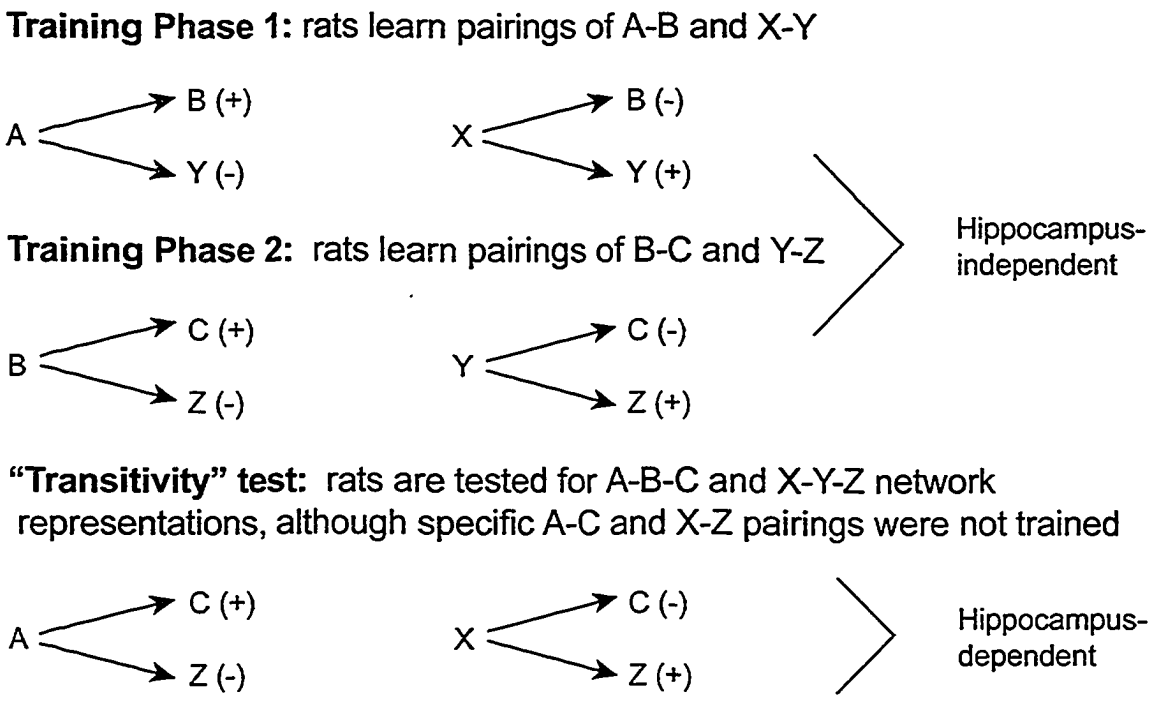

Figure 1.4: Testing and training procedures for the paired associate task (Bunsey and Eichenbaum, 1996). Letters indicate different odours, (+) indicates a rewarded choice, (-) indicates a non-rewarded choice. For example, in *training phase 1*, rats are presented first with odour "A" and subsequently are given a choice between odours "B" and "Y." If a rat chooses odour "B" it receives a reward, and if it chooses odour "Y" it does not receive a reward.

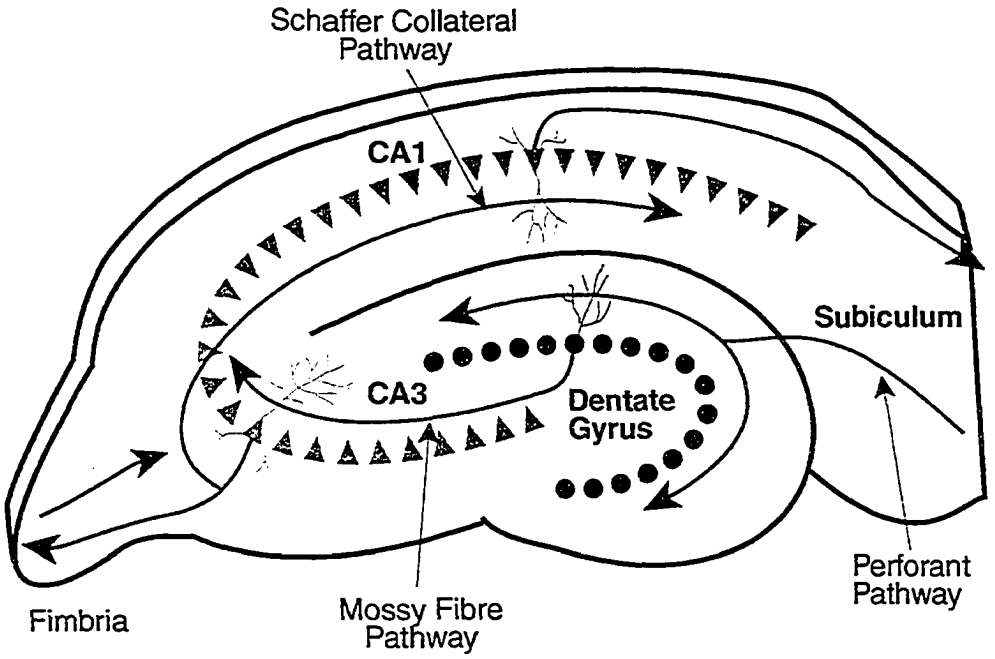

Figure 1.5: The trisynaptic pathway of the hippocampus. Projections enter the hippocampus from the entorhinal cortex via the perforant pathway, and synapse onto the dendrites of dentate granule cells. Dentate granule cells then send projections (the mossy fibres) to pyramidal neuron dendrites in area CA3. CA3 pyramidal neurons project to area CA1 (Schaeffer collateral pathway), then CA1 pyramidal neurons send efferents through the subiculum and back to the entorhinal cortex.

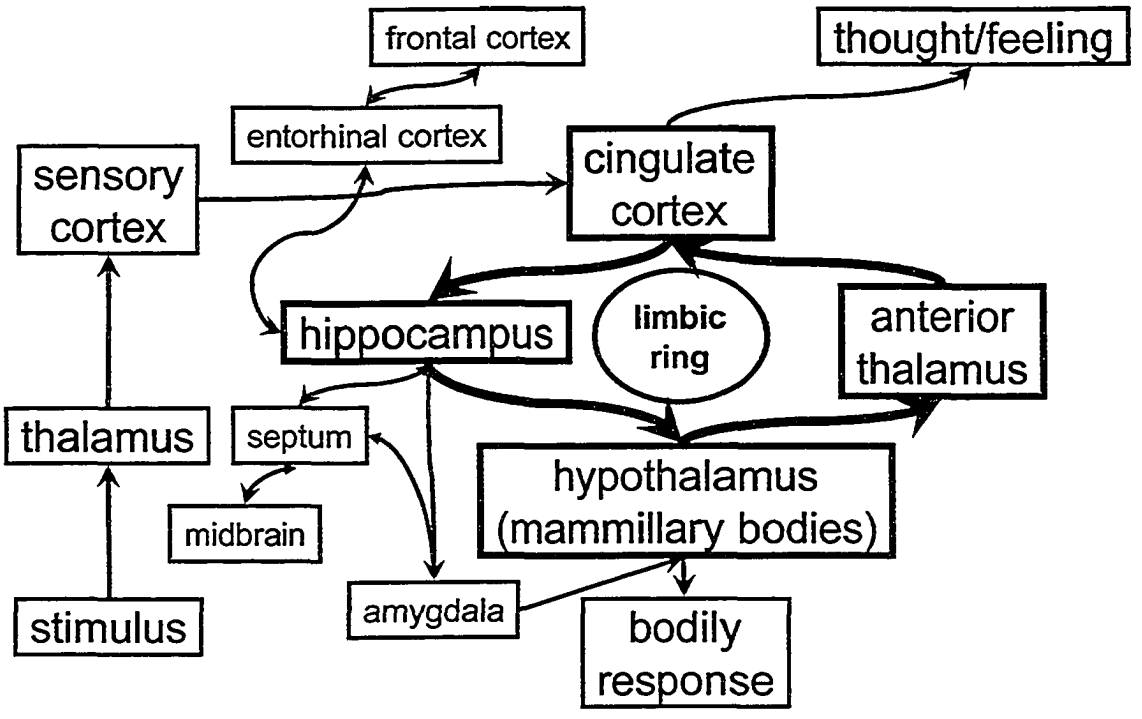

Figure 1.6: The limbic system. The cingulate cortex, hippocampus, mammillary bodies, and anterior thalamus are the core structures that comprise the limbic ring. The cingulate cortex receives sensory inputs, and also provides outputs that mediate changes in thoughts and feelings. The mammillary bodies provide outputs that elicit changes in the body in response to emotional stimuli. Many other brain structures interact with the limbic system, including the amygdala and entorhinal cortex.

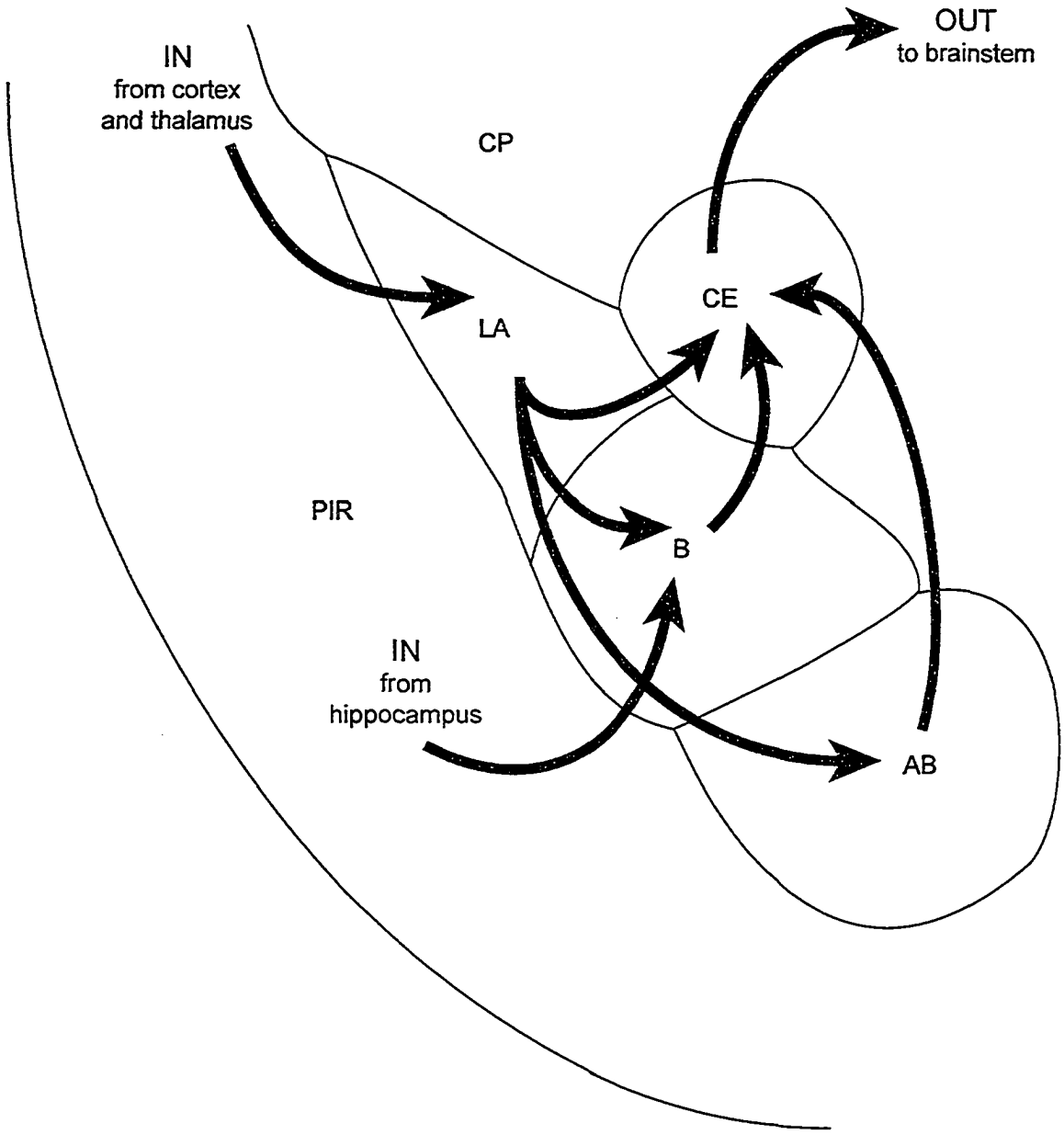

Figure 1.7: Inputs, outputs, and connectivity between nuclei of the rodent amygdala (adapted from LeDoux, 2000). This cartoon depicts the region of a coronal rodent brain section that includes the amygdala. The lateral amygdala (LA) receives inputs from the cortex and thalamus, and then projects to the central nucleus (CE) directly, or indirectly via the basal nucleus (B) or the accessory basal nucleus (AB). The hippocampus also provides input to the amygdala by influencing the basal nucleus. The CE provides outputs to the brainstem that can elicit emotional responses. CP, caudate putamen; PIR, piriform cortex.

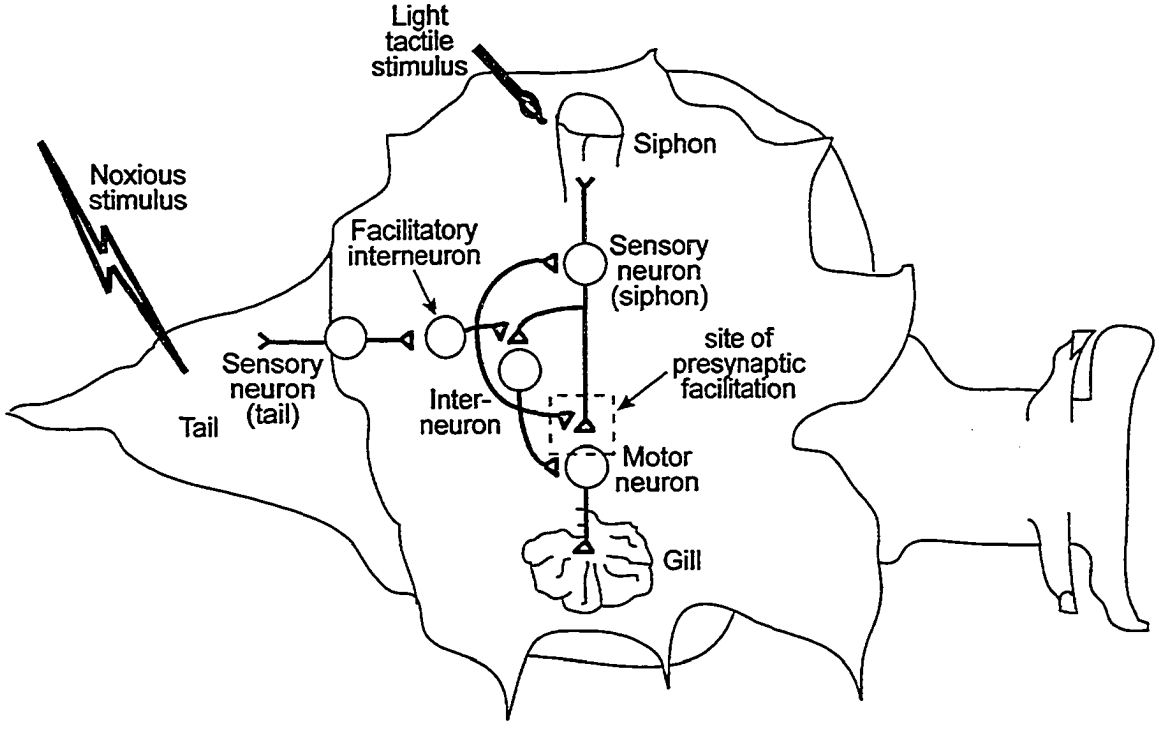

Figure 1.8: Circuits underlying the gill-withdrawal reflex and sensitization in Aplysia californica (adapted from Kandel, 2000). The gill-withdrawal reflex is mediated by the homosynaptic circuit of the siphon sensory neuron and the gill motor neuron. In this model, sensitization can be elicited by applying a noxious stimulus (like an electric shock) to the tail. An increase in gill contraction elicited by a light touch to the siphon would result. Synaptically, the tail shock activates the tail sensory neuron and facilitatory interneuron. The facilitatory interneuron secretes serotonin onto the presynaptic terminal of the siphon sensory neuron (*site of presynaptic facilitation*). Transmitter release from the siphon sensory neuron is enhanced, eliciting increased firing of the motor neuron, and increased gill contraction.

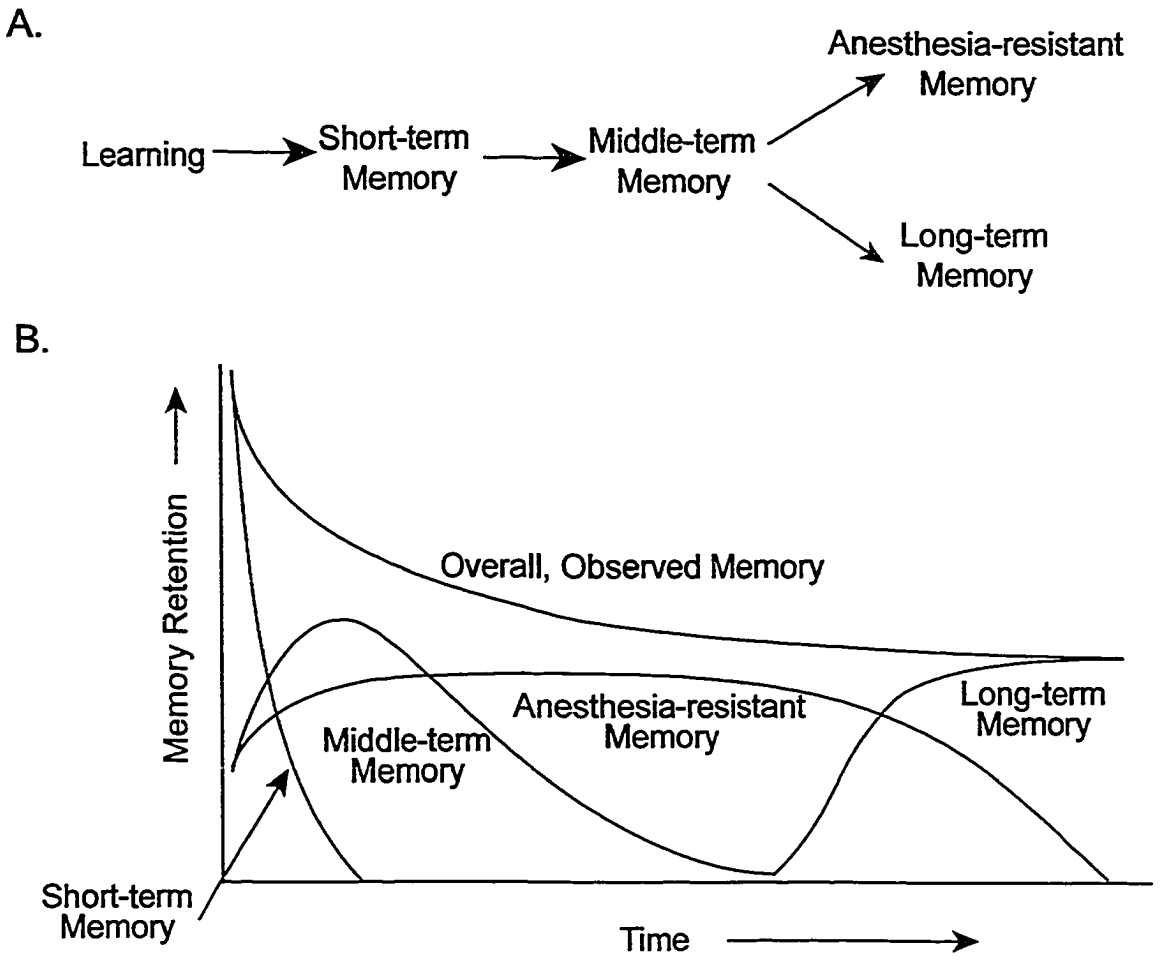

Figure 1.9: Memory phases in Drosophila melanogaster (adapted from Dubnau and Tully, 1998). A) Studies of single-gene mutants have elucidated several phases of memory in *D. melanogaster*. Short-term and middle-term memory operate in sequence, but anesthesia-resistant and long-term memory operate in parallel. Mutants in which anesthesia-resistant memory is impaired can still exhibit intact long-term memory, and vice versa. B) Schematic of the relative time courses of memory phases in *D. melanogaster*. Overall, observed memory is comprised of the four separate memory phases.

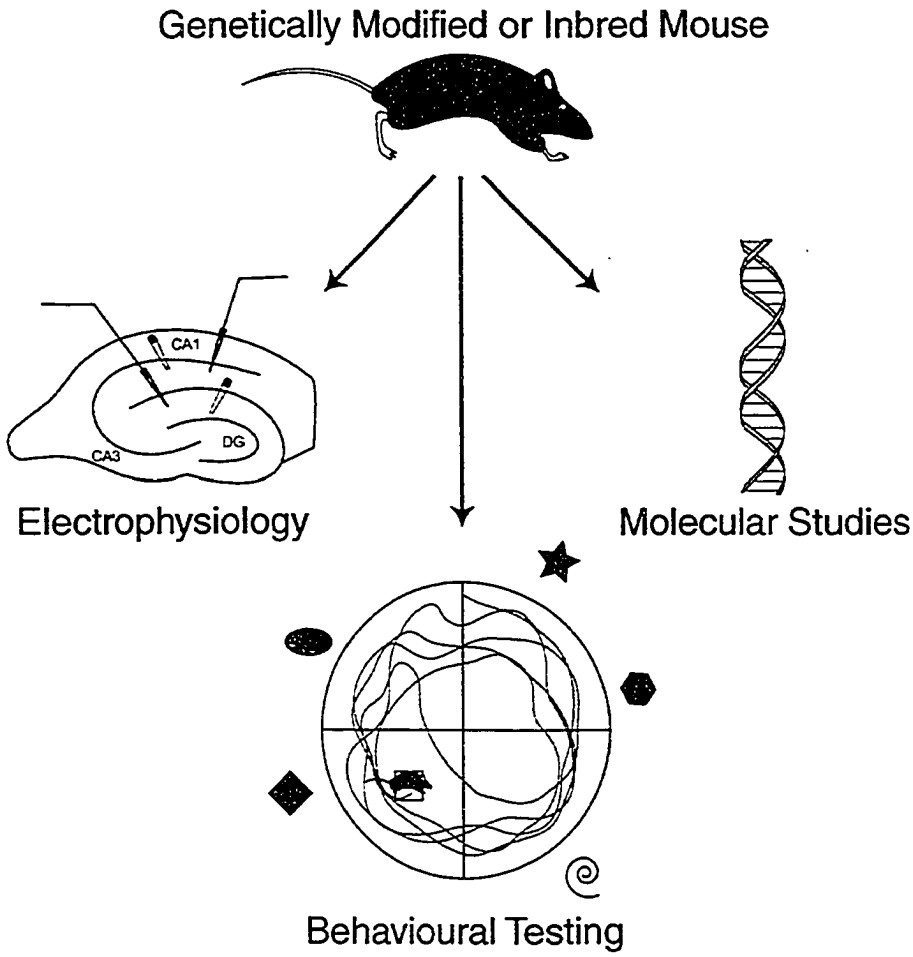

Figure 1.10: A three-pronged approach to studying the neurobiology of learning and memory in mice (from Schimanski and Nguyen, 2004). Genetically modified or inbred mice can serve as models of learning and memory impairments that can be examined for underlying neurophysiological mechanisms. Electrophysiological, behavioural, and molecular characteristics may be determined and compared to shed light on the neurophysiological causes of cognitive deficits.

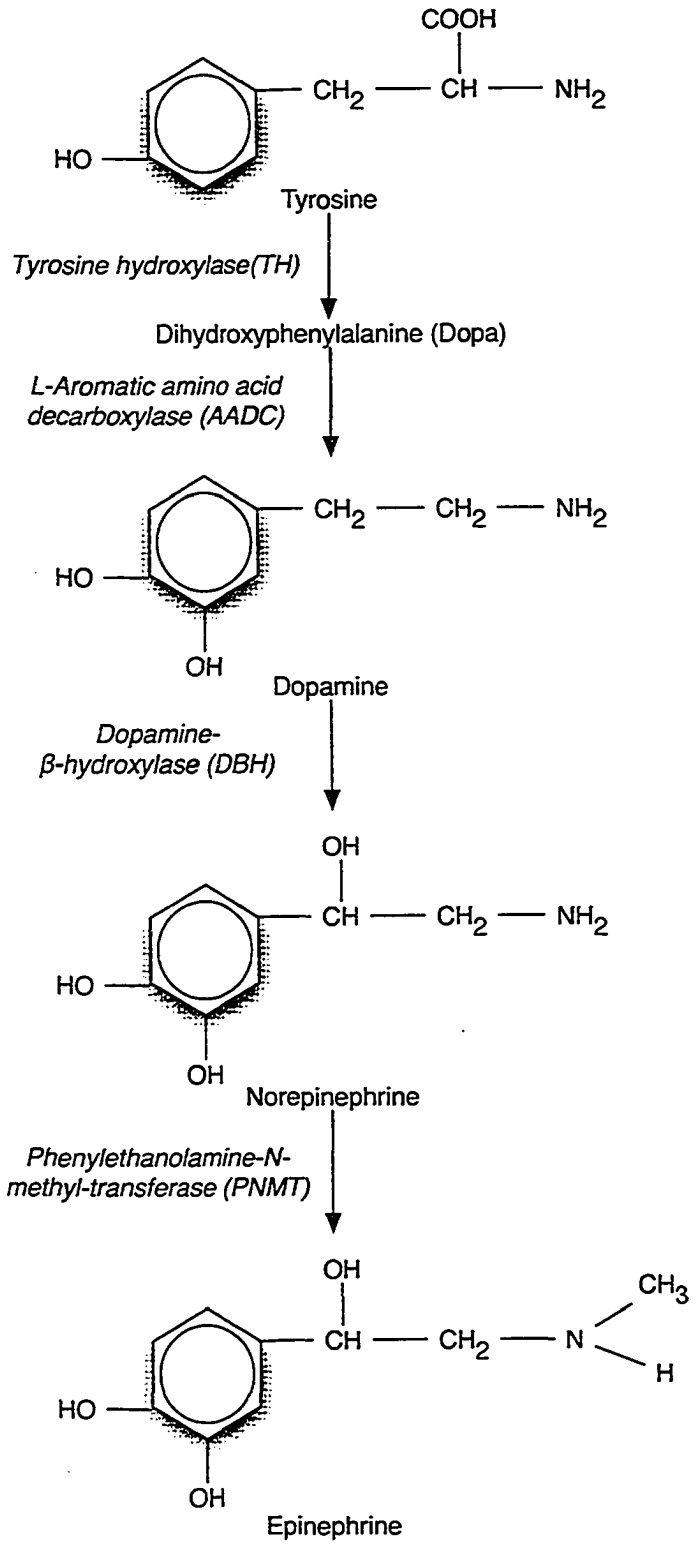

Figure 1.11: The synthesis pathway of dopamine, norepinephrine, and epinephrine (adapted from Nestler et al., 2001).

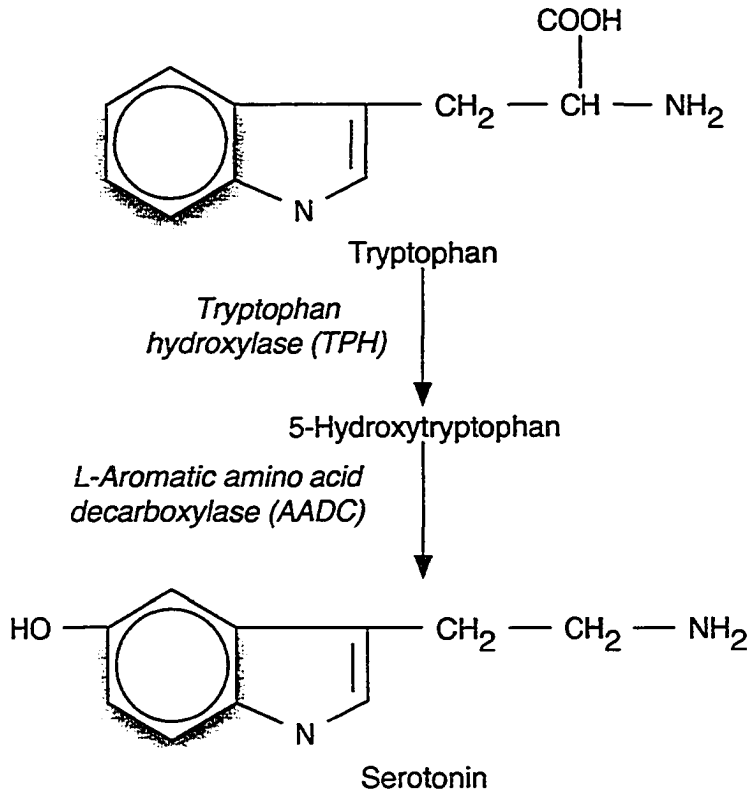

Figure 1.12: The synthesis pathway of serotonin (adapted from Nestler et al., 2001).

Table 1.1: Molecules Required for STM and LTM of Inhibitory Avoidance Memory

		Required for STM during:	**Required for LTM during:**
	Protein synthesis		Training, and 3-4 hrs after training
Glutamate Receptors	NMDARs	Training	Training
	AMPARs	Training	Training and next 3hrs
	mGluRs	Training	Training
Kinases	PKC	Training and next 1hr	Training and next 2hrs
	CaMKII		Training
	MAPK	Training	3-4 hrs after training
cAMP/PKA Signaling	Adenylyl cyclase	Training	3-6 hrs after training
	PKA	Training and next 2hrs	Training, and 3-6 hrs after training
	CREB (phosphorylated)		Training, and 3-6 hrs after training
	ß-adrenergic		Training, and 3-6 hrs after training
	D1/D5	Training	3-6 hrs after training
Retrograde Messengers	Nitric oxide		Training
	Carbon monoxide		Training

Adapted from Izquierdo and McGaugh (2000), p. 528

Table 1.2: Cued and Contextual Fear Conditioning in Inbred Mouse Strains

Inbred Strain	*Contextual Fear Conditioning*	*Cued Fear Conditioning*	*Notes*	*Reference(s)*
129S1/SvImJ	Good	Good		(Bolivar et al., 2001; Cook et al., 2002)
129S6/SvEv	Good	Good	Cook et al. (2002) show differences in cued fear conditioning performance across 129 substrains, but all performed at a high level.	(Balogh and Wehner, 2003; Cook et al., 2002; Holmes et al., 2002; Owen et al., 1997)
129T2/SvEmsJ	Good	Good		(Cook et al., 2002; Nguyen et al., 2000a)
129X1/SvJ	Good	Good		(Cook et al., 2002; Owen et al., 1997)
A	Good	Intermediate	Generalized freezing may elevate performance.	(Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997)
AKR	Poor	Good		(Balogh and Wehner, 2003)
BALB/c	Intermediate			(Chen et al., 1996; Radulovic et al., 1998)
BALB/cByJ	Intermediate	Good		(Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997)
BuB/Bn	Poor	Poor	High freezing to altered context.	(Owen et al., 1997)
C3H/He	Intermediate	Good	Required more training trials than C57BL/6 to show good contextual fear conditioning (Chaudhury and Colwell, 2002).	(Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Owen et al., 1997)
C57BL/6	Good	Good	Substrain C57BL/6N shows generalized freezing to new contexts after training. Less generalized freezing in C57BL/6J (Radulovic et al., 1998; Stiedl et al., 1999).	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Chen et al., 1996; Cook et al., 2002; Fitch et al., 2002; Francis et al., 2003; Gerlai, 1998; Holmes et al., 2002 (trace fear conditioning); Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Radulovic et al., 1998; Stiedl et al., 1999; Valentinuzzi et al., 1998; Young et al., 2000)

Table 1.2: Cued and Contextual Fear Conditioning in Inbred Mouse Strains (Ctd.)

Inbred Strain	*Contextual Fear Conditioning*	*Cued Fear Conditioning*	*Notes*	*Reference(s)*
C57BL/10	Good	Good	20% baseline freezing.	(Owen et al., 1997)
CBA	Poor	Poor	Performance increases with more training trials (Bolivar et al., 2001).	(Balogh and Wehner, 2003; Bolivar et al., 2001; Nguyen et al., 2000a; Nie and Abel, 2001)
DBA/2	Poor	Intermediate	Performance increases with more training trials.	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Fitch et al., 2002; Francis et al., 2003; Gerlai, 1998; Holmes et al., 2002 (trace fear conditioning); Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Stiedl et al., 1999; Valentinuzzi et al., 1998; Young et al., 2000)
FVB/N	Poor	Intermediate	Performance increases with more training trials (Bolivar et al., 2001).	(Bolivar et al., 2001; Owen et al., 1997)
LP	Good	Good		(Balogh and Wehner, 2003; Owen et al., 1997)
SJL	Good	Good		(Balogh and Wehner, 2003; Owen et al., 1997)

Adapted from Schimanski and Nguyen (2004).

Table 1.3: Hidden Platform Morris Water Maze Performance in Inbred Mouse Strains

Inbred Strain	*MWM Learning*	*MWM Probe Trial (Memory)*	*Notes*	*Reference(s)*
129S2/Sv	Good	Good		(Contet et al., 2001; Voikar et al., 2001)
129S6/SvEv	Good	Good		(Gerlai, 2002; Holmes et al., 2002; Owen et al., 1997)
129T2/SvEms	Good	Good	Slow swimmers (Fox et al., 1999; Wolff et al., 2002).	(Nguyen et al., 2000a; Wolff et al., 2002)
129X1/SvJ	Poor	Poor		(Leil et al., 2002; Owen et al., 1997)
129/Ola	Good	Good		(Royle et al., 1999)
A	Poor	Poor	Poor visible platform task – could be vision problem.	(Owen et al., 1997)
BALB/c	Intermediate	Intermediate	Might lack motivation (Yoshida et al., 2001). Better performance with more pretraining (Royle et al., 1999).	(Francis et al., 2003; Royle et al., 1999; Yoshida et al., 2001)
BALB/cByJ	Poor	Poor	8-20% of mice acquire and perform well (Francis et al., 1995). Poor visible platform performance (Upchurch and Wehner, 1988).	(Francis et al., 1995; Owen et al., 1997; Upchurch and Wehner, 1988)
BuB/BnJ	Poor	Poor	Poor visible platform – vision deficit likely.	(Owen et al., 1997)
C3H	Poor	Poor	Poor visible platform (Owen et al., 1997; Upchurch and Wehner, 1988).	(Gutekunst et al., 1993; Owen et al., 1997; Upchurch and Wehner, 1988)
C57BL/6	Good	Good		(Contet et al., 2001; Fox et al., 1999; Francis et al., 1995; Francis et al., 2003; Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Leitinger et al., 1994; Logue et al., 1997; Nguyen et al., 2000a; Owen et al., 1997; Royle et al., 1999; Upchurch and Wehner, 1988; Upchurch and Wehner, 1989; Voikar et al., 2001; Wolff et al., 2002; Yoshida et al., 2001)
C57BL/10J	Good	Good		(Owen et al., 1997)

Table 1.3: Hidden Platform Morris Water Maze Performance in Inbred Mouse Strains (Ctd.)

Inbred Strain	*MWM Learning*	*MWM Probe Trial (Memory)*	*Notes*	*Reference(s)*
CBA/J	Poor	Poor	Poor visible platform.	(Nguyen et al., 2000a)
DBA/2	Intermediate	Poor	Good visible platform (Nguyen et al., 2000a; Owen et al., 1997). Good performance on probe trial in selected studies (Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Owen et al., 1997).	(Francis et al., 1995; Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Logue et al., 1997; Nguyen et al., 2000a; Owen et al., 1997; Upchurch and Wehner, 1988; Upchurch and Wehner, 1989)
DBA/2J-I	Intermediate	Intermediate	Good visible platform.	(Owen et al., 1997)
FVB/N	Poor	Poor	Poor visible platform.	(Fox et al., 1999; Owen et al., 1997; Royle et al., 1999; Voikar et al., 2001)
LP	Intermediate	Intermediate	Good visible platform.	(Leil et al., 2002; Owen et al., 1997)
SJL	Poor	Poor	Poor visible platform.	(Owen et al., 1997)

Adapted from Schimanski and Nguyen (2004).

Table 1.4: Hippocampal Synaptic Transmission in Inbred Mouse Strains

Inbred Strain	*Intact*	*Impaired*	*Notes*	*Reference(s)*
129S6/SvEv		4 trains HFS (20sec ITI), PPF.	Hippocampal slices, area CA1.	(Gerlai, 2002)
129T2/SvEmsJ	4 trains HFS (3sec ITI), FSK+IBMX , LTD, I/O, PPF, general biophysical and electrophysiological attributes.	1 train HFS, TBS, 4 trains HFS (20sec ITI and 5 min ITI).	Hippocampal slices, area CA1.	(Nguyen et al., 2000a; Nguyen et al., 2000b)
129 Ola	HFS-induced LTP followed for one hour. PPF.		In vivo, medial perforant pathway.	(Bampton et al., 1999)
C3H Albino	HFS-induced LTP, PPF.		In vivo, medial perforant pathway.	(Bampton et al., 1999)
C57 Albino	HFS-induced LTP, PPF.		In vivo, medial perforant pathway.	(Bampton et al., 1999)
C57BL/6	LTP induced by 1 train or 3 trains HFS.		In vivo perforant path.	(Matsuyama et al., 1997)
	1 train HFS, 3 trains HFS, TBS LTP.		In vivo, dentate gyrus.	(Jones et al., 2001)
	4 trains HFS (20sec ITI), PPF.		Hippocampal slices, area CA1.	(Gerlai, 2002)
	1 train HFS, TBS, 4 trains HFS (3 sec, 20sec, and 5min ITI). FSK+IBMX, LTD, PPF, I/O. General biophysical and electrophysiological attributes.		Hippocampal slices, area CA1.	(Nguyen et al., 2000a; Nguyen et al., 2000b)
CBA/J	1 train HFS, LTD, PPF, I/O, general biophysical and electrophysiological attributes.	TBS, 4 trains HFS (3sec, 20sec, and 5min ITI), FSK+IBMX.	Hippocampal slices, area CA1.	(Nguyen et al., 2000a; Nguyen et al., 2000b)

Table 1.4: Hippocampal Synaptic Transmission in Inbred Mouse Strains (Ctd.)

Inbred Strain	*Intact*	*Impaired*	*Notes*	*Reference(s)*
DBA/2	TBS LTP.	1 train HFS, 3 trains HFS LTP.	In vivo, dentate gyrus.	(Jones et al., 2001)
	LTP induced by 3 trains HFS.	LTP induced by 1 train HFS.	In vivo perforant path.	(Matsuyama et al., 1997)
	PPF.	HFS-induced LTP.	In vivo, medial perforant pathway. Good induction, impaired maintenance.	(Bampton et al., 1999)
	PPF.	4 trains HFS (20sec ITI).	Hippocampal slices, area CA1. Good induction, impaired maintenance.	(Gerlai, 2002)
	1 train HFS, 4 trains HFS (5min ITI), LTD, I/O, general biophysical and electrophysiological attributes.	TBS, 4 trains HFS (3sec and 20sec ITI), FSK+IBMX, PPF.	Hippocampal slices, area CA1.	(Nguyen et al., 2000a; Nguyen et al., 2000b)
FVB/N	HFS-induced LTP, PPF.		In vivo, medial perforant pathway.	(Bampton et al., 1999)

Adapted from Schimanski and Nguyen (2004). *Abbreviations:* HFS, high frequency stimulation; ITI, inter-train interval; FSK+IBMX, application of a forskolin + 3-isobutyl-1-methylxanthine cocktail; I/O, input-output relation; PPF, paired-pulse facilitation; TBS, theta-burst stimulation.

Table 1.5: PKC Isozymes

	C57BL/6	*DBA/2*
α	132 ± 10	136 ± 10
β_I	156 ± 5	172 ± 8
β_{II}	235 ± 29	246 ± 6
γ	295 ± 32	200 ± 20*
δ	131 ± 15	118 ± 7
ε	174 ± 19	147 ± 15

Membrane-bound PKC isozymes were compared between C57BL/6 and DBA/2 hippocampi and are expressed as percentage of whole brain (means ± SEM of five to 10 mice). *p<0.05. Adapted from Bowers et al. (1995).

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CHAPTER 2:

General Methodology

This chapter will provide an overview of the primary methodologies used in *Chapters 3-6*. These include cued and contextual fear conditioning and extinction, and extracellular field recordings in the hippocampus and amygdala.

A. Animals

All mice used in this thesis research were inbred. The specific strains used will be specified in each chapter. Unless otherwise stated, only female mice were used because mice were group-housed, and males can express high levels of fighting and aggression when housed in groups. Strain differences in aggression (reviewed by Miczek et al., 2001) may confound neurobiological and behavioural results; as such, only females were used to minimize this variable. Behavioural testing was done at 8-10 weeks of age, and mice were killed for electrophysiology at 8-13 weeks of age. Unless otherwise stated, all mice were housed in groups of 3-5 in plastic cages (29 x 18 x 13 cm) filled with wood chip bedding (changed once per week). Mice were housed on a 12-hr light/dark cycle at the University of Alberta Animal Care Facility and had free access to tap water and to solid food (PMI Laboratory Rodent Diet 5001). Care and experimental procedures followed guidelines approved by the Canadian Council on Animal Care.

B. Fear conditioning and extinction

The general procedures for cued and contextual fear conditioning are illustrated in **Figure 2.1**. The tests require two environments (Med Associates, Georgia, VT, USA). One, which was used for training and contextual testing, has an electrifiable floor that is rectangular in shape and made of metallic rods, and walls and ceiling made of transparent Plexiglas. The second environment is quite different from the first. Its floor is triangular in shape, and is of area equal to that floor of the training environment. It is constructed of

different material: it has a smooth black plastic floor rather than metal rods, walls of black plastic (except for the side from which rodents are observed, which is transparent), and is scented differently (with lemon cleaning solution). In addition to the conditioning boxes, a shock delivery system is used, along with a sound source. A tone of 80-85 dB intensity is used as the conditioning stimulus.

During *training*, the mouse acclimated to the electrifiable chamber for 2 min. This was followed by a 30 sec presentation of the conditioned stimulus (CS), the tone, which co-terminated with a 2 sec footshock of 0.7 mA intensity (unconditioned stimulus, US). A single pairing of CS and US was adequate to induce strong learning of an association between CS and US in some inbred strains (e.g., Nguyen et al., 2000; Paylor et al., 1994; Valentinuzzi et al., 1998), but some labs have used up to 6 pairings of CS and US in training their mice (e.g., Chaudhury and Colwell, 2002). After the final CS-US pairing, the mouse was left in the training chamber for 30 sec longer (to measure immediate learning), and then removed to the home cage. The training chamber was wiped down with ethanol between trials. Throughout training and testing, the mouse was observed for freezing behaviour. This was done by an experimenter with a stopwatch and pad of paper. Freezing behaviour was assessed every 5 sec. A mouse was considered 'frozen' in the complete absence of motion (except for respiration). The percentage of time spent frozen during each interval was calculated for each mouse, and these data were averaged for each interval for each group of mice.

After 1 hr, to test short-term memory, or 24 hrs, to test long-term memory, the mouse was tested for *contextual fear conditioning*. It was placed in the training chamber for 5 min with no tone or shock presentation, and was observed for freezing behaviour.

30 min to one hour later, the mouse was tested for freezing in an *altered context*, and then for *cued fear conditioning*. For these tests, the mouse was placed in the second, different chamber and observed for freezing for 2 min (altered context). Then, the mouse remained in the chamber while the CS was presented for 3 min, during which freezing behaviour was also observed (cued fear conditioning). The chamber used for altered context and cued fear memory testing was wiped down with lemon cleaning solution between trials. The best indices of contextual and cued fear are freezing during the contextual and cued test, respectively, in relation to freezing in the altered context. This accounts for such factors as general activity level, stress, and handling.

Contextual extinction was tested by repeatedly exposing the mouse to the training chamber in the absence of further US presentations. Thus, mice were individually placed back in the electrifiable chamber without footshock for several trials (trials were 3 min each in duration). Mice were given one or two trials per day for several days following initial contextual fear conditioning. Freezing was monitored throughout the trials; freezing should lessen as the trials progress if mice are extinguishing the original context-US association.

Cued extinction was tested by repeatedly exposing mice to the auditory tone (CS) in the second, different chamber in the absence of further footshock. Mice were individually placed back in that chamber, and trials of CS presentation were given. Each trial was 2 min in duration, and the intertrial interval was much shorter (i.e., 5 sec). Many trials (30) were given all in one session in order to minimize the association of the experience of entering the alternate environment with the lack of US presentations. Fear

responses were monitored throughout the trials; freezing should lessen as more trials are given and the CS-US association is extinguished.

C. Slice electrophysiology: field excitatory postsynaptic potential (fEPSP) recordings

All electrophysiological experiments were done *in vitro*, using slice preparations maintained in an interface chamber for extended periods of recording.

i. Preparation and maintenance of hippocampal and amygdalar slices

For all electrophysiological experiments, 8-13 week old female mice were used. If mice were behaviourally tested before being killed for electrophysiological experiments, at least one week elapsed after fear conditioning before preparing amygdalar and hippocampal tissue from the same mice; LTP measured 24 hrs, but not one week, after contextual fear conditioning is altered in rat hippocampal CA1 *in vitro* (Sacchetti et al., 2002). Mice were killed by cervical dislocation then decapitation. Brains were removed quickly then placed into ice-cold artificial cerebrospinal fluid (ACSF). The ACSF contained (in mM): 125 NaCl, 4.4 KCl, 1.5 $MgSO_4$, 1 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, and 2.5 $CaCl_2$. After cooling in ACSF for about 1 min, hippocampal or amygdalar slices were prepared from the brain.

For hippocampal transverse slices, brains were first cut down the midline. The hippocampus was dissected from one brain hemisphere, then placed on a manual tissue chopper (Stoelting, Wood Dale, IL, USA). Transverse hippocampal slices (400 μm thick) were cut and placed in an interface chamber at 28°C. This procedure was then repeated with the second brain hemisphere. Within the humidified interface chamber, slices rested on mesh chambers, and were perfused (about 1 mL/min) with ACSF that was bubbled with carbogen gas (95% oxygen, 5% carbon dioxide).

For amygdala slices, the rostral and caudal ends of the cooled brain were removed, and the remaining tissue was prepared for slicing on a vibrotome by gluing the caudal surface to a block. During slicing, the blocked brain was surrounded by cooled, carbogen-bubbled ACSF. 400 μm thick coronal sections of the whole brain were cut. Sections that contained amygdalar tissue were transferred to a dissecting scope where the coronal sections of whole brain were cut along the midline to yield two amygdalar slices, one from each brain hemisphere. These slices were then transferred to interface chambers as described above. Both hippocampal and amygdalar slices rested for 60-90 min in the interface chambers before field EPSP recording began.

ii. Recording fEPSPs in hippocampal and amygdalar slices

Extracellular fEPSPs were recorded using glass microelectrodes (resistances, 2–4 MΩ) filled with ACSF, and were evoked using bipolar nickel-chromium electrodes (diameter 130 μm; pulse width 0.08ms). For Schaeffer collateral (SC)/CA1 fEPSP recordings, stimulating and recording electrodes were positioned in stratum radiatum (**Figure 2.2a**). For medial perforant pathway (MPP) recordings, electrodes were placed in the molecular layer of the dentate gyrus as illustrated in **Figure 2.2b**. MPP fEPSPs were verified by observing paired-pulse depression (PPD; 50 ms inter-pulse interval; McNaughton, 1980; Nguyen and Kandel, 1996); if PPD was not observed, the recording was discarded. For the mossy fibre (MF) pathway, stimulating electrodes were placed near the granule cell bodies of the dentate gyrus, and fEPSPs were recorded from the proximal apical dendrites of pyramidal cells in stratum radiatum of area CA3 (**Figure 2.2c**). To ensure that MF, and not associational/commissural, LTP was measured in area CA3, 50 μM D(-)-2-amino-5-phosphonopentanoic acid (AP5), an NMDA receptor

antagonist (Research Biochemicals Inc., Natick, MA, USA), was bath applied beginning 10 min before, and ending 10 min after, tetanization. AP5 was prepared as a 50 mM stock solution in distilled H_20, then diluted to 50 μM in ACSF for bath application. In amygdalar slices, fEPSPs were evoked by stimulating the lateral amygdala and recording from the basolateral amygdala. Other groups have illustrated that the thalamo- and cortico-amygdalar pathways exhibit robust LTP and likely have a role in fear conditioning in rats (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997; Tsvetkov et al., 2002). However, very few, if any, groups have been successful in recording from these pathways in *in vitro* slice preparations from mice. Reliable field potentials and LTP in the thalamo- and cortico-amygdalar pathways could not be elicited in the amygdalar slice preparation used.

Evoked fEPSPs from hippocampal and amygdalar slices were recorded, digitized, and analyzed using pClamp 7 software (Axon Instruments Inc., Union City, CA, USA). For hippocampal pathways, fEPSPs were elicited once per minute at a stimulus intensity that evoked fEPSP amplitudes 40% of the maximum size (0.08-ms pulse width). For amygdalar recordings, fEPSPs were elicited four times per minute at a stimulus intensity that evoked 50% of the maximal fEPSP amplitude (0.08-ms pulse width). Responses from four fEPSPs were averaged together to provide one amygdalar fEPSP measurement per minute.

Input-output (I/O) data were collected by varying stimulus intensity over five stimuli applied to the Schaeffer collateral pathway. Paired-pulse facilitation (PPF) was examined in the Schaeffer collateral pathway by applying two pulses at 50, 75, or 100ms inter-pulse intervals. Five recordings at each inter-pulse interval were taken per slice.

Various stimulation protocols were used to induce LTP in hippocampal and amygdalar slices; these are described in the *Results* section of *Chapters 3-6*.

iii. Data and statistical analysis

For electrophysiological experiments, the initial slope of the fEPSP was measured to gauge synaptic strength (Johnston and Wu, 1995). The average fEPSP slope during stable recordings before LTP induction was calculated for each experiment, and fEPSP slopes recorded after LTP induction were normalized in relation to these "baseline" averages and expressed as percentages of baseline slopes. fEPSP slopes were averaged within each strain and the resulting means were compared across strains.

fEPSP slopes were compared at 60 min (for amygdalar LTP, CA1 1 train and theta-burst stimulation, MF pathway, and MPP experiments), or at 120 min (for CA1 4 train) after tetanization. For chemically induced LTP (i.e., by application of forskolin and 3-isobutyl-1-methylxanthine), fEPSP slopes were compared 120 min after initial application of the drug cocktail. Student's t-test (two-tailed; for two groups), or analysis of variance (ANOVA) and Dunnett post-hoc tests (for three or more groups) were used to assess differences in potentiation levels between strains (Graphpad Instat software, San Diego, CA, USA).

For I/O analysis, hippocampal CA1 presynaptic fibre volley amplitudes and fEPSP slopes were plotted and a linear regression twas calculated for each strain. A two-tailed Student's t-test (for two groups) or a one-way analysis of covariance (ANCOVA) and Dunnett post-hoc tests (for three or more groups) were used to assess statistical significance between linear regression slopes. For PPF, the intial slopes of the fEPSPs elicited by the paired pulses were measured, and the slope of the second pulse was

normalized to that of the first pulse and then reported as % facilitation using the following formula: [(fEPSP slope from pulse 2/fEPSP slope from pulse 1) - 1]*100. Student's t-test or ANOVA followed by Dunnett post-hoc tests were calculated for each inter-pulse interval. Student's t-test was used for within-strain comparisons of MPP PPD before and after LTP induction.

Student's t-test, or ANOVA and Dunnett post-hoc tests were used to assess strain differences in the behavioural tests. For all ANOVA tests, if the standard deviations were determined to be significantly different between groups, a Kruskal-Wallis test (non-parametric ANOVA) was carried out instead, followed by Dunn's multiple comparisons test. A significance level of $p<0.05$ was used. All values shown are mean ± standard error (SEM), with n = number of animals for behavioural experiments, and n = number of slices for electrophysiological experiments (except for I/O experiments, in which n = number of test pulses).

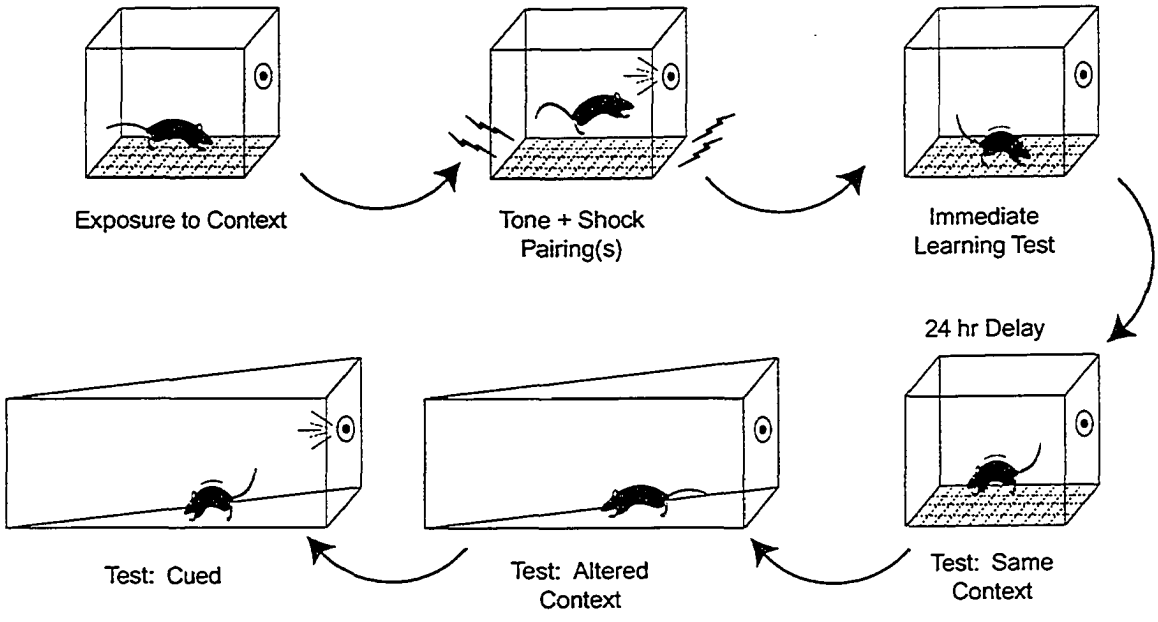

Figure 2.1: Methodology for cued and contextual fear conditioning (from Schimanski and Nguyen, 2004). Mice are first placed in the training context and allowed to explore for about 2 min. Then, they are presented with one to three pairings of an auditory cue (CS) and a footshock (US). The mice are left in the training context and their freezing behaviour is quantified for 30 sec after the last CS-US pairing (this is a measure of immediate learning for the association of the US with the context). 24 hours later, mice are tested for contextual, altered context, and cued fear memory by quantifying freezing behaviour in three different conditions. First, mice are returned to the training context for 5 min and tested for contextual fear memory. Next, mice are placed in a different environment for 2 min and tested for fear of a new environment. The last test is for cued fear memory, in which mice remain in the new environment while the CS is presented again for 3 min.

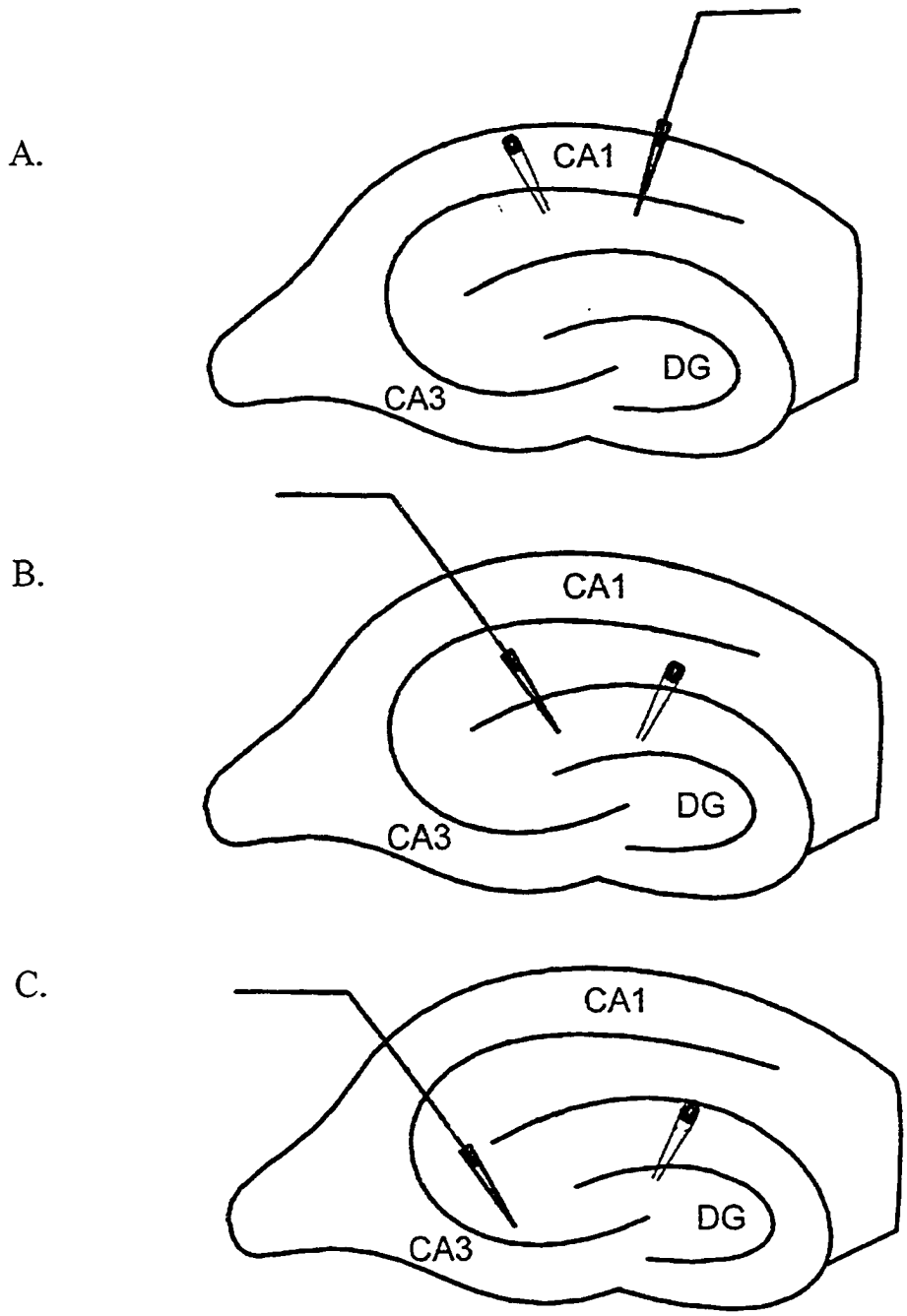

Figure 2.2: Diagrams of transverse hippocampal slices, indicating the recording and stimulating electrode placements used to measure fEPSPs in area CA1 (A), the medial perforant pathway (B), and the mossy fibre pathway (C) (adapted from Schimanski and Nguyen, 2005).

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*CHAPTER 3:

Selective Modification of Short-Term Hippocampal Synaptic Plasticity and Impaired Memory Extinction in Mice with a Congenitally Reduced Hippocampal Commissure

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A. Introduction

The classic case study of H.M., a patient with surgical transection of the medial temporal lobes, demonstrated that the hippocampus is critical for the formation of new declarative memories (Scoville and Milner, 1957). Studies of human commissurotomy patients indicate that the main interhemispheric communication pathway between hippocampi, the *hippocampal commissure* (HC), is important for normal memory function. Indeed, patients with a transected HC show greater recognition memory deficits than those with an intact HC (Phelps et al., 1991). Patients with transection of *both* the corpus callosum (CC) and the HC also showed poor memory performance (Zaidel and Sperry, 1974). These findings indicate that an intact CC and HC are required for normal memory function.

In two common inbred mouse strains, BALB/c and 129, agenesis of the CC occurs in less than 50% of these mice, whereas the remainder appear to have normal forebrain commissures (see **Figure 3.1**) (Wahlsten, 1989). This developmental variability within a genetically homogeneous strain provides a well-controlled experiment of nature to assess the behavioural effects of an absent CC. Surprisingly, hereditary absence of the CC has little or no impact on a wide range of mouse behaviours (Schmidt et al., 1991; Bulman-Fleming et al., 1992; Wahlsten et al., 2001).

By creating recombinant inbred lines from a cross of BALB/c and 129 mice, a new inbred strain (9XCA/Wah) has been generated that displays a more severe forebrain commissural defect than either progenitor strain: total CC deletion and a severely reduced HC are evident in every animal of this strain (Wahlsten and Sparks, 1995; D. Wahlsten and K.M. Bishop, unpublished observations). In a large sample of 162 mice, the

BALB/cWah1 strain showed partial or total absence of the CC in 44% of mice and reduction of the HC in only 4%. The HC was reduced only when the CC was totally absent. Thus, the principal difference between the BALB/cWah1 and 9XCA/Wah strains is the size of the HC, which is almost always normal in BALB/cWah1 but is always severely reduced in 9XCA/Wah. 9XCA/Wah is the only strain known to have a severely reduced HC, and it presents a unique opportunity for investigating the functional consequences of gross reduction of hippocampal commissural inputs.

Hippocampal long-term potentiation (LTP) is an enhancement of synaptic transmission that may be important for regulating the expression of specific types of learning and memory (reviewed by Bliss and Collingridge, 1993, and Martin et al., 2000). The absence of intact interhemispheric connections, such as the HC, may impair memory function by altering the expression of hippocampal LTP, but, to date, this hypothesis has not been tested. I present here the first conjoint assessment of hippocampal synaptic plasticity and behavioural learning and memory in mice with substantial reduction of the HC. I addressed two questions: Does absence of the CC and/or HC impair hippocampal synaptic plasticity? Are hippocampus-dependent learning and memory altered by agenesis of the CC and/or HC? My results reveal the importance of an intact HC in regulating both hippocampal synaptic plasticity and hippocampus-dependent memory function.

B. Materials and Methods

i. Mice

Males and females of two closely related strains of mice (aged >8 weeks), from the colony of D. Wahlsten, were compared in this study. BALB/cWah1 was derived from

BALB/cJ (Wahlsten, 1989), whereas 9XCA/Wah was created by inbreeding a line of mice from the F_2 hybrid cross of BALB/cWah1 and 129P1/ReJ (Wahlsten and Sparks, 1995; D. Wahlsten and K.M. Bishop, unpublished observations). The 9XCA strain has experienced more than 25 generations of full-sib inbreeding. Behavioural fear conditioning experiments were performed first, followed by LTP experiments using hippocampal slices from the same, previously conditioned, animals.

ii. Fear conditioning

Mice were trained for contextual and cued fear conditioning. Twenty-four hours later, mice were returned to the training chamber for a 5 min contextual memory test, then after a 1-hr waiting period, the mice underwent altered context and cued memory tests. For a subset of the mice, contextual extinction was assessed starting the day following testing. Mice were returned to the conditioning chamber for a 5 min trial on days 3, 5, 7, and 9 (day 1 = training day). A separate group of mice was tested for cued extinction only. These animals were placed in the same plexiglass conditioning chamber used for training and contextual testing. Following a 2-min acclimation period, a 2-min CS (85-dB tone) was presented that co-terminated with a 2-sec pulse of 0.7 mA footshock. The mice remained in the chamber for 30 sec following footshock. Twenty-four hours later, the mice were given 30 extinction trials of 2 min duration each (5-sec intertrial interval), during which the CS was presented. The next day, animals were returned to the chamber for a long-term memory (LTM) test consisting of 2-min acclimation followed by a 5-min CS. The percentage of time spent frozen was calculated for each mouse in discrete time intervals, and these results were pooled and averaged for each strain in each interval.

iii. Electrophysiology: extracellular field recordings

Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded in stratum radiatum of area CA1, and in the mossy fibre (MF) pathway of hippocampal slices. Various stimulation protocols were used to induce LTP; these are described at appropriate parts of the Results section. For some paired-pulse facilitation experiments in area CA1 the extracellular Ca^{2+} concentration was reduced to 0.5 mM. AP5, an NMDA receptor (NMDAR) antagonist (RBI, St. Louis, MO), was prepared as a 50 mM stock solution in dH_20, and it was diluted to 50 μM in ACSF prior to bath application.

iv. Data analysis

I assessed LTP, the input/output (I/O) relation between presynaptic fibre volley amplitude and fEPSP slope, and paired-pulse facilitation (PPF) in hippocampal slices from the two strains. The average fEPSP slope during stable recordings before LTP induction was calculated for each experiment, and unless otherwise stated, fEPSP slopes were measured at 45 min (for early-LTP and MF experiments), or at 120 min (for late-LTP experiments) after high-frequency stimulation (HFS). fEPSPs were averaged within each strain and the resulting means were compared across strains (Nguyen et al., 2000b). Student's t-test was used to determine whether there was a significant difference ($p<0.05$) between the two strains.

v. Anatomy

The myelin-stained brain sections shown in **Figure 3.1** were prepared from mice euthanized by i.p. injection of 120 mg/Kg pentobarbital sodium. Following euthanasia, animals were perfused with 20 mL of 4% paraformaldehyde, followed by 10 mL of physiological saline. For coronal images, frozen sections were cut at a thickness of 25-

μm and mounted on glass slides. Sections containing the center of the anterior commissure were selected for staining, and these were stained for myelin by using the gold chloride method (Schmued, 1990). For saggital images, brains were removed from animals following euthanasia and immersed in 10% formalin or 4% paraformaldehyde for fixation. Whole brains were bisected along the midsaggital plane, and one half was stained *en bloc* using Schmued's gold chloride method. Half-brains from some animals that received cued fear extinction were stained and analyzed for CC and HC integrity. No significant differences were found in cued fear extinction performance within strains, despite varying levels of CC integrity in BALB/cWah1 (data not shown), thereby allowing data to be combined within strains.

C. Results

i. Commissural anatomy

Although the procedure used to prepare the hippocampal slices often damaged the forebrain commissures, satisfactory anatomical descriptions of most animals used in this study were obtained. **Figure 3.2** compares the commissures of 13 BALB/cWah1 and 8 9XCA/Wah mice. Only one BALB/cWah1 mouse showed an abnormally small HC, whereas the HC was severely deficient in every 9XCA/Wah animal, including two in which there were almost no HC axons crossing the midline. There was a wide range of size of the CC in BALB/cWah1 mice, but this extreme size variation within BALB/cWah1 was not significantly related to fear conditioning (data not shown), which is consistent with the literature on behavioural sparing in CC agenesis (Schmidt et al., 1991; Bulman-Fleming et al., 1992; Wahlsten et al., 2001). As such, the focus of my

analysis was on the difference between strains that arose from grossly different sizes of the HC.

ii. Fear conditioning is comparable in strains BALB/cWah1 and 9XCA/Wah

Does loss of commissural input to the hippocampus alter memory function? Lesion studies have shown contextual fear conditioning to be dependent on the hippocampus (Kim and Fanselow, 1992; Chen et al., 1996) and on the amygdala (Phillips and Ledoux, 1992; Kim et al., 1993). In contrast, cued (tone) fear conditioning relies primarily on the amygdala (Fanselow and Kim, 1994). Both contextual and cued fear conditioning are dependent on NMDA receptor activation (Fanselow and Kim, 1994; Fanselow et al., 1994; Walker and Davis, 2002). Contextual fear conditioning was tested in order to determine whether a hippocampus-dependent deficit in fear memory was present in mice that display a reduced HC, and cued fear conditioning was tested in order to determine whether there was an amygdala-dependent deficit in fear memory in these mice.

As shown in **Figure 3.3a** and **Table 3.1**, the two strains exhibited comparable levels of freezing during the training session. Performance was not significantly different on the 24 hr contextual fear memory test (BALB/cWah1: 46 ± 6%, n = 12; 9XCA/Wah: 46 ± 6%, n = 12; $P > 0.05$). However, 9XCA/Wah exhibited greater freezing during acclimation (no CS) before the cued fear memory test (BALB/cWah1: 2 ± 1%, n = 12; 9XCA/Wah: 8 ± 2%, n = 12; $P < 0.01$). Therefore, freezing levels during acclimation were subtracted from freezing levels during cued testing for each animal in both strains. There was no significant difference between strains on the cued fear memory test (BALB/cWah1: 34 ± 7%, n = 12; 9XCA/Wah: 35 ± 6%, n = 12; $P > 0.05$).

iii. Mice with a reduced HC exhibit deficits in contextual extinction that are likely hippocampus-dependent

Extinction of fear entails repeated exposure of animals to a conditioned stimulus (CS) that they were previously trained to fear, until the fear response to the CS is reduced. Pavlov (1927) proposed that extinction involved the learning of new information, and not the erasure of old memories. Both strains were tested for contextual and cued fear extinction as described in the Methods. Mice exhibiting reduction of the HC (strain 9XCA/Wah) were deficient in contextual fear extinction (see **Figure 3.3b**). These mice showed significantly higher levels of freezing than BALB/cWah1 (see **Table 3.1** for data), such that they exhibited an increased latency to learn to dissociate the CS (context) from the US (footshock) and showed higher freezing levels at the end of testing trials (BALB/cWah1: 6 ± 1%, $n = 7$; 9XCA/Wah: 16 ± 4%, $n = 7$; $P < 0.05$). Linear regression analysis that included all four extinction trials yielded a regression slope that was significantly different from zero in strain BALB/cWah1 ($P < 0.01$) but not strain 9XCA/Wah ($P > 0.05$), reinforcing the notion that contextual extinction was impaired in 9XCA/Wah.

A separate group of mice was tested for *cued* extinction (see **Figure 3.3c**). Before the cued extinction trials, mice were given a 2-min acclimation period without CS. As in the cued fear conditioning test, 9XCA/Wah showed a significantly higher level of freezing during acclimation than BALB/cWah1 (BALB/cWah1: 4 ± 2%, $n = 12$; 9XCA/Wah: 20 ± 5%, $n = 8$; $P < 0.01$). Thus, individual acclimation scores were subtracted for each animal (in both strains). No significant difference was found between strains for any of the 6 cued extinction trial blocks (see **Table 3.1** for data). Because contextual, but not cued, extinction was impaired in 9XCA/Wah, these results suggest a

hippocampal deficit in learning to dissociate the CS from the US in mice that have a reduced HC.

iv. Paired-pulse facilitation is deficient in mice with a reduced HC

Does absence of an intact HC compromise short-term synaptic plasticity? One type of short-term plasticity that is prominent at hippocampal synapses is paired-pulse facilitation (PPF). PPF is an enhancement of synaptic transmission during the second of two closely spaced stimuli (Katz and Miledi, 1968). PPF occurs because the calcium entering the presynaptic terminal following the first stimulus pulse is not completely cleared prior to a closely spaced, subsequent stimulus pulse. The residual calcium left over after the first pulse sums with the second pulse's presynaptic calcium influx to elicit enhanced transmitter release during the second stimulus pulse (Katz and Miledi, 1968).

PPF was examined in the SC pathway at interpulse intervals of 50-, 75-, and 100-ms in both strains. In addition, PPF was examined in slices from both strains during perfusion with ACSF containing reduced levels of Ca^{2+} (see Methods). As shown in **Figure 3.4**, PPF in standard ACSF was significantly reduced in strain 9XCA/Wah in comparison to BALB/cWah1 at 75-ms (BALB/cWah1: 58± 3%, $n = 27$; 9XCA/Wah: 42 ± 2%, $n = 51$; $P < 0.05$) and 100-ms interpulse intervals (BALB/cWah1: 47 ± 2%, $n = 49$; 9XCA/Wah: 35 ± 2%, $n = 68$; $P < 0.01$). However, reduced extracellular Ca^{2+} rescued PPF in strain 9XCA/Wah, such that PPF was now not significantly different from BALB/cWah1-PPF measured in standard ACSF at interpulse intervals of 50-ms (BALB/cWah1: 60 ± 3%, $n = 49$; 9XCA/Wah ↓ $[Ca^{2+}]$: 70 ± 7%, $n = 20$; $P > 0.05$), 75-ms (BALB/cWah1: 58 ± 3%, $n = 27$; 9XCA/Wah ↓ $[Ca^{2+}]$: 57 ± 7%, $n = 20$; $P > 0.05$), and 100-ms (BALB/cWah1: 47 ± 2%, $n = 49$; 9XCA/Wah ↓ $[Ca^{2+}]$: 48 ± 6%, $n = 20$; $P >$

0.05). Decreasing the probability of transmitter release by reducing extracellular [Ca^{2+}] leaves more transmitter available to be released in response to the second pulse. Thus, in standard ACSF, 9XCA/Wah CA3 pyramidal neurons may possess altered intracellular calcium dynamics and/or higher basal transmitter release in comparison to BALB/cWah1. Consistent with these hypotheses, I found that in BALB/cWah1 neurons, reduced extracellular [Ca^{2+}] increased PPF substantially at all three interpulse intervals tested (**Figure 3.4**, $P < 0.001$ compared to normal ACSF). Thus, reduction of the HC impaired hippocampal PPF in area CA1, and this deficit in short-term synaptic plasticity was rescued by reducing the extracellular calcium concentration.

v. LTP in two pathways does not require fully intact interhemispheric connections

Hippocampal long-term potentiation (LTP) is an activity-dependent strengthening of synaptic transmission believed to be important for regulating information storage in the mammalian brain (Bliss and Lømo, 1973; Moser et al., 1998; Martin et al., 2000; Brun et al., 2001). Area CA1 of the hippocampus is particularly important for learning and memory in humans and in rodents (Zola-Morgan et al., 1986; Tsien et al., 1996), and thus, synaptic modifications in this subregion of the hippocampus may contribute to the modification of hippocampus-dependent aspects of memory function, such as contextual fear extinction. Does absence of an intact HC alter LTP in area CA1?

LTP was examined in the SC pathway in hippocampal slices from strains BALB/cWah1 and 9XCA/Wah. The "early" and "late" temporal phases of LTP (E-LTP and L-LTP) in the SC pathway differ in their optimal induction requirements (reviewed by Huang et al., 1996). Two patterns of stimulation were employed to induce E-LTP: twin bursts of 100-Hz (1 sec duration) delivered at a 20-sec interburst interval, and theta-

burst stimulation (TBS). TBS consisted of 15 bursts of 4 pulses at 100-Hz, delivered at an interburst interval of 200-ms. Twin-burst HFS resulted in a mean fEPSP slope value of 156 ± 16% (n = 10) at 45-min post-HFS in BALB/cWah1 slices, and a corresponding mean fEPSP slope value of 159 ± 13% (n = 9) in 9XCA/Wah slices ($P > 0.5$ for interstrain comparison; **Figure 3.5a**). There was also no significant difference between strains for TBS-LTP at 45-min post-HFS (BALB/cWah1: 151 ± 14%, n = 8; 9XCA/Wah: 141 ± 10%, n = 10, $P > 0.5$) (**Figure 3.5b**). However, both strains exhibited slightly lower potentiation than that observed in C57BL/6 mice in previous studies that employed identical methods (Nguyen et al., 2000a).

Is L-LTP affected by the absence of an intact HC? Multiple bursts of HFS induce L-LTP in mouse hippocampal slices (Nguyen et al., 2000a,b; Woo et al., 2000). A tetraburst pattern was employed in my experiments: four trains of 100-Hz (1-sec duration), with an interburst interval of 20-sec (Woo et al., 2000). The mean fEPSP slope in BALB/cWah1 slices measured 120 min after tetraburst HFS was 157 ± 19% (n = 5), and this was not significantly different from the corresponding mean fEPSP slope measured in 9XCA/Wah slices (147 ± 8%, n = 11, $P > 0.5$) (**Figure 3.5c**). However, these values were smaller than those previously reported for C57BL/6 mice (Nguyen et al., 2000b). The mean fEPSP slopes resulting from more temporally spaced tetraburst HFS (5-min interburst interval) also were not significantly different between strains (BALB/cWah1: 179 ± 19%, n = 4; 9XCA/Wah: 217 ± 27%, n = 9, $P > 0.1$) (**Figure 3.5d**), and they were similar to those previously reported for C57BL/6 mice (Nguyen et al., 2000a). Thus, reduction of the HC produced no significant impairments in either E-LTP or L-LTP in area CA1.

Pyramidal cells in hippocampal area CA3 receive two excitatory synaptic inputs from the mossy fibre (MF) and the associational-commissural (assoc-com) pathways (Andersen et al., 1966). It is possble that in strain 9XCA/Wah, which exhibits agenesis of the hippocampal commissure, MF LTP is altered as a result of the absence of the assoc-com input. Thus, I examined MF LTP in slices from both inbred strains, BALB/cWah1 and 9XCA/Wah.

MF LTP is NMDA receptor-independent (Harris and Cotman, 1986), but it is dependent on presynaptic Ca^{2+} influx (Zalutsky and Nicoll, 1990; see Nicoll and Malenka, 1995, for a review). To ensure that MF, and not assoc-com, LTP was measured in CA3, I bath-applied 50μM AP5 starting at 10-min pre-tetanization and ending at 10-min post-HFS. MF LTP was induced using two trains of 200-Hz (1-sec duration each), delivered at a 20-sec intertrain interval. The mean fEPSP slope at 45-min post-HFS was 114 ± 14% (n = 9) in BALB/cWah1 and 111 ± 13% (n = 17; $P > 0.5$) in 9XCA/Wah (**Figure 3.5e**). Thus, MF LTP was not significantly altered by the absence of an intact HC.

Does reduction of the HC change basal synaptic input-output relations? The presynaptic fibre volley amplitude and initial fEPSP slope were measured from single fEPSP sweeps elicited in the SC pathway of area CA1 of several hippocampal slices, using a range of stimulus intensities. The fibre volley size is proportional to the number of presynaptic axons recruited by stimulation, and the initial fEPSP slope is a measure of synaptic strength (Johnston and Wu, 1995). A trendline fit to plotted data points was generated using linear regression analysis (**Figure 3.5f**). These positive trendline slopes indicate that postsynaptic responses increased as the magnitude of presynaptic

stimulation increased. Both strains showed fEPSP slopes that were not significantly different from one another over a range of stimulation intensities and presynaptic volley sizes (BALB/cWah1: $y = 2.1808x + 1$, $R^2 = 0.5197$; 9XCA/Wah: $y = 2.0961x + 1$, $R^2 = 0.7164$; $P > 0.5$) **(Figure 3.5f)**. Thus, basal hippocampal synaptic transmission and presynaptic fibre recruitment in the SC pathway were unaffected by the absence of an intact HC.

vi. E-LTP and L-LTP are NMDA receptor-dependent in slices from mice with a reduced HC

Is the NMDA receptor-dependence of LTP in the SC pathway reduced by the absence of an intact HC? The NMDAR antagonist, AP5 (D(-)-2-amino-5-phosphopentanoic acid; 50 μM), was bath-applied for 15-min pre- and 10-min post-HFS. At 40-min post-HFS, twin-burst LTP in AP5-treated slices was significantly reduced in comparison to control slices in both BALB/cWah1 (control: 154 ± 13%, $n = 10$; AP5: 111 ± 8%, $n = 5$; $P < 0.05$) and 9XCA/Wah (control: 154 ± 11%, $n = 11$; AP5: 105 ± 9%, $n = 4$; $P < 0.05$) (see **Figure 3.6a,b**). At 120-min post-HFS, tetraburst L-LTP in AP5-treated slices was also significantly reduced in comparison to control experiments in both BALB/cWah1 (control: 173 ± 17%, $n = 5$; AP5: 104 ± 15%, $n = 4$; $P < 0.01$) and 9XCA/Wah (control: 150 ± 10%, $n = 11$; AP5: 98 ± 2%, $n = 4$; $P < 0.05$) (see **Figure 3.6c,d**). Thus, reduction of the HC does not compromise the NMDA receptor-dependence of early- and late-LTP in the SC pathway.

D. Discussion

Our study aimed to determine whether agenesis of the CC and/or HC results in memory impairments that were correlated with altered hippocampal synaptic plasticity. Contextual fear extinction was impaired in mice with a reduced HC (strain 9XCA/Wah).

Like fear conditioning, extinction has been proposed to be a form of new learning, rather than an erasure of existing memories (reviewed by Bouton, 1993), and it is dependent on hippocampal function (Wilson et al., 1995; Frohardt et al., 2000; see also Fox and Holland, 1998). In addition, extinction is NMDA-receptor dependent (Lee and Kim, 1998; Santini et al., 2001; but see Berman and Dudai, 2001) and requires protein synthesis (Flood et al., 1977; Berman and Dudai, 2001; Vianna et al., 2001), similar to fear conditioning. Furthermore, Berman and Dudai (2001) suggest that both fear conditioning and extinction rely on the same brain structures. Also, the prefrontal cortex (Quirk et al., 2000; Herry and Garcia, 2002) has been shown to play a role in the maintenance of cued fear extinction, indicating that extinction may be dependent on the interaction of the hippocampus with other brain regions. Thus, my observations need to be considered from a broader, more complex mechanistic perspective, one that likely involves brain regions outside of the hippocampus.

It is unclear which synaptic pathways within the hippocampus are critical for contextual extinction. However, the impaired contextual extinction in strain 9XCA/Wah was correlated with a deficit in PPF in the SC pathway. Experiments using reduced extracellular [Ca^{2+}] indicated that this deficient PPF may result from elevated basal transmitter release and/or altered calcium handling efficacy in neurons of the 9XCA/Wah strain. The lack of a significant difference in basal synaptic input-output relations between these two strains suggests that altered presynaptic calcium dynamics may contribute to the impaired PPF seen in 9XCA/Wah. Thus, contextual extinction in these strains is correlated with PPF in the SC pathway, and the HC may have an important role in the acquisition of contextual extinction. Hippocampal LTP has not been definitively

correlated with contextual fear extinction. My results suggest that contextual fear extinction is poorly correlated with certain types of hippocampal LTP.

Cued and contextual fear conditioning were unaltered in mice with a reduced HC, and LTP in the SC and MF pathways of the hippocampus remained intact. Hippocampal and amygdalal LTP may contribute (in ways still unclear) to consolidation of memory for fear conditioning (Clugnet and LeDoux, 1990; Rogan and LeDoux, 1995; McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997; Blair et al., 2001; Schafe et al., 2001). Several types of hippocampal LTP were intact in slices from 9XCA/Wah mice, and it is interesting that contextual fear conditioning, which is hippocampus-dependent, was also preserved in these mice. In contrast, gross reduction of commissural input to the hippocampus altered short-term synaptic plasticity (PPF), and selectively impaired one particular aspect of memory function, contextual memory extinction. It is noteworthy that cats with an HC transection show no transfer of conditioned learning to the opposite hemisphere when only one brain hemisphere was trained (Majkowski, 1967). Thus, one cerebral hemisphere, containing one hippocampus, can learn a CS-US association.

Interestingly, I found that the fear responses of 9XCA/Wah mice during acclimation or during exposure to a novel environment (measured after training but prior to cued testing) were significantly enhanced as compared to BALB/cWah1 mice. This enhanced response to a novel environment was a possible contaminant in testing for cued memory and extinction; hence, freezing levels during acclimation were subtracted from cued memory and extinction data for each animal. Exposure to novel environments has been correlated with expression of long-term depression (LTD) in the hippocampus of specific strains of rats. Manahan-Vaughan and Braunewell (1999) showed that

hippocampal low-frequency stimulation *in vivo* during exploration of a novel environment induced LTD in the SC pathway of the hippocampus, and that such stimulation increased exploratory behaviour. Altered LTD may be a cellular correlate for the increased fear in a novel environment that was seen in strain 9XCA/Wah, but LTD was not examined in the present study.

The altered contextual fear extinction found in 9XCA/Wah may be caused by 129P1/ReJ alleles at loci unrelated to defects of the HC. However, Cook et al. (2002) found that contextual and cued fear conditioning were normal in 129P1/ReJ mice as compared to C57BL/6J mice. Thus, it is unlikely that the genetic contribution of 129P1/ReJ to the 9XCA/Wah strain is the sole cause of altered hippocampal synaptic transmission and impaired memory extinction. Because the strains studied here may differ in more than one gene that is pertinent to a reduced HC, I cannot rule out the possibility that these genes are essential for LTP and for contextual fear extinction. Definitive evidence will require identification of the specific genes involved in formation of the HC, so that they can be backcrossed onto different inbred strain backgrounds. Alternatively, causality may be established when there is extreme phenotypic variation within a genetically uniform inbred strain. To date, however, all recombinants with defective HC showed 100% abnormality (D. Wahlsten and K.M. Bishop, unpublished observations). The I/LnJ strain shows extremely variable HC but has several other abnormalities that render interpretation difficult. A recent discovery of a new inbred strain with no CC and severely reduced HC (D. Wahlsten, unpublished data) should aid the search for gene identity and the analysis of physiological mechanisms of LTP and memory.

Some consideration of the human literature is needed to place my findings in a broader functional framework. Split-brain patients tend to have minimal cognitive dysfunction associated with transection of the CC. Indeed, split-brain surgery results in a surprising lack of severe memory dysfunction in humans, and it produces no significant behavioural changes in monkeys (Springer and Deutsch, 1993). Patients with transection of the CC generally exhibit normal memory function (Phelps et al., 1991; also reviewed by Clark and Geffen, 1989), although Finlay et al. (2000) suggest that such patients may display some minor, but specific, cognitive deficits. Impairments associated with split-brain surgery can include difficulties in facial recognition and solution of geometric problems, as well as interhemispheric competition (Springer and Deutsch, 1993). Split-brain patients may retain the ability to perform everyday tasks because of hemispheric specialization, and also because each hemisphere remains functional. Patients with callosal agenesis also show relatively few cognitive deficits (Sauerwein et al., 1994), perhaps because they develop compensatory behavioural strategies to cope with the absence of interhemispheric communication (Springer and Deutsch, 1993).

A more detailed study of split-brain patients that analyzed the extent of callosal and commissural transection in patients, as well as memory function, indicated that transection of the HC (in addition to the CC) elicits more profound memory deficits than in patients with callosal transection *per se* (Phelps et al., 1991). Memory deficits in HC-transected patients are limited to specific types of memory, and these deficits seem to encompass complex aspects of memory function (Phelps et al., 1991; Zaidel, 1995). Interestingly, a human patient with an astrocytoma spanning the hippocampal commissure displayed amnesia (Gillespie et al., 2000). My present data are in agreement

with these findings, in that basic fear conditioning remained intact in mice lacking an intact HC, whereas contextual fear extinction was impaired.

The study of mice exhibiting agenesis of the CC and/or HC may provide exciting new animal models that may prove useful for the development of treatments for syndromes associated with reduced CC and/or HC size. For example, MRI studies show that patients with fetal alcohol syndrome display numerous brain abnormalities, including agenesis of the CC and HC (Bhatara et al., 2002). Rat studies have found that alcohol ingestion during pregnancy can reduce HC size (Livy and Elberger, 2001). Also, reduced CC size in children may be correlated with attention-deficit hyperactivity disorder (ADHD) and dyslexia (Hynd et al., 1991; Baumgardner et al., 1996). Magara et al. (2000) utilized hybrid crosses of the acallosal mouse strain I/LnJ as a potential animal model of ADHD, and they found that acallosal mice were more active than control mice and exhibited greater variability in activity than controls. However, it is unlikely that general activity levels influenced the results of the present study. Freezing levels of 9XCA/Wah and BALB/cWah1 were not significantly different upon initial placement into the training environment (**Figure 3.3a, pre CS**).

In summary, my findings indicate that the HC contributes importantly to the regulation of both hippocampal short-term (but not long-term) synaptic plasticity and the acquisition of contextual fear extinction in the mouse. Three novel conclusions arise from my results: 1) lack of associational-commissural fibres to hippocampal area CA3 alters selected properties of the hippocampal trisynaptic pathway, such as PPF in the SC pathway; 2) normal contextual fear conditioning can occur in the absence of an intact HC, and is correlated with robust expression of LTP in the SC and MF pathways of mice

exhibiting a reduced HC; and 3) contextual fear extinction is impaired in mice with a reduced HC, despite normal expression of LTP in the SC and MF pathways.

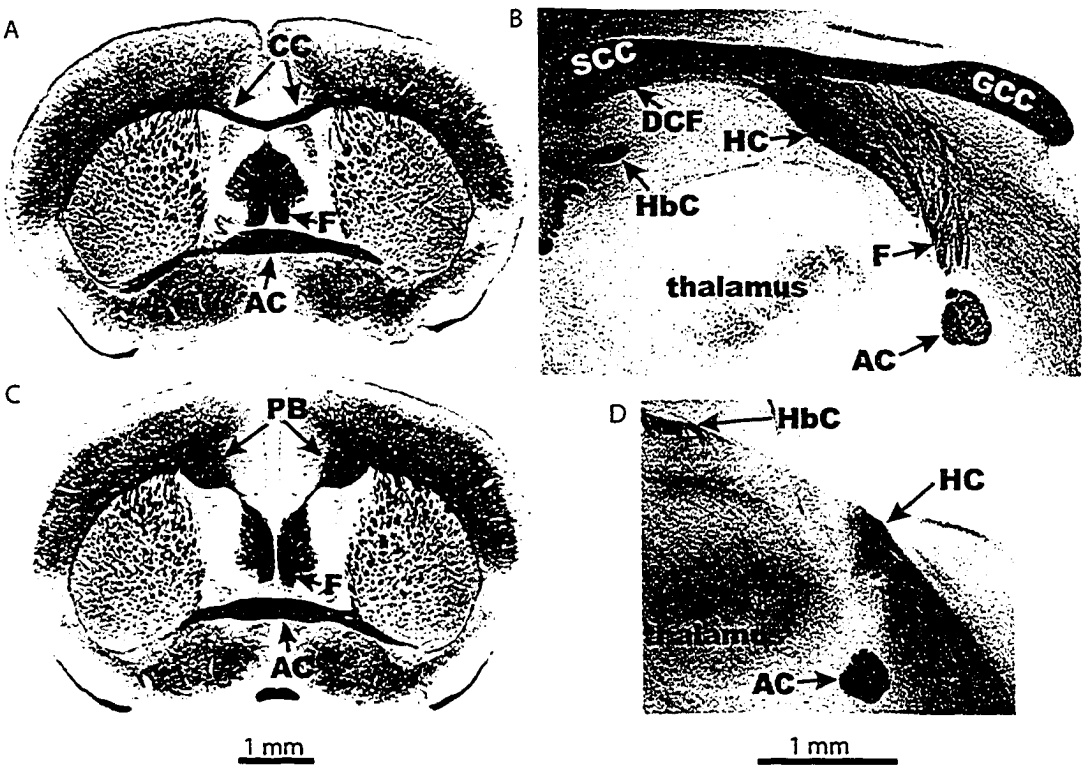


Figure 3.1: Brain morphology of normal and acallosal mice. A) Coronal section, normal brain: the corpus callosum spans the cerebral hemispheres. B) Saggital section, normal brain: the corpus callosum extends rostrocaudally along the midline of the brain. C) Coronal section, acallosal brain: there is no corpus callosum, and fibres have turned to form the Probst bundle. D) Saggital section, acallosal brain: there is no corpus callosum, and the hippocampal commissure is reduced in size. (AC, anterior commissure; CC, corpus callosum; DCF, dorsal commissure of the fornix; F, columns of the fornix; GCC, genu of the CC; HbC, habenular commissure; HC, hippocampal commissure; PB, Probst bundle; SCC, splenium of the CC)

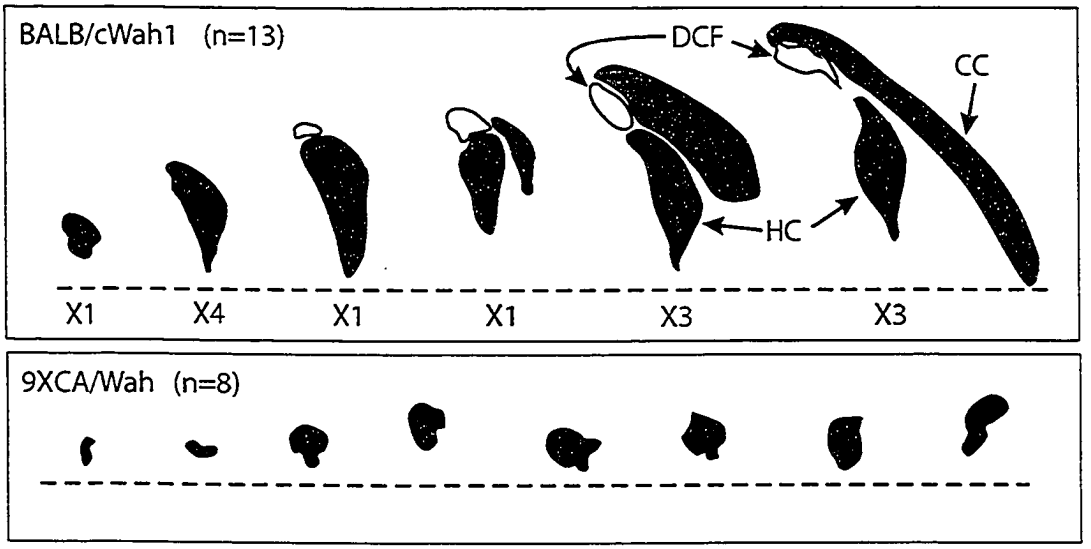
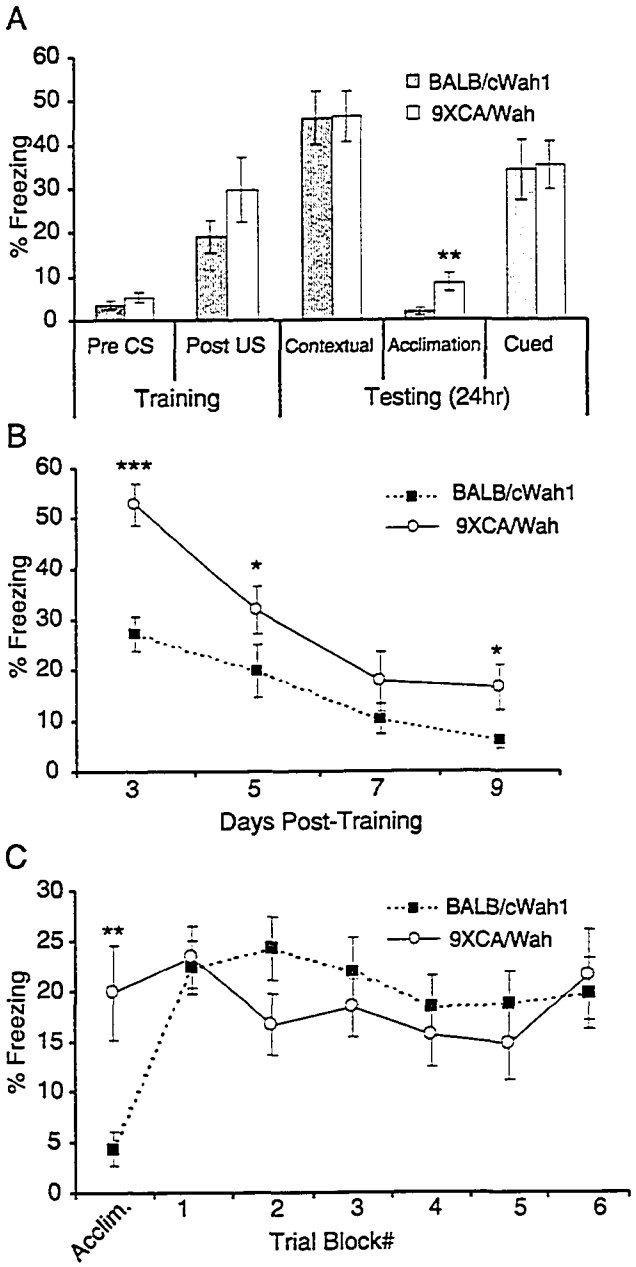


Figure 3.2: Diagrams of the corpus callosum (CC), hippocampal commissure (HC) and dorsal commissure of the fornix (DCF) in BALB/cWah1 and 9XCA/Wah mice. The vertical axis in each case was established by a line drawn through the centers of the HC and the anterior commissure (AC), whereas the vertical level was established by the dashed line perpendicular to the vertical axis and 1.5-mm above the center of the AC, which is not shown in these diagrams. In the 9XCA/Wah strain, every animal showed total absence of the CC and DCF as well as severe reduction of the HC. In BALB/cWah1 several patterns were seen, and the frequency of mice with each type is indicated by X_. For example, X4 means 4 mice had a normal HC but no DCF. Only one BALB/cWah1 mouse had an HC as small as the largest HC among 9XCA/Wah mice.

Figure 3.3: A reduced HC impairs contextual fear extinction, but does not affect cued and contextual fear conditioning. A) BALB/cWah1 (n = 12) and 9XCA/Wah (n = 12) performed normally on tests of 24-hr contextual and cued memory. Both strains displayed significantly different levels of freezing for "acclimation" during a 2-min test interval in a novel context, prior to presentation of the CS for the cued memory test. Thus, acclimation freezing values were subtracted for each mouse (both strains) to calculate % freezing for the cued memory test. Cued memory was not significantly different between strains. B) Both strains received 5-min contextual extinction trials 3, 5, 7, and 9 days post-training. 9XCA (n = 7) exhibited delayed contextual extinction in comparison to BALB/cWah1 (n = 7), and they still showed less extinction during the final trial on day 9. C) Separate groups of mice from both strains received a 2-min acclimation interval, followed by thirty 2-min cued extinction trials (5-sec intertrial interval) the day after training. Cued extinction trials were grouped into 6 blocks of 5 trials each. 9XCA/Wah again showed significantly greater freezing during acclimation, so acclimation values for each mouse (both strains) were subtracted for all subsequent cued extinction trials. There was no significant difference between BALB/cWah1 (n = 12) and 9XCA/Wah (n = 8) for any trial block during cued extinction. Asterisks indicate significance values derived from Student's t-test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.0001$). CS, conditioned stimulus (tone); US, unconditioned stimulus (footshock).



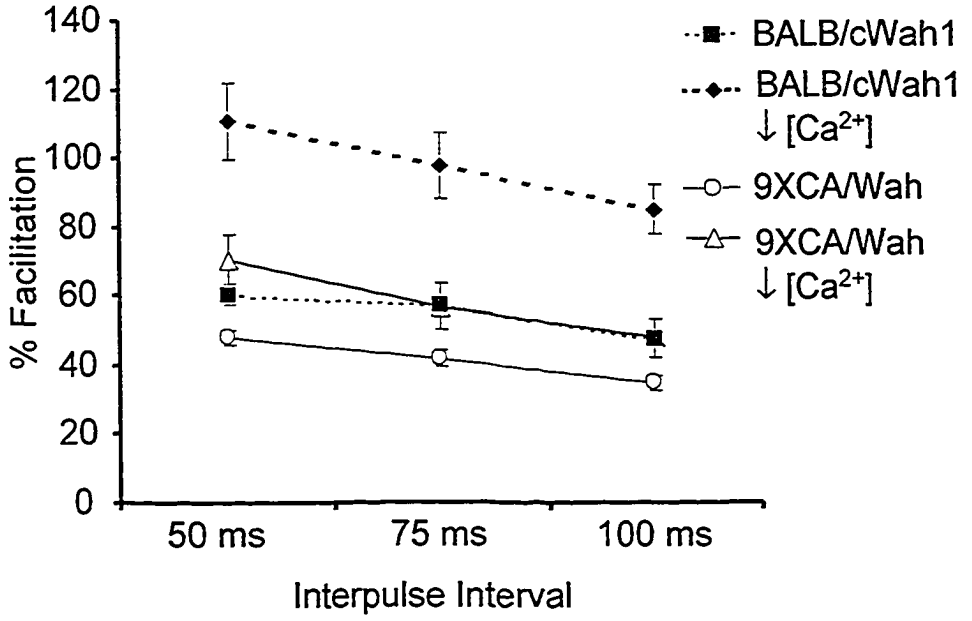


Figure 3.4: Paired-pulse facilitation is decreased in mice with a reduced HC, and is rescued by reduced extracellular [Ca2+]. Plot of % facilitation [ratio of fEPSP slopes: ((pulse 2/pulse 1) −1) x 100] vs. interpulse interval for strains BALB/cWah1 and 9XCA/Wah, In standard ACSF, 9XCA/Wah exhibited significantly less facilitation than BALB/cWah1. When measured under conditions of reduced extracellular [Ca^{2+}], facilitation in 9XCA/Wah was not significantly different from that seen in BALB/cWah1 slices in standard ACSF. Reduced extracellular [Ca^{2+}] also significantly increased facilitation in BALB/cWah1.

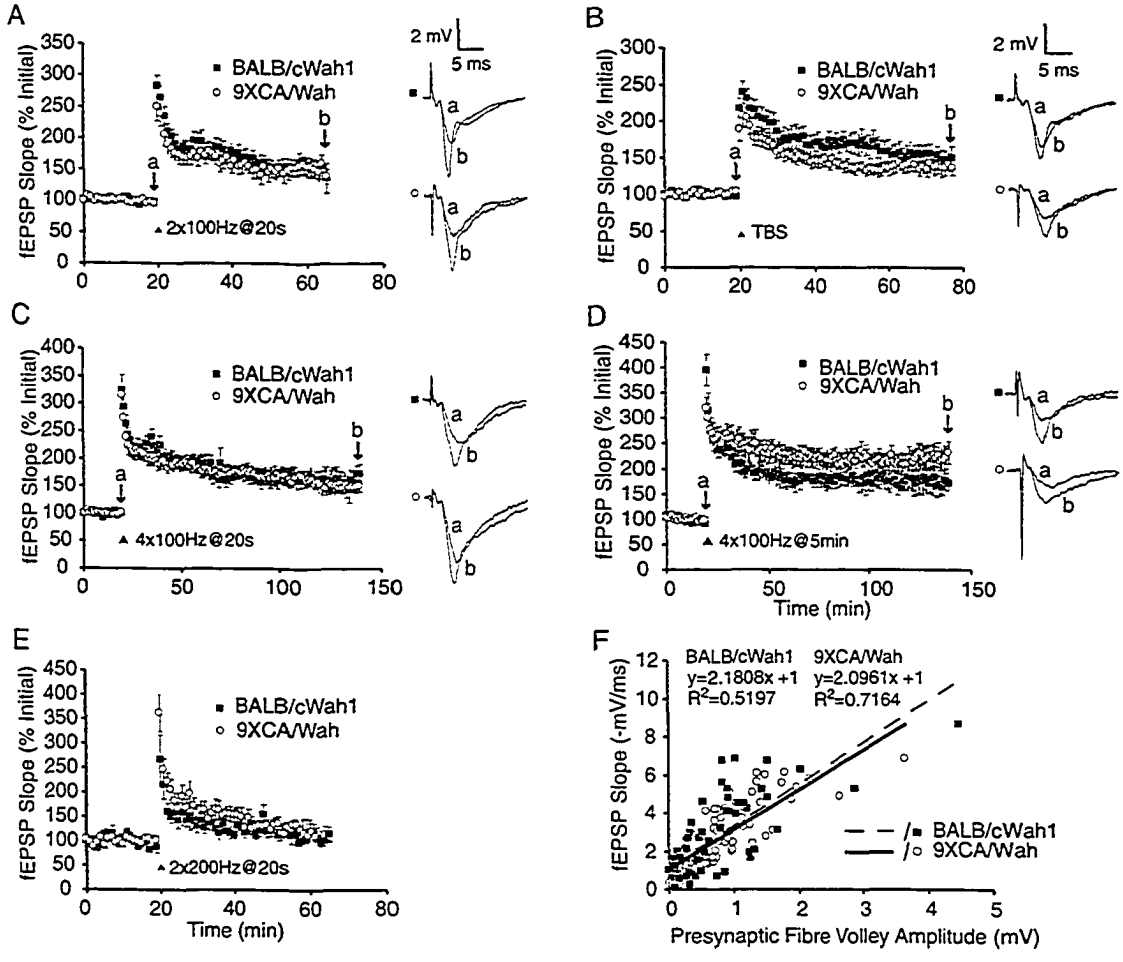


Figure 3.5: Hippocampal LTP in two pathways is normal in mice with a reduced HC. A and B: E-LTP in the Schaffer collateral pathway is not significantly different between strains. A: Plot of fEPSP slope vs. time, comparing averaged responses from BALB/cWah1 (n = 10 slices) and 9XCA/Wah (n = 9 slices). LTP was elicited by twin bursts of 100-Hz ("2x100Hz@20s"). B: Average responses from BALB/cWah1 (n = 8) and 9XCA/Wah (n = 10) for theta-burst stimulation ("TBS"). C and D: L-LTP in the Schaffer collateral pathway is not significantly different between strains. C: LTP was elicited by four bursts of 100-Hz ("4x100Hz@20s") in BALB/cWah1 (n = 5 slices) and 9XCA/Wah (n = 11 slices). D: LTP was elicited by four spaced bursts of 100-Hz, interburst interval of 5-min ("4x100Hz@5min") in BALB/cWah1 (n = 4 slices) and 9XCA/Wah (n = 9 slices). E: E-LTP in the mossy fibre pathway, elicited by twin bursts of 200-Hz ("2x200Hz@20s") in BALB/cWah1 (n = 9 slices) and 9XCA/Wah (n = 17 slices), was not significantly different between strains. F: Input-output curve slopes (fEPSP slope vs. presynaptic fibre volley amplitude) are not significantly different between BALB/cWah1 (n = 11 slices, 5 data points per slice) and 9XCA/Wah (n = 17 slices, 5 data points per slice). Data are shown as mean ± SEM. Sample fEPSP traces were recorded at times "a" and "b" indicated on the graphs.

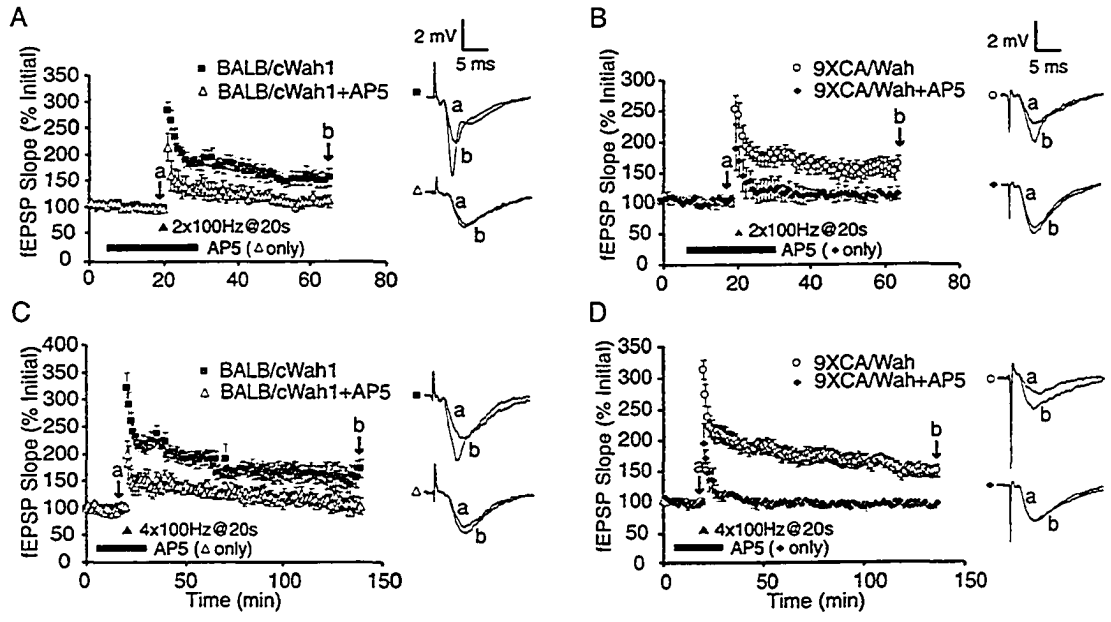


Figure 3.6: Early- and late-LTP in the Schaffer collateral pathway are NMDA receptor-dependent in both strains. A and B: E-LTP elicited by twin bursts of 100-Hz ("2x100Hz@20s") is significantly reduced by application of 50 μM AP5 in BALB/cWah1 (control $n = 10$; AP5 $n = 5$) and 9XCA/Wah (control $n = 11$, AP5 $n = 4$). A and B: L-LTP elicited by four bursts of 100-Hz ("4x100Hz@20s") is significantly reduced by AP5 in BALB/cWah1 (control $n = 5$; AP5 $n = 4$) and 9XCA/Wah (control $n = 11$, AP5 $n = 4$). Sample fEPSP traces were recorded at times "a" and "b" indicated on the graphs.

Table 3.1. Summary of Cued and Contextual Fear Conditioning and Extinction

		BALB/cWahl	*9XCA/Wah*	*P*
Training (Day 1)	Pre CS	3 ± 1 (n=12)	5 ± 1 (n=12)	ns
	Post US	19 ± 4 (n=12)	30 ± 7 (n=12)	ns
Testing (Day 2)	Contextual	46 ± 6 (n=12)	46 ± 6 (n=12)	ns
	Acclimation	2 ± 1 (n=12)	8 ± 2 (n=12)	< 0.01
	Cued	34 ± 7 (n=12)	35 ± 6 (n=12)	ns
Contextual Extinction	Day 3	27 ± 3 (n=7)	53 ± 4 (n=7)	< 0.001
	Day 5	20 ± 5 (n=7)	32 ± 5 (n=7)	< 0.05
	Day 7	10 ± 3 (n=7)	18 ± 6 (n=7)	ns
	Day 9	6 ± 1 (n=7)	16 ± 4 (n=7)	< 0.02
Cued Extinction (Day 2)	Acclimation	4 ± 2 (n=12)	20 ± 5 (n=8)	< 0.01
	Trial Block 1	22 ± 3 (n=12)	23 ± 3 (n=8)	ns
	Trial Block 2	24 ± 3 (n=12)	17 ± 3 (n=8)	ns
	Trial Block 3	22 ± 3 (n=12)	18 ± 3 (n=8)	ns
	Trial Block 4	18 ± 3 (n=12)	16 ± 3 (n=8)	ns
	Trial Block 5	19 ± 3 (n=12)	15 ± 4 (n=8)	ns
	Trial Block 6	20 ± 4 (n=12)	21 ± 5 (n=8)	ns

Values reported are mean % freezing ± SEM, followed by *n* mice tested. See methods for explanation of intervals tested. A separate group of mice was used for cued extinction testing. *P* values were derived from Student's t-test, significance was considered $P < 0.05$. (CS, conditioned stimulus – tone; US, unconditioned stimulus – footshock; ns, not significantly different)

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*CHAPTER 4:

Impaired Fear Memories are Correlated with Subregion-Specific Deficits in Hippocampal and Amygdalar LTP

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A. Introduction

A primary focus of neuroscience research is to elucidate the cellular and molecular mechanisms that underlie learning and memory. To this end, many studies have examined activity-dependent synaptic plasticity in the hippocampus. The hippocampus is a prime locus of study for several reasons. Lesion studies and human case studies have indicated that absence of an intact hippocampus impairs spatial memory in rodents (Morris et al., 1982) and causes amnesia in humans (Scoville and Milner, 1957). In addition, electrical stimulation of identified hippocampal pathways can induce long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission. LTP and LTD comprise the activity-dependent enhancement and reduction, respectively, of excitatory synaptic strength, and both can be induced at hippocampal Schaeffer Collateral-CA1 (SC) and medial perforant pathway-dentate gyrus (MPP) synapses (Bliss and Lomo, 1973; Dudek and Bear, 1992; Dudek and Bear, 1993; Wu et al., 2001). Both types of synaptic plasticity may be involved in encoding certain types of memories (including spatial and fear memory) in the mammalian brain (Martin et al., 2000).

Hippocampal area CA1 is crucial for encoding long-term memory in mice and in humans (Tsien et al., 1996; Zola-Morgan et al., 1986). In area CA1 of the mouse hippocampus, genetic modification of key signaling molecules impairs normal expression of long-term memory and LTP (reviewed by Chen and Tonegawa, 1997; Micheau and Riedel, 1999). Also, LTP in the perforant pathway-dentate gyrus synapse relies upon some of the same molecular mechanisms as CA1 LTP, including dependence on postsynaptic NMDA receptors (Chen and Tonegawa, 1997; Colino and Malenka, 1993; Collingridge et al., 1983; Hanse and Gustafsson, 1992; Nguyen and Kandel, 1996; Wu et

al., 2001). However, few studies have examined correlations between dentate gyrus LTP and hippocampal learning and memory.

Genetically modified mice have been used to study the roles of hippocampal genes and proteins in synaptic plasticity, learning, and memory (Chen and Tonegawa, 1996; Martin et al., 2000; Micheau and Riedel, 1999; Picciotto and Wickman, 1998; Wehner et al., 1996). However, another way of elucidating the mechanisms of hippocampal learning and memory is to examine mouse strains in which learning and memory are defective. Such strains constitute mouse models that might be screened for a variety of hippocampal factors, and can provide some insight into which alterations might contribute to amnesia. For instance, hippocampal anatomy (e.g., total volume, intactness of subregions, cell density, neurogenesis), neurochemistry, and synaptic plasticity might be examined. Also, gene expression may be surveyed using microarray techniques. Carrying out such analyses on mouse strains that display amnesia would help identify the genes, molecules, and cellular mechanisms that underlie memory deficits.

Inbred mouse strains are generated by mating sibling mice for at least 20 generations (Lyon and Searle, 1989). This results in a population of genetically homogeneous animals within a strain. However, many phenotypic differences between strains are evident, and these could result from genetic divergence (Lyon and Searle, 1989; but see Francis et al., 2003). Numerous inbred strains have been phenotyped for hippocampus-dependent learning and memory (e.g., Bolivar et al., 2001; Crawley et al., 1997; Owen et al., 1997). Several strains have reduced hippocampal memory function as determined from the results of multiple behavioural tests (see Schimanski and Nguyen, 2004). These include DBA/2 (Nguyen et al., 2000a; Owen et al., 1997; Paylor et al.,

1994; Upchurch and Wehner, 1988) and BALB/c (Chen et al., 1996; Francis et al., 2003; Radulovic et al., 1998; Royle et al., 1999; Yoshida et al., 2001). Some other strains have shown mixed results on tests of hippocampal learning and memory, such as contextual fear conditioning and the Morris water maze. These strains (including C3H: Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Gutekunst et al., 1993; Owen et al., 1997; Upchurch and Wehner, 1988) might have more subtle, or restricted, alterations in hippocampal function; however, further research is needed to define these alterations.

In this article, I examined contextual and cued fear conditioning, several forms of hippocampal synaptic plasticity, and amygdalar LTP in four inbred mouse strains (C57BL/6NCrlBR, 129S1/SvImJ, C3H/HeJ, and DBA/2J). C57BL/6 exhibits high levels of hippocampus-dependent learning and memory (e.g., Nguyen et al., 2000a) and is the mouse strain most often used to study the cellular and molecular mechanisms of hippocampal synaptic plasticity. I chose three strains to compare with C57BL/6. C3H/HeJ and DBA/2J have selective impairments of hippocampal memory (Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Gutekunst et al., 1993; Nguyen et al., 2000a; Owen et al., 1997; Paylor et al., 1994; Upchurch and Wehner, 1988). I confirmed their poor performance on the contextual fear conditioning task, and then examined LTP in the hippocampal Schaeffer collateral (CA1-LTP) and medial perforant (MPP-LTP) pathways in hippocampal slices from these mice to determine whether these strains display robust LTP comparable to C57BL/6. I carried out the same experimental procedures on the strain,129S1/SvImJ, which performs well on contextual fear conditioning (Bolivar et al., 2001; Cook et al., 2002), but has not been

examined for hippocampal synaptic plasticity. I also examined cued fear conditioning and amygdalar LTP in the four inbred strains, in order to assess whether alterations in amygdalar synaptic physiology correlate with fear memory impairments. I found that strains that have reduced fear memory also have reduced amygdalar LTP. Interestingly, I also found that expression of CA1 LTP, but not MPP LTP, was correlated with contextual fear memory expression in all four inbred strains. This result supports the notion that in the hippocampus, LTP in the area CA1 subregion, but not in the MPP, strongly correlates with expression of hippocampus-dependent memory.

B. Methods

i. Subjects

Four inbred strains of mice were compared in this study (C57BL/6NCrlBR, 129S1/SvImJ, C3H/HeJ, and DBA/2J). C57BL/6 mice were obtained from Charles River Canada (Saint-Constant, Quebec), and the other three strains were purchased from The Jackson Laboratory (Bar Harbor, ME) at 5-7 weeks of age. The same mice underwent both behavioural and electrophysiological experimentation.

ii. Fear conditioning

Separate groups of mice were used to test short-term memory (STM) one hour after training and long-term memory (LTM) 24 hours after training. Thus, either one or 24 hours after training, mice were returned to the chamber for a 5-min *contextual* memory test in the absence of the tone or footshock. One hour after the contextual test, the mice were placed in an alternate chamber for a 5-min *cued* test interval, in which the CS tone was presented during the final 3 min. An unbiased human observer quantified conditioning throughout training and testing by assessing freezing behaviour every 5s.

iii. Shock threshold testing

Groups of mice separate from those used for fear conditioning were individually placed in the same chamber used for fear conditioning. After a 30-s delay, a 2-s footshock of 0.1 mA intensity was applied. Subsequent footshocks were applied to each mouse at 30-s intervals, and were increased by 0.1 mA with each application. Shocks of increasing intensity were given until each mouse exhibited flinching, vocalization, and jumping. The highest intensity applied was 0.8 mA. These sessions were videotaped. Each mouse was scored from video for its first incidence of flinching, vocalization, and jumping in response to shock application.

iv. Electrophysiology: extracellular field recordings

Amygdalar LTP experiments were conducted on brain tissue obtained from mice that underwent shock threshold testing, and hippocampal LTP experiments were conducted on tissue from mice that were tested for fear conditioning. Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded in stratum radiatum of area CA1 and in the medial perforant pathway (MPP) of hippocampal slices; approximate electrode placements for the MPP are shown in **Figure 4.6a.** fEPSPs were also evoked in amygdalar slices by stimulating the lateral amygdala and recording from the basolateral amygdala. Various stimulation protocols were used to induce LTP; these are described at appropriate parts of *section C.* In addition, hippocampal SC LTP was chemically induced by bath application of a cocktail of 50 μM forskolin (FSK) and 50μM 3-isobutyl-1-methylxanthine (IBMX) in ACSF (drugs obtained from Sigma-Aldrich Canada Ltd, Oakville ON). Fresh cocktail was prepared daily from individual concentrated stock

solutions for each drug, prepared at 50mM in dimethylsulfoxide (DMSO) and frozen until use.

v. Data analysis

I assessed LTP, the input/output relation between presynaptic fibre volley amplitude and fEPSP slope (I/O), and paired-pulse facilitation (PPF) in hippocampal slices, and LTP in amygdalar slices, from the four strains. The average fEPSP slope during stable recordings before LTP induction was calculated for each experiment, and fEPSP slopes recorded after high-frequency stimulation (HFS) or drug application were normalized in relation to these "baseline" averages. fEPSP slopes were averaged within each strain and the resulting means were compared across strains (Nguyen et al., 2000b).

Hippocampal CA1 presynaptic fibre volley amplitudes and fEPSP slopes, measured by varying the stimulus intensity, were plotted and a linear regression was done for each strain. To confirm the validity of the linear regression analyses because not all data points were independent (i.e., 5 data points were used from each hippocampal slice), approximate randomization was carried out (Noreen, 1989; formulas required for linear regressions and other tests were obtained from Zar, 1984). The Y variable (postsynaptic fEPSP slope) was randomized across all four groups (1000 iterations, run 10 times), and linear regressions for each group were carried out for each iteration. The significance of the linear regression was assessed using two test statistics: the regression coefficient (slope) and coefficient of determination (r^2). Significance was calculated using the ratio (nge+1)/(NS+1), where nge = the number of iterations that yielded a test statistic value greater than that calculated for the non-shuffled data, and NS = the number of shuffles. If the ratio yielded a value of less than or equal to 0.05, the regression was considered to be

valid. The significance ratios for the 10 runs were averaged together, and the mean is reported. A one-way analysis of covariance (ANCOVA) and Dunnett post-hoc tests (with B6 as the control value) were used to assess statistical significance between linear regression slopes. Again, approximate randomization was used to assess whether the results were valid or due to chance alone. Data were shuffled in the same manner as for linear regression testing, and an ANCOVA and post-hoc tests were carried out for each iteration. The test statistic used for the ANCOVA was the F ratio, and for the Dunnett post-hoc tests was the q value. Significance was calculated again using the ratio (nge+1)/(NS+1), and a significance level of 0.05 was used. A custom program written in C++, which utilized the standard C pseudorandom number generator, was employed to carry out the approximate randomization analyses. For PPF, data were analysed as described in *Chapter 2*. To determine which strains exhibited significant differences from B6 in behavioural tests, I/O, and after LTP induction, I used ANOVAs followed by a Dunnett post-hoc test in which B6 served as the control strain (Graphpad Instat software, San Diego, CA, USA). Student's t-test was used for within-strain comparisons of MPP PPD before and after LTP induction.

C. Results

i. C3H and D2, but not 129, have impaired hippocampus-dependent memory

Lesion studies have shown that contextual fear conditioning relies on hippocampal (Kim and Fanselow, 1992; Chen et al., 1996) and amygdalar (Phillips and LeDoux, 1992; Kim et al., 1993) function. However, cued fear conditioning depends primarily on amygdalar function (Fanselow and Kim, 1994). Both types of fear conditioning were tested in my selected inbred mouse strains. This allowed me to gauge

whether hippocampal and/or amygdalar function is compromised (evidenced by contextual and/or cued memory deficits, respectively) in these strains. By testing at two time points after training (1hr and 24hr), I was able to determine whether memory for the cue or the context is intact for the short-term (1hr) and/or the long-term (24hr). Because STM can rely on different molecular mechanisms from LTM (Izquierdo and McGaugh, 2000), the results from these two time delays could suggest which proteins might have altered expression or function in inbred strains.

Previous studies have established that particular inbred strains show varying performance on tests of hippocampus-dependent memory. My cued and contextual fear conditioning results are consistent with previous data. As shown in **Figure 4.1a**, all four strains showed negligible freezing while placed in the conditioning chamber before presentation of the CS or US, $F(3,60) = 0.9406$, $p > 0.5$. Time spent freezing increased in all strains after presentation of the US, but significantly more so in D2 compared with B6 (**Figure 4.1b**, $F(3,60) = 3.084$, $p < 0.05$; D2 vs. B6: $p < 0.05$). Strain differences were evident for expression of contextual fear memory at both 1 hr, $F(3,28) = 14.532$, $p < 0.0001$; and 24 hr, $F(3,27) = 22.063$, $p < 0.0001$. Both C3H and D2 exhibited lower levels of freezing to the context at 1 hr (**Figure 4.1c**, C3H: $p < 0.01$; D2: $p < 0.01$) and at 24 hr (**Figure 4.1d**, C3H: $p < 0.01$; D2: $p < 0.01$) after training, as compared to B6. Strain differences were also evident in the expression of cued fear memory at 1hr: $F(3,28) = 10.461$, $p < 0.0001$; and at 24 hr: $F(3,27) = 10.417$, $p < 0.0001$. C3H and D2 froze less during the cued test at 1hr (**Figure 4.1e**, C3H: $p < 0.01$; D2: $p < 0.01$) and at 24hr (**Figure 4.1f**, C3H: $p < 0.01$; D2: $p < 0.01$) than B6. 129 performed comparably to B6 on all 1hr and 24hr tests of contextual and cued fear memory (all $p > 0.05$).

These behavioural results generally agree with what has been previously published for contextual fear conditioning in inbred strains (see review by Schimanski and Nguyen, 2004). Several studies have shown that C3H has impaired performance on contextual fear conditioning (Balogh and Wehner, 2003; Chaudhury and Colwell, 2002; Owen et al., 1997), although it can perform well if more training trials are given (Bolivar et al., 2001; Chaudhury and Colwell, 2002). Although C3H carries the retinal degeneration (*rd*) mutation that impairs vision (The Jackson Laboratory, 2002), Bolivar et al. (2001) determined that this mutation does not result in fear conditioning deficits in strain C3H by conducting experiments on the congenic strain, C3.BliA-+^{Pde6b}. This congenic strain does not exhibit retinal degeneration like C3H does, but it did not exhibit superior contextual fear conditioning performance to C3H. Furthermore, Adams et al. (2002) suggest that visually-impaired mice are capable of learning a context-US association using non-visual cues (e.g., olfactory, auditory, or tactile cues). Also, many studies have shown that D2 has impaired memory for contextual fear conditioning (e.g., Nguyen et al., 2000a; Owen et al., 1997; Paylor et al., 1994). Therefore, I suggest that these two strains, C3H and D2, are models of impaired hippocampal function, as measured by contextual fear conditioning. In contrast, I found that 129 has intact memory for contextual fear conditioning, in agreement with Bolivar et al. (2001) and Cook et al. (2002). Importantly, the 129 substrains have been shown to vary in performance on the fear conditioning paradigm (Cook et al., 2002). Thus, I can only state that the substrain 129S1/SvImJ exhibits intact contextual fear memory.

ii. All strains exhibited observable responses to 0.7 mA footshock

Different inbred strains might have different sensitivities to 0.7mA footshock. It is possible that the reduced expression of freezing to the context and/or the tone in strains D2 and C3H results from low sensitivity to the applied footshocks and thus reduced fear of the environment and/or cue. In order to determine whether a 0.7mA footshock was adequate to serve as an effective US in all four inbred strains, a shock threshold sensitivity test was carried out. Three types of reactions were observed in order to determine whether the mice were sensitive to the applied footshocks: flinching, vocalizations, and jumping. **Figure 4.2** illustrates the average amperage at which mice of each strain started to elicit these types of reactions to footshock. Although the amperages at which the different types of reactions differed somewhat between strains, all four strains elicited all three types of reactions at less than 0.7mA. Thus, differing sensitivities to footshock probably did not affect my tests of fear memory in these four inbred mouse strains.

Although all four strains exhibited reactions to footshock below an intensity of 0.7mA, statistical analysis revealed significant differences between strains for all three types of reaction to footshock (flinching: $F(3,12) = 14.8$, $p < 0.001$; vocalization: $F(3,12) = 8.526$, $p < 0.01$; jumping: $F(3,12) = 23.684$, $p < 0.0001$). Strains 129 and C3H exhibited all three types of reaction to footshock beginning at higher intensity than strain B6 (strain 129: flinching, $p < 0.01$; vocalization, $p < 0.05$; jumping, $p < 0.01$; strain C3H: flinching, $p < 0.01$; vocalization, $p < 0.01$; jumping, $p > 0.01$). Strain DBA differed from B6 only in that vocalization began at a higher footshock intensity ($p < 0.01$).

iii. Strains with impaired hippocampus-dependent memory have deficits in SC LTP

Hippocampal LTP is an activity-dependent strengthening of synaptic transmission that can regulate memory storage in the mammalian brain (Bliss and Lomo, 1973; Moser et al., 1998; Martin et al., 2000; Brun et al., 2001). LTP induced by different types of tetanic stimulation can rely on different cellular and molecular mechanisms for induction and maintenance. For example, in area CA1 of hippocampal slices, one train of high-frequency stimulation (HFS) yields LTP that decays to baseline within 1-2 hours and is dependent on the activation of particular kinases (such as protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II: Malinow et al., 1989; Silva et al., 1992), as well as NMDA receptors (Collingridge et al., 1983). This early phase of LTP (E-LTP) does not require protein synthesis or activation of PKA (Huang and Kandel, 1994; Huang et al., 1996). However, if three or more trains of HFS are delivered to CA1 within a particular time frame, late-LTP (L-LTP) can be induced, which can last for several hours in slices (Andersen et al., 1977; Huang and Kandel, 1994). The maintenance of L-LTP in CA1 requires protein synthesis and the activation of other molecules and processes, including PKA and CRE-mediated gene expression (Abel et al., 1997; Bourtchouladze et al., 1994; Frey et al., 1988; Frey et al., 1993; Huang and Kandel, 1994; Impey et al., 1996; Matsushita et al., 2001; Matthies and Reymann, 1993; Nguyen et al., 1994).

Area CA1 of the hippocampus is important for particular types of learning and memory in humans (Zola-Morgan et al., 1986) and in rodents (Tsien et al., 1996). Therefore, alterations in synaptic plasticity in area CA1 might underlie impairments in contextual fear conditioning. Therefore, I examined several forms of LTP in the SC pathway of the hippocampi from the four inbred strains to determine whether altered

synaptic plasticity in area CA1 might correlate with the contextual fear memory impairments found in some of these strains.

Three patterns of electrical stimulation were used to elicit LTP in the SC pathway. One train of 100Hz (1s duration) and theta-burst stimulation (TBS) were used to induce E-LTP. TBS consisted of 15 bursts of 4 pulses at 100Hz, with an inter-burst interval of 200ms. fEPSPs were measured for one hour after tetanization with these two protocols. To elicit L-LTP I used four trains of 100Hz (1s train duration, 5 min inter-train interval), and fEPSPs were measured for two hours post-tetanization. These stimulation protocols were chosen because they elicit robust LTP in hippocampal slices from the strain B6 (Nguyen et al., 2000a; Nguyen et al., 2000b).

SC pathway LTP impairments were found only in those strains that had defective contextual fear memory (**Figure 4.3**). ANOVA tests showed that the strains differed in the expression of SC LTP: 1 train LTP: $F(3,24) = 3.096$, $p < 0.05$; TBS LTP: $F(3,23) = 3.121$, $p < 0.05$; 4 train LTP: $F(3,21) = 5.002$, $p < 0.01$. Post-hoc testing using the Dunnett multiple comparisons test (with B6 as the control) revealed strain-specific deficits for all three stimulation protocols. Strain C3H exhibited impaired 1 train LTP compared with B6 (**Figure 4.3b**; $p < 0.05$). C3H also showed reduced 4 train LTP (**Figure 4.3h**; $p < 0.05$), but intact TBS LTP (**Figure 4.3e**). Strain D2 showed reduced TBS LTP at one hour after induction (**Figure 4.3f**; $p < 0.05$) but intact 1 train (**Figure 4.3c**) and 4 train (**Figure 4.3i**) LTP. All types of electrically-induced SC LTP were intact in strain 129 (**Figure 4.3a,d,g**).

Interestingly, the two strains with contextual fear memory deficits, C3H and D2, exhibited a range of CA1 LTP deficits, none of which overlapped. This contrasted to

strain 129 in which contextual fear memory and all types of electrically-induced CA1 LTP were intact. This indicates that there may not be a single type of LTP that unambiguously correlates with learning and memory. Rather, several types of CA1 LTP likely contribute to multiple forms of memory. Furthermore, these data suggest that alterations in all three forms of LTP that I examined might reflect hippocampal dysfunction that leads to learning and memory impairment.

iv. Chemically-induced LTP is intact in all strains examined

In transgenic mice, reduced hippocampal protein kinase A (PKA) activity resulted in impaired maintenance of LTP (Abel et al., 1997). Because C3H and D2 exhibit L-LTP deficits that could result from reduced PKA activity, I examined cAMP-dependent synaptic facilitation in the inbred strains. If increased concentrations of cAMP are unable to induce synaptic facilitation in C3H and D2 mice selectively, it might indicate that cAMP is unable to activate the PKA signaling cascade in these strains, thus preventing the induction of stable PKA-dependent L-LTP (Nguyen and Woo, 2003).

Transient co-application of FSK, an adenylyl cyclase activator, and IBMX, a phosphodiesterase inhibitor, elicited increases in fEPSP slope in the SC pathway in all strains examined. The increase in fEPSP slope 120 min after the start of FSK+IBMX application was equivalent between all four strains (**Figure 4.4**; $F(3,21) = 0.8491$, $p > 0.4$). Thus, I conclude that cAMP-dependent synaptic facilitation is intact in these four inbred strains.

v. Altered postsynaptic excitability in some inbred strains might contribute to LTP differences

Do basal synaptic input/output (I/O) relationships differ between inbred strains? In the SC pathway, the stimulus intensity was varied to elicit five different fEPSPs from each hippocampal slice. The presynaptic fibre volley amplitude and initial fEPSP slope of each sweep were measured, and a trendline plot for each strain was generated using linear regression analysis with y intercept=0 (**Figure 4.5a-d**). The presynaptic fibre volley amplitude reflects the number of axons recruited by stimulation, and the initial fEPSP slope is a measure of synaptic strength (Johnston and Wu, 1995). Validity of these linear regression analyses was assessed using approximate randomization testing, because not all data points were independent. Using the regression coefficient (slope) as the test statistic, I found that the linear regressions calculated for all four strains were not due to chance alone (B6, $p < 0.001$; 129, $p < 0.001$; C3H, $p < 0.01$; D2, $p < 0.02$). The results were similar when the coefficient of determination (r^2) was used as the test statistic (B6, $p < 0.001$; 129, $p < 0.001$; C3H, $p < 0.001$; D2, $p < 0.001$). The positive regression slopes indicate that the synaptic response increases as the number of stimulated axons increases.

An ANCOVA test on the linear regression slopes between the four inbred strains was significant: $F(3,272) = 13.105$, $p < 0.001$. Approximate randomization tests showed that the significant result of the ANCOVA test was not due to chance alone ($p < 0.001$). Dunnett post-hoc testing revealed that 129 had a significantly greater I/O slope than B6 (**Figure 4.5e**; $p < 0.01$) and that C3H had a significantly lower I/O slope than B6 ($p < 0.01$), whereas the I/O slope for the D2 strain was not significantly different from B6 ($p > 0.05$). The significant differences in the post-hoc test were again verified using

approximate randomization. In this case, the significant differences between B6 and 129 ($p < 0.01$) and between B6 and C3H ($p < 0.001$) were not due to chance alone.

In order to determine whether these I/O alterations in inbred strains are likely to have a pre- or post-synaptic locus, paired-pulse facilitation (PPF) was measured in area CA1 in hippocampal slices from inbred strains. PPF is an enhancement of synaptic transmission resulting from two closely spaced stimuli (Katz and Miledi, 1968). This facilitation occurs because calcium ions that enter the presynaptic terminal in response to an electrical stimulus are not completely cleared from the terminal before the next stimulus pulse is given. This residual calcium sums with the presynaptic calcium influx elicited by the second pulse, and enhances transmitter release in response to the second pulse (Katz and Miledi, 1968). Thus, the magnitude of PPF is an indirect measure of the capacity of the presynaptic terminal to increase transmitter release probability as a result of residual calcium. Reduced PPF could indicate that transmitter release in response to the first pulse is elevated such that less transmitter is available for release in response to the second pulse. Such elevated transmitter release could also affect the I/O response such that the I/O slope is steeper.

PPF was measured at three inter-pulse intervals: 50, 75, and 100ms (**Figure 4.5f**). The ANOVA tests were significant at 50ms: $F(3,57) = 3.660$, $p < 0.02$; but not at 75ms: $F(3,57) = 1.313$, $p > 0.2$; or 100ms: $F(3,57) = 1.646$, $p > 0.1$. However, post-hoc testing indicated that none of the inbred strains differed significantly from B6 at the 50ms inter-pulse interval. Thus, 129, C3H, and D2 do not significantly differ from B6 on PPF measured at these three intervals. This indicates that presynaptic transmitter release capabilities in these three strains do not significantly differ from B6.

From the I/O relationships and PPF results in four inbred strains, I can infer that strains 129 and C3H have altered neuronal excitability in response to electrical stimuli in the SC pathway, but that no strains significantly differ from B6 in presynaptic facilitation elicited by paired-pulse stimulation. Thus, it is likely that the altered neuronal excitability in 129 and C3H has a postsynaptic locus. Furthermore, the increased I/O relationship in 129 correlates with intact SC LTP in this strain, whereas the decreased I/O relationship in C3H correlates with deficits in SC LTP induced by one or 4 trains of electrical stimulation. These data indicate that a decreased postsynaptic response to electrical stimulation of the presynaptic fibres may contribute to impairments of specific forms of LTP.

vi. MPP LTP is intact in inbred strains with hippocampus-dependent memory deficits

The MPP provides a major input to the trisynaptic pathway of the hippocampus from the entorhinal cortex. Also, the MPP-dentate gyrus synapse exhibits LTP that shares many properties with SC LTP. For instance, MPP LTP is NMDA receptor-dependent and exhibits a late phase that is protein synthesis dependent (Colino and Malenka, 1993; Hanse and Gustafsson, 1992; Nguyen and Kandel, 1996; Wu et al., 2001). Thus, if MPP LTP were altered by genetic background, it might have a profound influence on information processing in the hippocampus. Accordingly, I characterized MPP LTP in my four selected inbred strains.

Figure 4.6a shows the recording and stimulating electrode placements in a hippocampal slice that were used to elicit fEPSPs in the MPP-dentate gyrus synapse. To confirm that the field recordings were of the MPP and not contaminated by lateral perforant pathway responses, I applied paired pulses at an interval of 50ms, and only

those responses that displayed depression of the second pulse were accepted (McNaughton, 1980). This paired-pulse depression (PPD) was measured prior to and after LTP induction in all MPP experiments (**Figure 4.6b**). An ANOVA calculated for PPD between strains before LTP induction showed a significant effect of strain: $F(3,25) = 3.391$; but post-hoc testing revealed that no strains differed significantly from B6. There were no strain differences in MPP PPD after LTP induction: $F(3,25) = 2.677$, $p > 0.05$. All strains showed a trend towards increased PPD after LTP induction, although only C3H exhibited significantly more depression after LTP induction compared with before LTP induction: $t(12) = 3.639$, $p < 0.004$. These findings are consistent with the notion that enhanced transmitter release following LTP induction should increase PPD, and thus support a presynaptic locus for expression of MPP LTP.

MPP LTP was elicited by four 100Hz trains of 500-ms duration with an inter-train interval of 20s. The stimulus intensity was increased to 1.5 times that used to elicit baseline, and the pulse width was increased to 0.1ms during delivery of tetani. This stimulation protocol resulted in robust LTP in all four strains examined (**Figure 4.6c-e**). One hour after tetanization, all strains exhibited equivalent levels of potentiation: $F(3,25) = 2.121$, $p > 0.1$. Thus, MPP LTP is not impaired in inbred strains that exhibit reduced hippocampal memory, whereas some types of SC LTP are impaired in these strains.

vii. Induction of amygdalar LTP is reduced in C3H and D2, but intact in 129

The amygdala has a role in learning and memory for fear conditioning. This brain structure is necessary in rodents for both contextual and cued fear memory, although the hippocampus also has a primary role in contextual fear memory (Chen et al., 1996; Fanselow and Kim, 1994; Kim and Fanselow, 1992; Kim et al., 1993; Phillips and

LeDoux, 1992). Thus, the ability of the amygdala to undergo synaptic plasticity will most likely affect cued fear memory, and may have a more limited influence on contextual fear memory as well.

Figure 4.7a shows LTP induced by applying five trains of high-frequency stimuli to the lateral amygdala with recordings made from the basolateral amygdala in the four inbred strains. Sample traces are illustrated in **Figure 4.7b**. Statistical analyses were carried out at time points immediately after tetanization (*induction*) and 1 hr after tetanization (*maintenance*). Non-parametric ANOVA analyses (Kruskal-Wallis tests, used because of unequal standard deviations between groups) revealed that there were significant differences between strains for induction ($p = 0.01$) but not for maintenance ($p = 0.43$). A post-hoc Dunn's multiple comparisons test showed that strains C3H ($p < 0.05$) and D2 ($p < 0.01$) had significantly lower levels of amygdalar LTP induction than B6, whereas amygdalar LTP induction in strain 129 was not significantly different from B6 ($p > 0.05$). Thus, strains C3H and D2, which exhibit impaired cued and contextual fear memory, also exhibit deficits in amygdalar LTP induction. Strains B6 and 129 display intact amygdalar LTP and fear memory.

viii. Summary

Overall, my results indicate that memory impairments in selected inbred strains correlate with defective LTP in hippocampal area CA1 and the amygdala, but not in the dentate gyrus (see **Table 4.1**). The reduced hippocampal LTP observed in area CA1 in C3H mice was correlated with a reduced I/O relationship with intact PPF. This outcome suggests that genetic variation can result in a range of capacities to sustain SC LTP, which might be related in part to varying degrees of excitability of the SC-CA1 synapse.

Strains that exhibit impaired SC LTP are more likely to perform poorly on hippocampus-dependent memory tests. Thus, SC LTP is highly correlated with hippocampal memory function, whereas MPP LTP is not.

D. Discussion

Because STM, LTM, and LTP rely on related molecular mechanisms, I examined these three processes in four genetically distinct inbred mouse strains. The primary finding of these experiments is that hippocampal SC LTP, but not MPP LTP, is strongly correlated to memory for fear conditioning in different inbred mouse strains. Additionally, the induction of amygdalar LTP was reduced in those strains that exhibit poor memory for fear conditioning. Because the hippocampus has a role in mediating contextual fear memory, deficits in contextual fear memory might be correlated with the hippocampal LTP deficits observed in particular strains. Similarly, poor cued memory performance in the strains might be correlated with the amygdalar LTP deficits observed in the same strains. I have identified two inbred mouse strains, D2 and C3H, that exhibit impaired short-term and long-term fear memory as well as particular SC and amygdalar LTP impairments. In contrast, 129 and B6 have intact cued and contextual fear memory along with robust hippocampal and amygdalar LTP. All strains exhibited intact MPP LTP. Thus, LTP in the MPP is unlikely to be a process that underlies the memory alterations observed in C3H and D2. I cannot rule out the possibility that synaptic transmission in the MPP might still importantly shape hippocampal information processing.

The MPP pathway terminates in the dentate gyrus (DG) and exhibits bidirectional synaptic plasticity (Bramham and Srebro, 1987). The role of the DG in hippocampus-

dependent memory has garnered some attention recently, although no definitive role has been determined for the DG in mediating specific aspects of learning and memory. Although LTP saturation in the DG impairs spatial learning (Brun et al., 2001; McNaughton et al., 1986; but see Sutherland et al., 1993), LTP induced in such a manner may affect synaptic transmission in other subregions of the hippocampus, making it difficult to localize exactly where the disruption of learning and memory occurred. Additionally, other studies have indicated that DG LTP does not correlate with spatial memory (Nosten-Bertrand et al., 1996; Saucier and Cain, 1995). Okada et al. (2003) suggest that both DG and area CA1 LTP can have different functional roles in spatial learning. For instance, the DG exhibits place cell activity (Jung and McNaughton, 1993). Furthermore, cells in CA1 can establish place fields without input from area CA3 (and the DG); this lack of input does not affect spatial learning but seems to impair the recollection of spatial memory (Brun et al., 2002). Others have suggested that area CA1 and the DG can mediate memory for different tasks, such as temporal and spatial discrimination, respectively (Gilbert et al., 2001). My data indicate that LTP in the MPP is not as strongly correlated with memory for contextual fear conditioning as SC LTP. This suggests that area CA1 may be more involved than the DG in the integration of information that is needed for successful contextual fear conditioning. I also note that the direct projection from the entorhinal cortex to area CA1 (the temporoammonic pathway) makes it possible for some hippocampal input to bypass the DG altogether.

All four strains showed similar performance in the two memory retention periods tested (1hr STM and 24hr LTM). This was the case for both contextual and cued fear memory; all strains either performed well on both the STM and the LTM tests, or

performed poorly on both the STM and the LTM tests. Specifically, D2 and C3H exhibited impaired STM and LTM of cued and contextual fear associations. The memory impairments identified in strains D2 and C3H likely result from alterations in function of the cells and molecules that have a role in both STM and LTM, as opposed to those that participate in either STM or LTM only.

Strains C3H and D2 showed little freezing during cued and contextual memory testing at both 1 and 24 hrs after training, which indicates that they likely have impairments in both hippocampal and amygdalar function. However, one may question whether the impairments seen in these two strains reflect a memory deficit, decreased sensitivity to footshock, or inability to exhibit the measured freezing response. I suggest that the lack of freezing observed in these strains results from memory impairment. I can rule out decreased sensitivity to footshock because my shock threshold experiments showed that all four strains reacted to footshocks of less than 0.7mA (**Figure 4.2**), the intensity used in this study. There is also evidence from other studies that the C3H and D2 strains can sense footshocks of less than 0.7mA, and can express a fearful freezing response. Owen et al. (1997) used two training trials with a 0.35mA footshock to train these strains, and they exhibited high levels of freezing to the tone 24 hours after training. Bolivar et al. (2001) applied three training trials with a 0.5 mA footshock, and observed freezing to the context and to the tone in both C3H and D2. Also, Balogh and Wehner (2003) applied two training trials with a 0.7mA footshock (the same intensity used in my study) and observed freezing to the tone in C3H and D2. These studies illustrate that strains C3H and D2 are capable of expressing a freezing response to a fearful situation, and that they are sensitive to footshocks of intensity 0.35 mA and greater. Therefore, my

footshock intensity of 0.7 mA is sufficient to serve as a US. The primary difference between my study and the other three cited here is that I used only one training trial, whereas the other studies employed two or three. Thus, I probably conducted a more sensitive test of fear learning and/or memory, which may explain why I observed memory impairments for contextual and cued fear at both 1 and 24 hrs after training.

Several molecules are likely to be involved in mediating both STM and LTM of contextual fear conditioning. By examining a variety of molecular/behavioural studies on inhibitory avoidance learning, a form of conditioned fear, Izquierdo and McGaugh (2000) identified a number of molecular mechanisms, in CA1 hippocampus, that contribute to STM, to LTM, or to both STM and LTM. Interestingly, many molecules in area CA1 that were found to contribute to STM also contribute to LTM. These include the three major classes of glutamate receptors, protein kinases C and G, the protein kinase A (PKA) signaling pathway (including PKA, adenylyl cyclase, D1/D5 receptors, and the 5-HT_{1A} receptors), and MAP kinase (Izquierdo and McGaugh, 2000). Thus, at least one of these molecules that have a role in both STM and LTM might be altered in C3H and D2 mice. There is evidence that protein kinase C (PKC) levels are altered in the D2 hippocampus (Bowers et al., 1995; Paylor et al., 1996; Wehner et al., 1990). Because hippocampal PKC contributes both to STM and LTM for inhibitory avoidance, it may underlie the STM and LTM deficits observed in D2 mice. Currently, it is unknown whether any of these molecular mechanisms for hippocampal STM and LTM are altered in the strain C3H. However, the molecular mechanisms that underlie hippocampal STM and LTM impairments in C3H may be different from those in D2 because these two strains exhibited differences in SC LTP impairments.

All four strains either had intact memory for both contextual and cued fear conditioning, or poor memory for both contextual and cued fear conditioning. These results indicate that similar molecular and/or cellular mechanisms might underlie these two forms of memory in inbred mice. Thus, the molecular mechanisms that contribute to plasticity in both the hippocampus and the amygdala should be the focus of further investigation aimed at elucidating the causes of impaired memory in the D2 and C3H strains.

Amygdalar and hippocampal LTP share many underlying molecular mechanisms. These include the activation of both NMDA receptors and voltage-gated calcium channels (VGCCs), although the amygdala may rely more on VGCCs than hippocampal area CA1 does (reviewed by Chapman et al., 2003). Because the amygdalar LTP impairments observed in strains C3H and D2 in this study were restricted to reduced LTP induction (and not maintenance), the molecular and/or cellular alterations responsible for these impairments might have a role in regulating the influx of ions during or immediately following tetanic stimulation. This hypothesis can be tested by measuring specific ionic currents (e.g., glutamatergic receptor currents and calcium currents) during tetanization and LTP induction in the basolateral amygdala. Also, L-LTP in both the hippocampus and the amygdala requires PKA and extracellular-regulated kinase/mitogen-activated protein kinase (ERK/MAPK; reviewed by Schafe et al., 2001). The Ras/MAPK/CREB signaling pathway, which may be involved in initiating protein synthesis, is also likely involved in LTP maintenance in both the hippocampus and the amygdala (reviewed by Adams et al., 2000; Chapman et al., 2003). Behavioural pharmacological studies that aim to block or restore the actions of these molecules in

relevant brain structures might be of value in determining the causes of particular fear memory impairments in inbred mice. Intracellular electrophysiological experiments will also help determine whether particular signaling cascades or ion channels contribute to the altered amygdalar function found in strains C3H and D2.

An alternate explanation is that the contextual and cued fear memory impairments observed in strains C3H and D2 result from impaired communication between the amygdala and the hippocampus. There is evidence that the basolateral amygdala can modulate hippocampal synaptic plasticity and hippocampus-dependent memory, especially for emotional events (Abe, 2001; Akirav and Richter-Levin, 2002; Almaguer-Melian et al., 2003; Frey et al., 2001; Richter-Levin and Akirav, 2003). Although most studies have determined that the basolateral amygdala can influence dentate gyrus LTP, the circumstances under which this can occur are not well defined. Thus, although I did not find impairments in dentate gyrus LTP in the inbred strains that exhibited altered amygdalar LTP, I cannot rule out the possibility that altered amygdalar synaptic plasticity can influence contextual fear memory in these inbred strains.

All types of hippocampal LTP induced in B6 and 129 were comparable in magnitude. However, strains C3H and D2 exhibited varied deficits in hippocampal SC LTP induced by electrical stimulation. Interestingly, C3H showed reduced potentiation in response to either one or four trains of high-frequency stimulation, whereas D2 had intact one- and four-train LTP but reduced LTP in response to TBS. Thus, both strains have genetic alterations that influence SC LTP, but these alterations must be different between strains. In strain C3H, the altered gene expression that influences both early- and late-LTP could take two forms: either one gene that affects both early- and late-LTP

is altered in expression, or multiple genes have altered expression in strain C3H. C3H has been identified as having the *rd* mutation – that is, C3H is homozygous for the retinal degeneration allele *Pde6b*^{rd1} (The Jackson Laboratory, 2002). *Pde6b*, which encodes a phosphodiesterase, is inactivated in C3H mice. Wimer et al. (1991) showed that the *rd* mutation elicits a decrease in hippocampal granule cells in the DG. Thus, the *rd* mutation might influence hippocampal synaptic plasticity, learning, and memory in strain C3H. In D2, because one- and four-train LTP were intact but TBS LTP was reduced, I can conclude that TBS LTP may depend on different molecular and cellular mechanisms than one- and four-train LTP. D2 has been identified as having reduced levels of ?-PKC (Bowers et al., 1995; Paylor et al., 1996; Wehner et al., 1990). This correlates with hippocampus-dependent memory deficits in D2 (Paylor et al., 1996; Wehner et al., 1990), and might also contribute to this selective deficit for TBS LTP.

The level of facilitation in the SC pathway induced by forskolin and IBMX application was comparable between the four strains examined. This suggests that the cAMP/PKA signaling pathway was recruited successfully by FSK+IBMX application in these inbred strains, and that the pathway is functionally intact. This result contrasts with that of Nguyen et al. (2000b). Nguyen et al. found that several inbred strains, including D2, exhibit reduced SC pathway facilitation in response to the application of FSK+IBMX at the same concentration used in the present study. Nguyen et al. used male mice, whereas the current study used female mice – it is possible that the conflicting results reflect sex differences. More importantly, Nguyen et al. housed their mice singly to avoid intra-species fighting among male mice. My present study examined group-housed female mice. Social isolation and sex differences can combine to significantly influence

many types of behaviours and synaptic plasticity in rodents (Bronzino et al., 1996; Guo et al., 2004; Roberts and Greene, 2003). Regardless of the reasons for the different results, the present study indicates that the cAMP/PKA signaling pathway can be successfully recruited in females of the four inbred strains tested, but does not exclude the possibility that the pathway may be less robust in some strains.

I found that two inbred strains exhibited significantly different SC pathway input/output curves from B6. The I/O linear regression slope was steeper in strain 129 than in B6, indicating that the area CA1 pyramidal cells are more excitable in 129 than in B6. Alternately, in strain C3H, the I/O linear regression slope was less steep than in B6. Thus, CA1 pyramidal cells are less excitable in C3H than in B6. Reduced postsynaptic depolarization in response to electrical stimulation of the Schaeffer collaterals has been shown to impair the induction of SC LTP in young rats (Liao and Malinow, 1996). Interestingly, SC LTP was intact for all induction protocols in strain 129, but C3H exhibited impairments in SC LTP induced by one or four trains of HFS. Thus, in area CA1, reduced postsynaptic depolarization in response to presynaptic activation may underlie defective SC LTP elicited by one or four trains of HFS in C3H, whereas increased postsynaptic excitability is not harmful for SC LTP in 129.

Alternately, SC LTP deficits in C3H may be caused by a genetic mutation of *Pde6b* in this strain. C3H carries this *rd* mutation that results in vision deficits. *Pde6b* encodes phosphodiesterase (PDE) 6, an enzyme that catabolizes cGMP (Soderling et al., 1998). mRNA for PDE6B is expressed in the mouse hippocampus (Razzaque et al., 2002). Increasing cGMP concentrations can affect hippocampal synaptic transmission, either by causing synaptic depression or by enhancing potentiation elicited by high-frequency

stimulation (Boulton et al., 1994; Zhuo et al., 1994a; Zhuo et al., 1994b). The effect of the *rd* mutation on hippocampal synaptic plasticity was recently investigated by Collingridge and colleagues (Kuenzi et al., 2003). Transgenic mice expressing the *rd* mutation were found to exhibit intact SC LTP elicited by TBS stimulation, as did the C3H strain in the present study. Thus, Kuenzi et al. (2003) concluded that the *rd* mutation does not have an effect on SC LTP. However, the authors did not examine SC LTP induced by one or four trains of HFS so it remains to be seen whether the *rd* mutation might have a selective effect on these types of SC LTP. Because strain C3H carries the *rd* mutation and has impaired one and four train SC LTP, I suggest that PDE6 may have a selective role in these two forms of LTP. Further examination of SC LTP in the rd/rd transgenic mouse might be valuable in elucidating whether specific SC LTP deficits might result from the altered expression of PDE6.

Several neurochemical differences between DBA/2 and C57BL/6 reported in the literature might begin to account for the differences in hippocampal learning, memory, and synaptic plasticity between these two strains. Compared with C57BL/6 mice, DBA/2 mice exhibit reduced levels hippocampal protein kinase C (PKC) (Bowers et al., 1995; Paylor et al., 1996; Wehner et al., 1990). PKC is required for the induction of LTP (Malinow et al., 1989), and it has a role in hippocampal learning and memory (Abeliovich et al., 1993; Olds and Alkon, 1991; Van der Zee et al., 1992). Beginning at 24 days of age, hippocampal γ-PKC expression is significantly reduced in DBA/2 mice compared with C57BL/6 mice (Paylor et al., 1996). Interestingly, C57BL/6 mice also begin to outperform DBA/2 mice on spatial learning in the Morris water maze at 24 days of age (Paylor et al., 1996). Consistent with reduced PKC concentrations, hippocampal

growth associated protein 43 (GAP-43; a substrate for PKC) is less phosphorylated following contextual fear conditioning training in DBA/2 than in C57BL/6 mice (Young et al., 2000). McNamara et al. (2003) also found that hippocampal MARCKS (myristoylated alanine-rich C kinase substrate) mRNA and protein expression (primarily in the cytosolic fraction) is greater in DBA/2 than in C57BL/6 mice. Because PKC downregulates and destabilizes MARCKS mRNA and protein independent of transcription (Brooks et al., 1992), the higher level of PKC in the C57BL/6 hippocampus may inhibit MARCKS function, resulting in the strain difference between C57BL/6 and DBA/2. The altered expression of PKC and of related neurochemicals in the DBA/2 hippocampus may cause the LTP and memory deficits that this strain exhibits.

Other neurochemical alterations in the DBA/2 hippocampus (compared with C57BL/6) include lower basal expression of *Zif/268* (Fordyce et al., 1994), an immediate-early gene that is upregulated during particular types of associative learning, and by LTP-inducing tetanic stimuli (Davis et al., 2003). Also, Zilles et al. (2000) found that poor spatial learning on the radial arm maze in DBA/2 mice is correlated with a reduced density of muscimol binding sites (i.e., $GABA_A$ receptors) in hippocampal area CA1 and the dentate gyrus, as well as reduced density of AMPA receptors and kainate binding sites in all hippocampal subregions. My data indicate that hippocampal SC LTP that is induced by TBS, but not necessarily one or four trains of HFS, might be linked to these neurochemical alterations in DBA/2. One or several of the previously identified neurochemical alterations might contribute to this LTP deficit in DBA/2, including PKC-related signaling changes, altered inhibition via the $GABA_A$ receptor, or less AMPA receptor- or kainate receptor-mediated currents.

Some groups have asked whether the hippocampus is as important for contextual fear conditioning in the DBA/2 mouse as it is in C57BL/6. Logue et al. (1997) and Ammassari-Teule et al. (2000) have conducted studies that indicate that contextual fear memory is less hippocampus-dependent in DBA/2 than in C57BL/6. These studies indicated major impairments in hippocampal function in DBA/2 mice, which may also compromise the ability of the DBA/2 hippocampus to integrate its function with other interconnected brain regions.

The most important conclusion from this study is that hippocampal SC, but not MPP, LTP correlates with memory for contextual fear conditioning in inbred mouse strains. The inbred strains B6 and 129 exhibit STM and LTM for contextual and cued fear conditioning, and have intact SC, MPP, and amygdalar LTP. The inbred strains C3H and D2 display little STM and LTM for contextual and cued fear conditioning with intact MPP LTP and specific impairments of SC and amygdalar LTP. Previously identified genetic/molecular alterations in C3H and D2 (*rd* mutation and reduced hippocampal ?-PKC levels, respectively) may account for the hippocampal deficits identified in this study, but further research is necessary to test this hypothesis. On a cellular level, I suggest that reduced excitability of CA1 cells may contribute to the SC LTP deficits in C3H. I suggest that the cAMP/PKA signaling pathway is intact and can be recruited in female mice from the four strains examined. Finally, although my results indicate that strains D2 and C3H have altered function of the hippocampus and the amygdala, they do not rule out the possibility that other brain regions that are involved in learning and memory also function differently in these strains.

I emphasize that in order to generalize my findings, a more thorough examination of numerous other inbred mouse strains is necessary. Further studies should also investigate the frequency-dependence of electrically-induced LTP in inbred strains. Nonetheless, my data provide an important foundation for probing the synaptic mechanisms that may contribute to information processing by specific subregions of the mammalian hippocampus. Synaptic plasticity in areas CA1 and the dentate gyrus may be correlated with additional cognitive processes other than behavioural fear conditioning, but this idea can only be rigorously tested after clear-cut cognitive roles for these hippocampal subregions have been identified. In rats, there is evidence that specific computational functions, such as pattern association and temporal pattern completion, may be mediated by particular hippocampal subregions (e.g., see review by Kesner et al., 2000). However, it is unclear whether similar processes can be ascribed to hippocampal subregions in mice. More broadly, my findings demonstrate that inbred mouse strains can serve as models of memory impairment that can be used to elucidate the cellular and molecular mechanisms that underlie specific memory deficits.

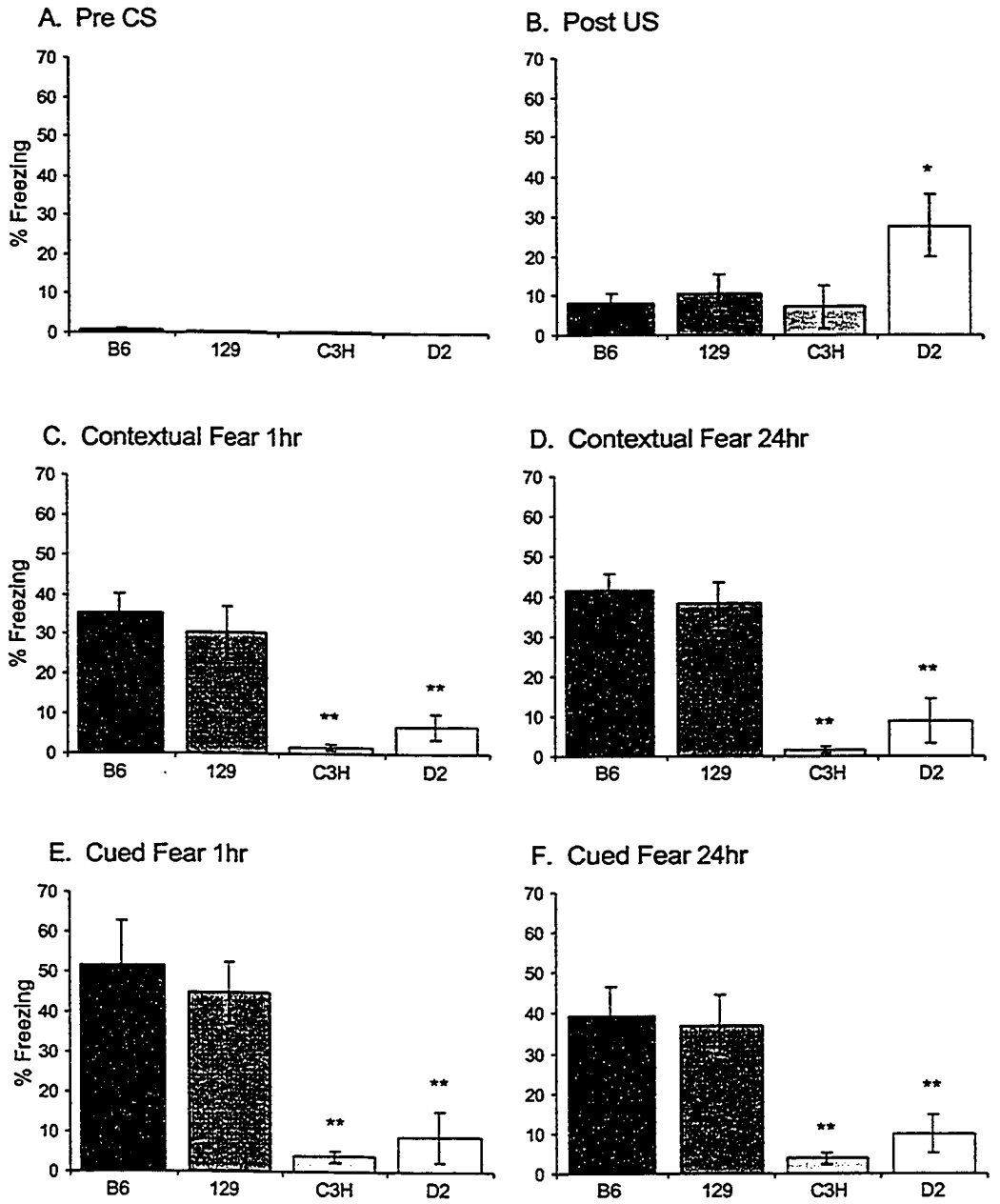

Figure 4.1: Short- and long-term memory for contextual and cued fear conditioning are impaired in inbred mouse strains C3H and D2, but not 129, compared with B6. A) All four strains tested (n = 16 for each strain) showed low levels of freezing upon initial placement into the training context for 2 minutes. B) Strain D2 exhibited significantly more freezing after the single training trial than B6 (n = 16 for all strains). C,D) C3H and D2 have reduced short-term (1hr; n = 8 for all strains) and long-term (24 hr; n = 8 for all strains except D2, n = 7) memory for the context-US association. E,F) C3H and D2 have reduced short-term (1hr; n = 8 for all strains) and long-term (24 hr; n = 8 for all strains except D2, n = 7) memory for the CS-US association. Data are shown as means ± SEM. CS, tone; US, footshock. Asterisks indicate significance values derived from the Dunnett post-hoc test with B6 as the control value (*$p < 0.05$; **$p < 0.01$).

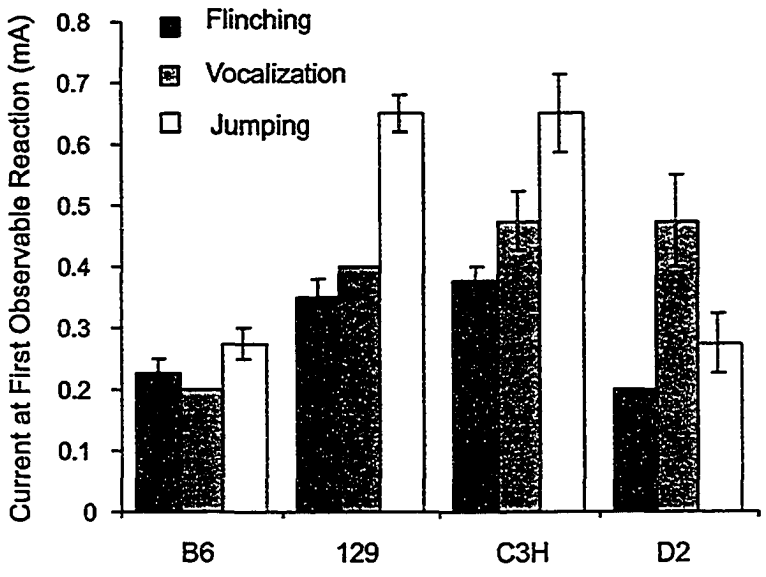

Figure 4.2: All four inbred strains exhibit flinching, vocalizations, and jumping in reponse to footshocks of less than 0.7mA. Although there are differences in the amperages at which some inbred mouse strains exhibit particular types of reactions to footshock, the four inbred strains tested (n = 4 for all strains) elicited all three types of measured reactions at currents less than 0.7mA.

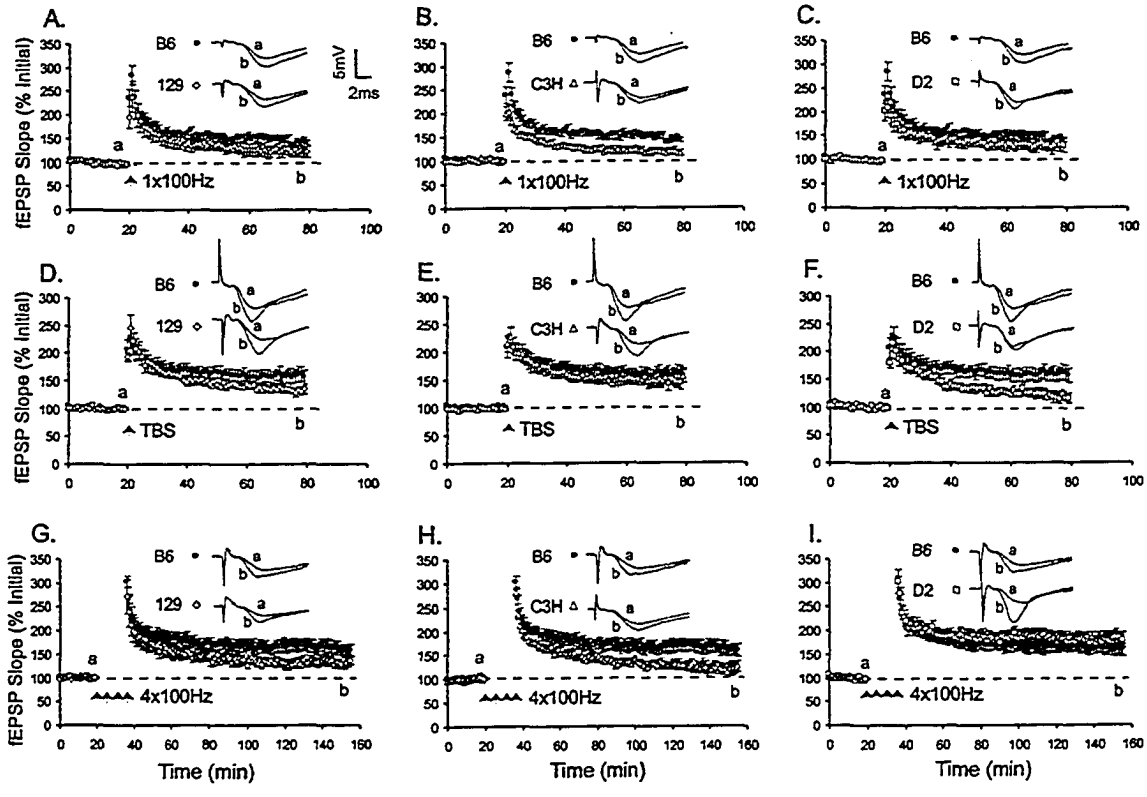

Figure 4.3: Strains C3H and D2, but not 129, have reduced hippocampal area CA1 LTP compared with B6, that correlates with contextual and cued fear memory performance. A,B,C) LTP induced by one train of high-frequency electrical stimulation (*1x100Hz*; duration 1s) is significantly reduced one hour after tetanization in strain C3H (B6, n = 11; 129, n = 6; C3H, n = 5; D2, n = 6). D,E,F) LTP induced by theta-burst stimulation (*TBS*; 15 bursts of four pulses at 100Hz, inter-burst interval 200ms) is significantly reduced one hour after tetanization in strain D2 (B6, n = 10; 129, n = 6; C3H, n = 6; D2, n = 5). G,H,I) LTP induced by four trains of high-frequency stimulation (4x100Hz; duration 1s, inter-pulse interval 5 min) is significantly impaired in strain C3H two hours after tetanization (B6, n = 8; 129, n = 6; C3H, n = 5; D2, n = 6). Sample fEPSP traces were recorded at times *a* and *b* as indicated on the graphs.

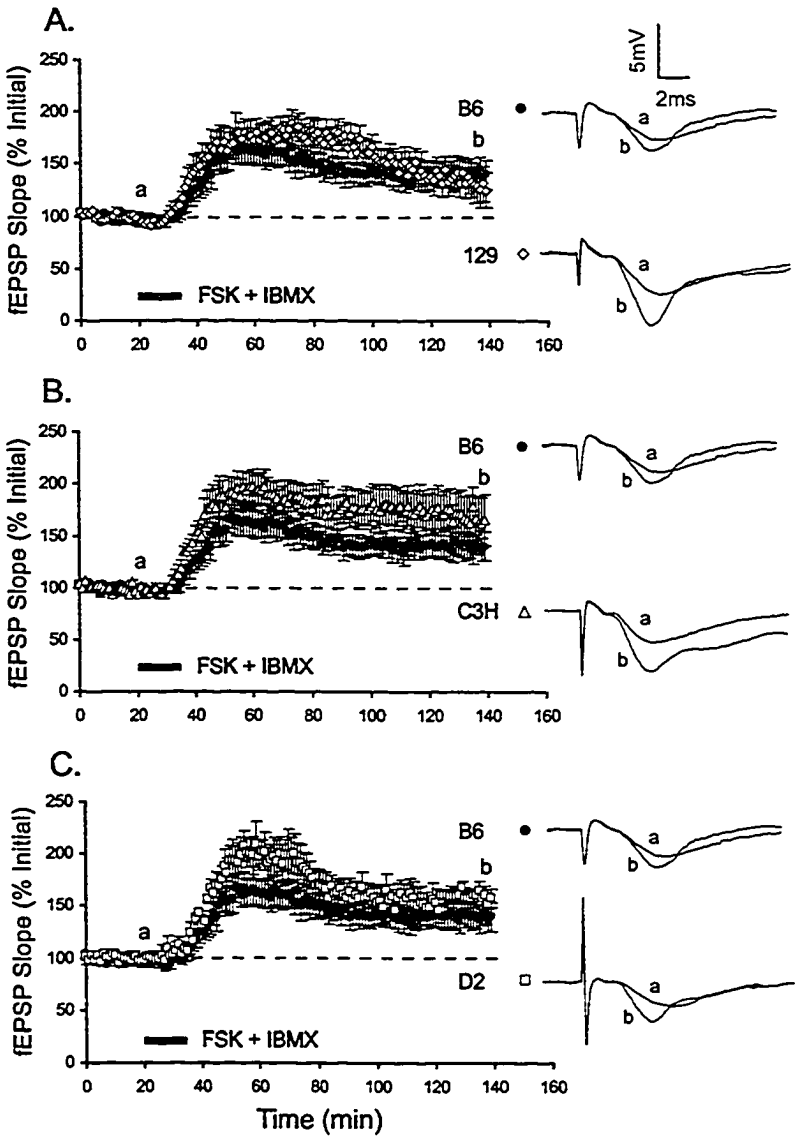

Figure 4.4: Area CA1 LTP induced by the application of chemicals that increase cyclic AMP levels and recruit the PKA signalling pathway is intact in all four inbred strains. A,B,C) A cocktail of forskolin (50μM), an adenylyl cyclase activator, and IBMX (50μM), a phosphodiesterase inhibitor, was applied to the bath for 15 min, and induced facilitation in slices from all four inbred strains. See *section B* for details on drug solution preparation. The level of facilitation two hours after the start of drug application in strains 129 (A, n = 6), C3H (B, n = 6), and D2 (C, n = 5) was similar to that in B6 (n = 8).

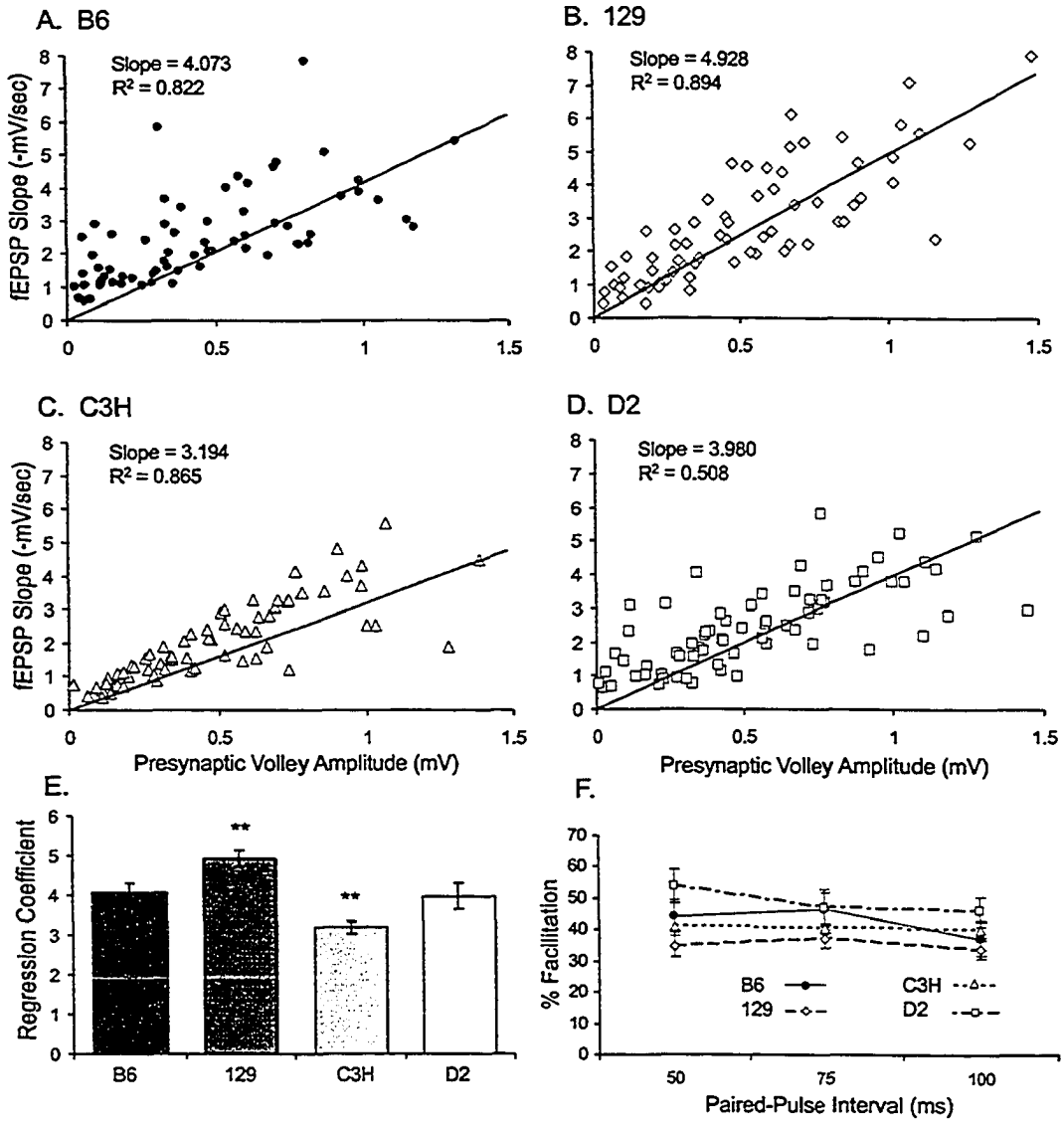

Figure 4.5: Area CA1 input-output curve (I/O) slopes are altered in two inbred strains, but paired-pulse facilitation (PPF) is similar between all four strains. A,B,C,D) I/O points were obtained by varying the stimulus intensity in area CA1 and measuring the amplitude of the presynaptic volley and the slope of the fEPSP. Linear regressions were calculated for each inbred strain. A) B6, n = 14 slices; B) 129, n = 14 slices; C) C3H, n = 14 slices; D) D2, n = 14 slices, 5 data points per slice for all strains. E) I/O linear regression slopes (± 95% confidence intervals) for the four inbred strains. 129 exhibited a greater I/O slope than B6, indicating stronger depolarization of the area CA1 cells in response to synaptic input. C3H exhibited a lower I/O slope than B6, indicating weaker depolarization of area CA1 cells in response to synaptic input. F) PPF in area CA1 of hippocampal slices from the four inbred strains at 50, 75, and 100ms inter-pulse intervals. Values are means ± SEM. Strains 129 (n = 16), C3H (n = 15), and D2 (n = 16) exhibited similar facilitation to B6 (n = 14) at all three inter-pulse intervals. Asterisks indicate significance values derived from the Dunnett post-hoc test with B6 as the control (**$p <$ 0.01).

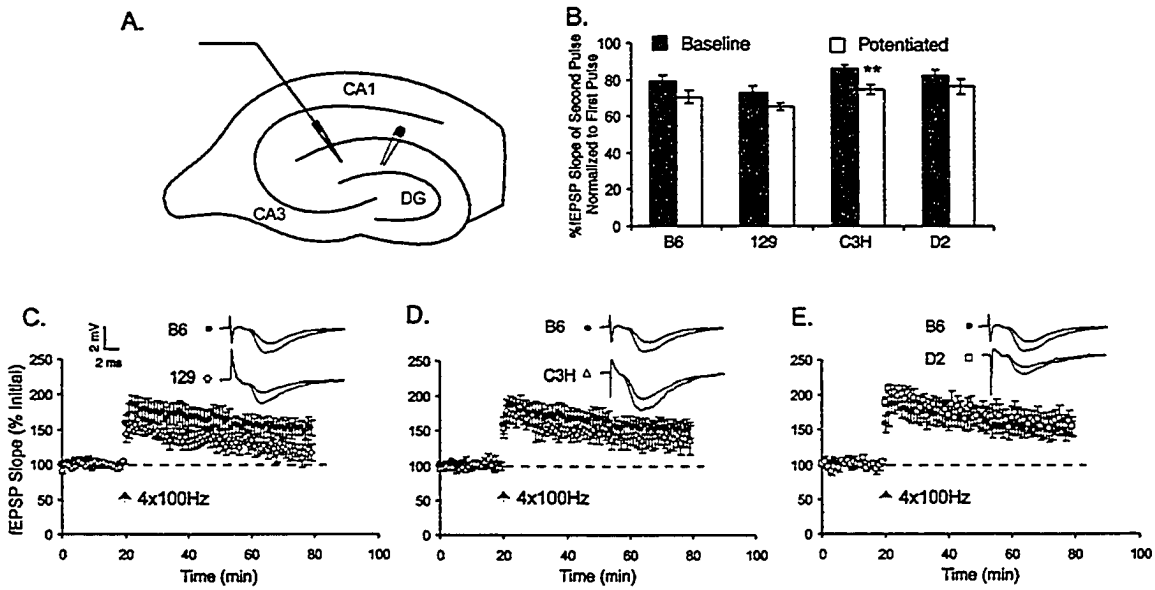

Figure 4.6: LTP in the medial perforant pathway (MPP) in hippocampal slices is similar between the four inbred strains, and does not correlate with contextual and cued fear memory performance. A) Diagram of a transverse hippocampal slice, indicating the recording and stimulating electrode placements used to measure fEPSPs in the MPP. B) Paired-pulse depression (PPD) recorded in the MPP with a 50ms inter-pulse interval, before (*Baseline*) and after (*Potentiated*) LTP induction. All strains exhibited comparable levels of PPD both before, and after, LTP induction, compared with B6. Only strain C3H exhibited significantly more PPD after LTP induction compared with before LTP induction, although all strains showed this trend. B6, n = 8; 129, n = 8; C3H, n = 7; D2, n = 6. C,D,E) LTP induced by four trains of high-frequency stimulation (*4x100Hz*; four trains of 100Hz stimulation, 500ms duration, 20s inter-train interval, 1.5X stimulus intensity, 0.1ms pulse width) is of similar magnitude one hour after tetanization in all four strains (B6, n = 8; 129, n = 8; C3H, n = 7; D2, n = 6). Asterisks indicate significantly different values as determined by Student's t-test (**$p < 0.02$).

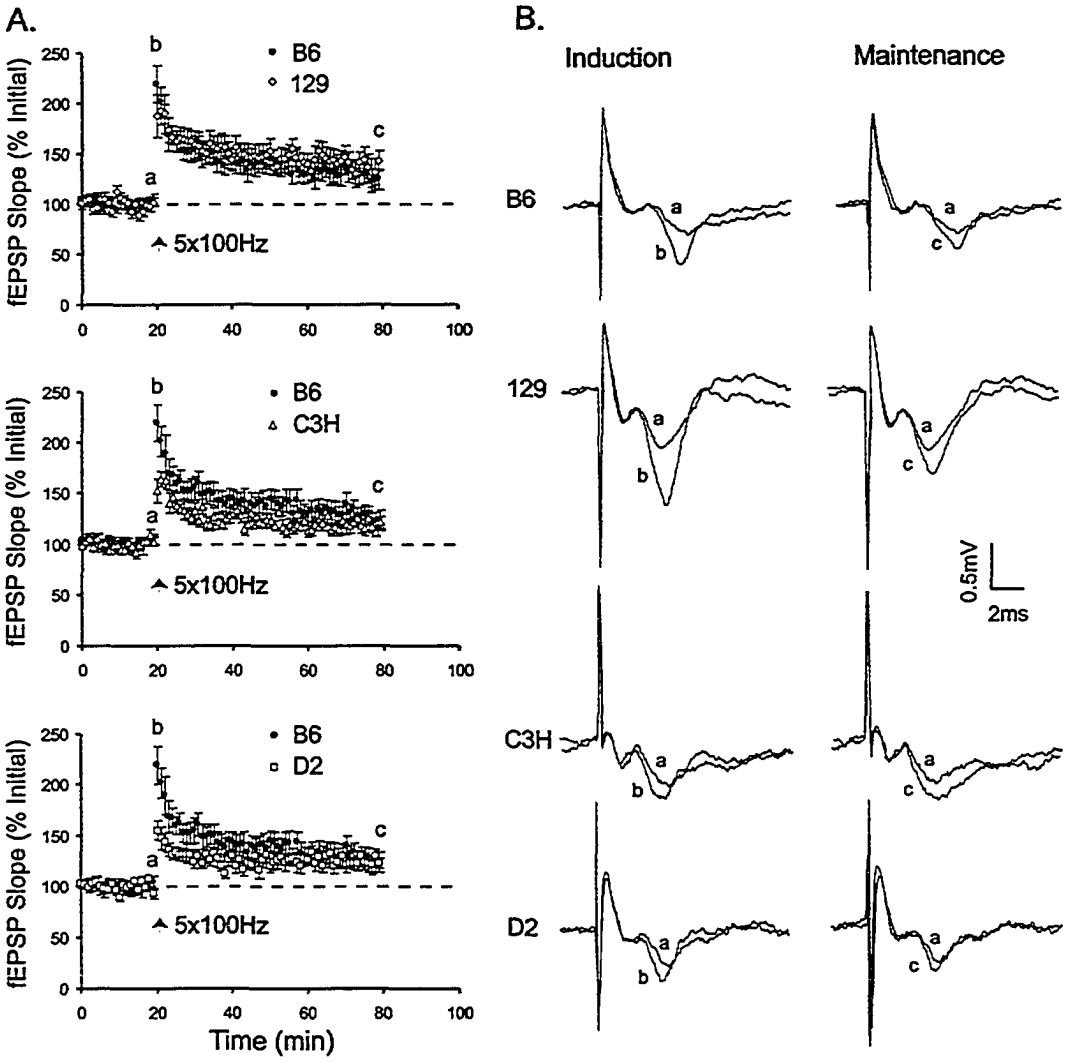

Figure 4.7: Amygdalar LTP induction is impaired in strains C3H and D2. A) LTP induced by 5 trains of high-frequency stimulation (*5x100Hz*; five trains of 100Hz stimulation, 1s duration, 10s inter-train interval) displays different levels of induction, but similar maintenance at 60 min post-tetanus, in the inbred strains (B6, n = 12; 129, n = 14; C3H, n = 12; D2, n = 12). Strains C3H and D2 exhibited lower LTP induction than B6, whereas LTP induction in strain 129 was not significantly different from B6. B) Sample traces of LTP induction and maintenance in the four inbred strains.

Table 4.1: Hippocampal SC and amygdalar, but not hippocampal MPP, LTP correlate with fear conditioning expression in four inbred mouse strains.

	Fear Conditioning		SC LTP			MPP LTP	Amyg LTP	CA1	
Strain	Contextual	Cued	1 train	TBS	4 train	2 train	5 train	I/O	PPF
B6	✓	✓	✓	✓	✓	✓	✓	✓	✓
129	✓	✓	✓	✓	✓	✓	✓	↑	✓
C3H	↓	↓	↓	✓	↓	✓	↓	↓	✓
D2	↓	↓	✓	↓	✓	✓	↓	✓	✓

SC, Schaeffer collateral pathway; MPP, medial perforant pathway; Amyg, amygdalar; TBS, theta-burst stimulation; I/O, input-output relation slope; PPF, paired-pulse facilitation; ✓ indicates comparable to B6; ↑ indicates greater than B6; ↓ indicates less than B6.

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*CHAPTER 5:

Mouse Models of Impaired Fear Memory Exhibit Deficits in Amygdalar LTP

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A. Introduction

It is unclear whether specific forms of synaptic plasticity underlie behavioural expression of particular forms of learning and memory. Long-term potentiation (LTP) is one type of synaptic plasticity that can critically influence expression of some forms of learning and memory in the mammalian brain (Bliss and Collingridge, 1993; Shors and Matzel, 1997; Moser et al., 1998; Micheau and Riedel, 1999; Brun et al., 2001). LTP is an activity-dependent enhancement of synaptic transmission that is induced by electrical stimulation (Lømo, 1966; Bliss and Lømo, 1973), and it can be elicited at many excitatory synapses in the brain, including the trisynaptic pathway of the hippocampus and in the amygdala (see review by Martin et al., 2000). Compelling evidence that LTP may underlie some forms of learning and memory has been obtained from studies of hippocampal and amygdalar synaptic plasticity (Morris et al., 1986; Barnes et al., 1994; McKernan and Shinnick-Gallagher, 1997; Maren, 2000; Martin et al., 2000).

Interestingly, the hippocampus and the amygdala have roles in different aspects of learning and memory. In rodents, hippocampal damage impairs spatial memory (Morris et al., 1982) and fear memory for a context (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Kim et al., 1993; Chen et al., 1996; Stiedl et al., 2000), although the amygdala also contributes to contextual fear memory (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Kim et al., 1993). Further studies have shown that area CA1 of the hippocampus is particularly important for encoding long-term memory (humans: Zola-Morgan et al., 1986; mice: Tsien et al., 1996). In particular, genetic modification of key signalling molecules in area CA1 can alter expression of both LTP and long-term memory (reviewed by Chen and Tonegawa, 1997; Micheau and Riedel, 1999). Although

it is reasonably well established that hippocampal area CA1 has a role in encoding long-term memory, the contributions of the other hippocampal subregions to memory function are less clear. For instance, plasticity at the perforant pathway-dentate gyrus synapse relies on many of the same molecular mechanisms as that in area CA1 (Collingridge et al., 1983; Hanse and Gustafsson, 1992; Colino and Malenka, 1993; Nguyen and Kandel, 1996; Chen and Tonegawa, 1997; Wu et al., 2001), but few studies have examined correlations between dentate gyrus LTP and hippocampus-dependent memory function. Jones et al. (2001) examined a mutant mouse in which the immediate-early gene *Zif/268* was disrupted; the mutant mice exhibited reduced dentate LTP *in vivo*, as well as impaired long-term, but not short-term, memory on several tasks. However, it is unclear whether the effect of the targeted mutation of *Zif/268* was solely due to impaired plasticity in the dentate gyrus, because other subregions of the hippocampus also exhibited reduced expression of *Zif/268*.

On the other hand, the amygdala is essential for expression of emotional memories, including those associated with fearful situations (LeDoux, 2000; McGaugh, 2004; Richter-Levin, 2004). Amygdalar lesions impair fear memory for a cue that is associated with an unpleasant stimulus (Fanselow and Kim, 1994). Furthermore, the amygdala can modulate memory expression in other brain regions, including the hippocampus (reviewed by Abe, 2001; McGaugh et al., 2002; Richter-Levin, 2004). In addition, recent experiments have shown that both the lateral amygdala (Doyere et al., 2003) and the basal amygdala (Yaniv et al., 2003) can express late-LTP *in vivo*. The late phase of LTP is strongly correlated with consolidation of long-term memory in rodents (Doyere and Laroche, 1992; Bourtchouladze et al., 1994; Abel et al., 1997; Jones et al.,

2001; Genoux et al., 2002). Some of the strongest evidence that LTP is causally linked to memory was presented by McKernan and Shinnick-Gallagher (1997). They showed that in fear-conditioned rats, excitatory post-synaptic currents in the lateral amygdala were enhanced 24hr after conditioning, compared with naïve control rats. Thus, synaptic plasticity in the amygdala is likely associated with its known role in mediating behavioural expression of fear memories.

Mouse models in which hippocampus- or amygdala-dependent memory is impaired can be used to determine whether synaptic plasticity in these regions has a role in mediating memory expression. Although genetically-modified mice have been used to identify genes and proteins (and their specific roles) that contribute to synaptic plasticity, learning, and memory (Chen and Tonegawa, 1997; Micheau and Riedel, 1999), I propose that inbred mouse strains are also valuable resources for achieving the same end. Some inbred strains exhibit impairments of specific forms of memory (e.g., see Schimanski and Nguyen, 2004), and thus may be used as models for probing the mechanisms of memory dysfunction. Importantly, inbred mouse strains are commonly used to produce mutant mice. As such, the choice of inbred strain(s) should be carefully considered.

Within an inbred mouse strain, all animals are genetically homogeneous, and they are generated by breeding siblings for a minimum of 20 generations (Lyon and Searle, 1989). Many inbred strains have been phenotyped for their performance on specific behavioural learning and memory tasks (e.g., Crawley et al., 1997; Owen et al., 1997; Bolivar et al., 2001). Particular strains have impaired hippocampus-dependent memory, validated by the results of multiple types of behavioural tests (reviewed by Schimanski and Nguyen, 2004). These strains include DBA/2 (Upchurch and Wehner, 1988; Paylor

et al., 1994; Owen et al., 1997; Nguyen et al., 2000a) and BALB/c (Chen et al., 1996; Radulovic et al., 1998; Royle et al., 1999; Yoshida et al., 2001; Francis et al., 2003). Study of strain DBA/2, in particular, has yielded some insight into molecular mechanisms that might underlie altered hippocampal function (Wehner et al., 1990; Bowers et al., 1995; Paylor et al., 1996). Interestingly, Manahan-Vaughan and Braunewell (1999) demonstrated differences in hippocampal synaptic plasticity between rat strains, although these rat strains were qualitatively different from inbred mouse strains because they were *outbred*, meaning that within strains, there were individual genetic differences (see also Manahan-Vaughan 2000a, 2000b). However, the mnemonic traits of many inbred mouse strains have not yet been characterized, and synaptic physiology has been defined in very few inbred strains (for review see Schimanski and Nguyen, 2004).

Here, I examined hippocampus- and amygdala-dependent learning and memory in five inbred mouse strains. I used contextual and cued fear conditioning to gauge memory functions that involve the hippocampus and amygdala, respectively. I tested retention at 1hr (to test short-term memory, or STM) and at 24hr (to test long-term memory, or LTM) after training. Both time delays were tested because STM and LTM require different molecular mechanisms and brain structures (Izquierdo and McGaugh, 2000; Eichenbaum and Cohen, 2001), and thus, deficits isolated to either STM or LTM could help identify which signalling pathways or brain regions are altered in these strains. I also characterized LTP, in area CA1 and in the dentate gyrus, as well as in the amygdala, to determine whether LTP in these regions correlates with behavioural memory mediated by these regions.

Strain C57BL/6NCrlBR (B6) has been widely used to characterize the mechanisms of LTP. B6 generally exhibits robust learning, memory, and synaptic plasticity. In addition, B6 is often used as a genetic background for the production of genetically modified mice. B6 was used as a control here because of it exhibits high levels of learning and memory performance, and because it has been well-characterized and is widely used. I chose four other inbred strains (A/J, BALB/cByJ, C57BL/10J, and SM/J) to compare with B6 that have not previously been characterized for synaptic plasticity. Strain A/J exhibits high levels of contextual and intermediate cued fear memory, but does not learn the Morris water maze (Owen et al., 1997; Bolivar et al., 2001; Balogh and Wehner, 2003). Strain BALB/cByJ (BALB) shows intermediate levels of contextual memory and high levels of cued fear memory, but has poor spatial memory (Upchurch and Wehner, 1988; Francis et al., 1995; Owen et al., 1997; Bolivar et al., 2001; Balogh and Wehner, 2003). Strain C57BL/10J (B10) is a C57BL substrain, as is strain B6. As such, these strains were derived from the same initial mating, but the accumulation of genetic mutations, as well as possible residual heterozygosity, have resulted in several genetic differences (Festing, 1998). The strains have been found to differ at the *H9*, *Igh2*, and *Lv* loci (Festing, 1998), and McClive et al. (1994) found differences at multiple loci on chromosome 4. Strain B10 performed well on fear and spatial memory tasks (Owen et al., 1997), but strain SM/J (SM) has not previously been examined. Interestingly, I found multiple impairments of contextual and cued fear memory in these four strains. However, contextual memory impairments were restricted to STM. I also found that these strains all exhibited intact hippocampal LTP in area CA1 and in the dentate gyrus, except for BALB, which displayed a selective impairment of

CA1 LTP induced by multiple trains of high-frequency stimulation. However, amygdalar LTP was reduced in strains A/J and BALB.

I suggest that deficient amygdalar LTP underlies fear memory impairments in strains A/J and BALB, and that hippocampal LTP does not always correlate with STM for contextual fear. Intact LTM for contextual fear correlates with intact hippocampal LTP in all strains examined, except for BALB. MPP LTP does not correlate with fear memory impairments in these strains. I conclude that contextual fear LTM correlates with hippocampal LTP, and cued fear LTM correlates with amygdalar LTP in some inbred mouse strains. My study has revealed valuable new data on the mnemonic and synaptic traits of specific mouse strains. These data establish some of these strains as murine models of amnesia that can be used in future studies to shed additional light on the cellular and molecular mechanisms of memory dysfunction.

B. Materials and Methods

i. Subjects

Female mice of five inbred mouse strains (C57BL/6NCrlBR, A/J, BALB/cByJ, C57BL/10J, and SM/J) were examined in this study. C57BL/6 mice were obtained from Charles River Canada (Saint-Constant, Quebec), and the other four strains were purchased from The Jackson Laboratory (Bar Harbor, ME), all at 5-7 weeks of age. Mice underwent behavioural testing between the ages of 8 and 10 weeks and then were killed for electrophysiological experimentation between the ages of 9 and 13 weeks.

ii. Cued and contextual fear conditioning and extinction

The fear conditioning methodology used here is described in *Chapter 2*. Either one hour or 24 hours after training (separate groups were used for each delay), mice

underwent a contextual fear memory test, and then one hour later, the same mice were tested in an altered context and then cued fear memory. The groups of mice tested for 24-hr contextual and cued fear memory were subsequently assessed for contextual fear extinction. Mice received two extinction trials per day, starting 24 hours after the contextual fear memory test. Extinction trials were conducted for 3 days, and on each day, the trials were 30 min apart. For each trial, mice were placed in the conditioning chamber for 3 min.

An unbiased human observer quantified freezing behaviour throughout all training and testing. The percentage of time spent frozen during each interval was calculated for each mouse, and these data were averaged for each interval for all mice within each strain.

iii. Electrophysiology: extracellular field recordings

Synaptic physiology was assessed a minimum of one week after fear conditioning in the same mice. Extracellular fEPSPs were recorded from hippocampal and amygdalar slices. Input-output (I/O) data were collected by varying stimulus intensity over five stimuli applied to the Schaeffer collateral pathway. Paired-pulse facilitation (PPF) was examined in the Schaeffer collateral pathway by applying two pulses at 50, 75, and 100ms inter-pulse intervals. Various stimulation protocols were used to induce amygdalar (lateral to basolateral pathway) and hippocampal (Schaeffer collateral and medial perforant pathways) LTP; these are described at appropriate parts of *Section C.*

iv. Data analysis

I assessed LTP, the I/O relation between presynaptic fibre volley amplitude and fEPSP slope, and PPF in hippocampal slices, and LTP in amygdalar slices, from the five

strains. For LTP experiments, fEPSP slopes were averaged within each strain and the resulting means were compared across strains (Nguyen et al., 2000b). For I/O analysis, hippocampal CA1 presynaptic fibre volley amplitudes and fEPSP slopes were plotted and a linear regression through origin was calculated for each strain. A one-way analysis of covariance (ANCOVA) and Dunnett post-hoc tests (with B6 as the control value) were used to assess statistical significance between linear regression slopes. For PPF, data were analysed as described in *Chapter 2*. To determine which strains exhibited significant differences from strain B6 in PPF and after LTP induction, I used ANOVAs followed by Dunnett post-hoc tests in which B6 served as the control group (Graphpad Instat software, San Diego, CA, USA). Student's t-test was used for within-strain comparisons of MPP PPD before and after LTP induction.

ANOVAs and Dunnett post-hoc tests were used to assess strain differences in the behavioural tests. Freezing values for cued and contextual memory tests, and extinction, were adjusted using the freezing values for the altered context test. For each strain, the average altered context freezing value was subtracted from cued and contextual memory freezing values, and from extinction values. For contextual fear extinction trials, freezing levels were normalized to those obtained for the 24-hr contextual fear memory test for each strain, and Student's t-test was used to compare freezing levels between trial 1 and trial 6 for each strain.

C. Results

i. Inbred strains express selective amygdala- and hippocampus-dependent memory deficits

Contextual and cued fear conditioning are dependent on different brain regions. Contextual fear memory depends on hippocampal function, but the amygdala has a role

as well (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Kim et al., 1993; Chen et al., 1996). Cued fear conditioning relies primarily on amygdalar function (Fanselow and Kim, 1994). Thus, by examining both contextual and cued fear conditioning in the same animals, we can look for potential deficits in amygdalar and hippocampal functions. Furthermore, short-term memory (STM; 1 hr retention) and long-term memory (LTM; 24hr retention) depend on different physiological processes (Izquierdo and McGaugh, 2000; Izquierdo et al., 2002). STM relies on the covalent modification of existing proteins, whereas LTM relies on long-term changes in synaptic structure and requires new protein synthesis (Bailey et al., 1996; Kandel, 2001). By examining both STM and LTM we may gain insight into what types of cellular processes are altered if deficits are found.

The average freezing values for the pre-CS and post-US intervals during training (for both the 1hr and 24hr retention groups combined) are shown in **Figure 5.1a**. An ANOVA conducted on pre-CS freezing levels for the five strains yielded a significant difference: $F(4,77) = 38.701$, $p < 0.0001$. Post-hoc testing revealed that strain A/J exhibited significantly more pre-CS freezing in the conditioning apparatus than strain B6 ($p < 0.01$). Post-US freezing levels also differed between strains: $KW = 14.049$, $p < 0.01$. Strain BALB froze significantly less than B6 after the CS-US pairing ($p < 0.01$).

Figure 5.1b shows data collected from the five strains one hour after training. An ANOVA of freezing during the altered context test showed that there were strain differences: $KW = 27.232$, $p < 0.0001$. Thus, the freezing values for the altered context test were subtracted from those for the contextual and cued memory tests to control for alterations in freezing that result from handling and training procedures prior to memory

testing. These corrected freezing levels are shown in **Figure 5.1b**. There were significant strain differences in freezing during the 1-hr contextual fear memory test: $F(4,35) = 4.394$, $p < 0.01$. Post-hoc tests showed that strains BALB ($p < 0.01$), SM ($p < 0.05$), and B10 ($p < 0.01$) froze significantly less than strain B6 during the contextual memory test. The strains also differed in freezing levels during the 1-hr cued fear memory test: $F(4,35) = 12.142$, $p < 0.0001$. All four strains (A/J, BALB, SM, and B10; all $p < 0.01$) froze less than strain B6 when the CS was played.

Figure 5.1c shows data from fear memory tests conducted 24hr after training on a separate group of mice. Similar to the results at 1hr after training, the strains exhibited different levels of freezing during the altered context test: $KW = 22.025$, $p < 0.001$. Thus, altered context freezing levels were subtracted from the 24-hr contextual and cued fear memory test freezing values. An ANOVA on the corrected contextual memory data was not significant: $F(4,37) = 2.133$, $p > 0.05$. Although the strains did not show differences in expression of contextual memory, an ANOVA revealed that there were strain differences in freezing during the cued memory test: $F(4,37) = 7.236$, $p < 0.001$. Post-hoc testing showed that strains A/J, BALB, and SM froze significantly less than B6 (all $p < 0.01$) when the CS was played.

Fear extinction is a new form of learning, not merely an erasure of previously learned associations (Pavlov, 1927; reviewed by Bouton, 1993). Furthermore, extinction may rely on the same brain regions that are involved in learning the initial association (Berman and Dudai, 2001). Following 24hr memory testing, the same group of mice was tested for contextual fear extinction. The values reported in **Figure 5.1d** have been corrected by subtraction of the 24-hr altered context freezing levels, and have been

normalized to the corrected freezing values for the 24-hr contextual memory test. In order to determine whether each strain exhibited extinction of contextual fear memory over six trials, Student's t-test was used to compare freezing during trial 1 vs. trial 6 in each strain. B6 ($t(18) = 4.140$, $p < 0.001$), A/J ($t(7) = 2.832$, $p < 0.05$), and B10 ($t(7) = 2.604$, $p < 0.05$, Welch correction applied) froze significantly less during trial 6 than during trial 1, indicating that expression of fear memory in these strains was extinguished. Strains BALB ($t(14) = 0.128$, $p = 0.9$) and SM ($t(7)= 2.342$, $p = 0.052$) did not exhibit significant differences in freezing levels between trial 1 and trial 6. Strain SM exhibited less and less freezing as it progressed through the extinction trials, but BALB exhibited varying levels of freezing throughout with no trend towards extinguished fear. Linear regression analysis was carried out on the corrected freezing values for each strain throughout the six extinction trials. Extinction in strains B6, B10, and SM yielded a linear regression slope that was significantly different from zero: B6, $p < 0.01$; B10, $p < 0.001$; SM, $p < 0.01$. Extinction in strain A/J nearly yielded a significant slope ($p = 0.0597$), whereas that in strain BALB was not significantly different from zero ($p > 0.9$). Thus, we can conclude that contextual fear extinction was successful in strains B6 and B10, and most likely successful in strains A/J and SM. Strain BALB did not express contextual extinction, and exhibited a learning deficit on this task.

These behavioural data indicate that inbred strains can vary in expression of memory depending on the retention interval tested. Although strains BALB, SM, and B10 exhibited reduced freezing during the 1-hr contextual memory test, they did not significantly differ from B6 during the 24-hr contextual memory test. Thus, contextual LTM is intact in all of the inbred strains tested here. However, more widespread cued

memory deficits were found in these inbred strains. Strains A/J, BALB, and SM exhibited impaired cued fear memory during both the 1- and 24-hr tests, and B10 was impaired for the 1-hr cued test. These results indicate that it is more likely that the inbred strains tested here have deficits in amygdalar function rather than hippocampal function, although the 1hr contextual memory deficits and contextual extinction deficits indicate that hippocampal function may be mildly altered in some of these strains.

ii. Amygdalar LTP is impaired in strains A/J and BALB one hour after induction

To test whether amygdalar synaptic plasticity impairments are correlated with amygdala-dependent cued memory deficits in inbred strains A/J, BALB, B10, and SM, I induced LTP in the basolateral amygdala of coronal brain slices by electrically stimulating the lateral amygdala. LTP was elicited by applying five trains of 100-Hz electrical stimulation (train duration of 1s) at a 10-s inter-train interval. LTP data for the five inbred strains are shown in **Figure 5.2**. An ANOVA conducted on the level of potentiation 60min after LTP induction was significant: $F(4,65) = 3.182$, $p < 0.05$. Post-hoc testing showed that LTP in strains A/J ($p < 0.05$) and BALB ($p < 0.01$) was significantly lower than that in BL6 at 60min post-tetanization. Amygdalar LTP in strains B10 and SM was not significantly different from that in B6 60min after induction. Thus, amygdalar LTP deficits in A/J and BALB correlate with impaired cued fear memory at 1 and 24hrs after training.

iii. Hippocampal LTP is intact in all five strains, except for 4 train L-LTP in BALB

LTP that relies on different cellular and molecular mechanisms can be elicited using distinct patterns of electrical stimulation. For instance, in hippocampal slices, one train of high-frequency stimulation (e.g., 100-Hz for 1s duration) results in early-LTP (E-

LTP). E-LTP decays to baseline within 1-2 hours of induction, and requires activation of protein kinases (e.g., protein kinase C and Ca2+/calmodulin-dependent protein kinase II: Malinow et al., 1989; Silva et al., 1992) and NMDA receptors (Collingridge et al., 1983). However, E-LTP does not require activation of protein kinase A (PKA) or protein synthesis (Huang and Kandel, 1994; Huang et al., 1996). If three or more trains of 100-Hz stimulation are applied, late-LTP (L-LTP) can be induced (Huang et al., 1996). L-LTP can last for several hours in vitro (Andersen et al., 1977; Huang and Kandel, 1994), and its maintenance requires protein synthesis, as well as the activation of PKA and CRE-mediated gene expression (Stanton and Sarvey, 1984; Deadwyler et al., 1987; Frey et al., 1988; Frey et al., 1993; Matthies and Reymann, 1993; Bourtchouladze et al., 1994; Huang and Kandel, 1994; Nguyen et al., 1994; Impey et al., 1996; Abel et al., 1997; Matsushita et al., 2001). If E-LTP or L-LTP deficits are found in particular inbred strains, future studies may then probe for impairments of signalling pathways and other molecular mechanisms known to underlie normal expression of E-LTP and L-LTP.

Particular types of learning and memory in rodents (Tsien et al., 1996) and in humans (Zola-Morgan et al., 1986) depend on area CA1 of the hippocampus. Therefore, altered synaptic plasticity in area CA1 could influence memory function in the inbred strains. To test this hypothesis, I induced E-LTP in the Schaeffer collateral-commissural pathway using one train of 100-Hz stimulation (1s duration), and L-LTP using four trains of 100-Hz stimulation (1s duration, 5min inter-train interval). In addition, I examined LTP induced by theta-burst stimulation (TBS). TBS resembles hippocampal theta rhythm (5-10 Hz) and is a more physiological induction protocol than longer trains of 100-Hz, because TBS mimics spike discharge patterns in the hippocampus of rodents

during exploration (Larson et al., 1986; Otto et al., 1991; Capocchi et al., 1992). TBS consisted of 15 bursts of 4 pulses at 100-Hz, with an inter-burst interval of 200ms.

I tested these three types of hippocampal LTP in the five inbred strains. E-LTP elicited by one train of HFS yielded comparable potentiation between all five strains (**Figure 5.3a,d,g,j**). An ANOVA showed that there were no strain differences in the level of potentiation 60min after tetanic stimulation: KW = 4.423, p = 0.352. TBS stimulation also resulted in intact LTP in all five strains (**Figure 5.3b,e,h,k**). One hour after LTP induction, potentiation was comparable between strains: KW = 1.422, p = 0.840. However, an ANOVA determined that there were strain differences in potentiation 120min after induction of L-LTP by four trains of HFS: $F(4,28) = 2.804$, $p < 0.05$ (**Figure 5.3c,f,i,l**). Post-hoc testing revealed that the level of potentiation in hippocampal slices from strain BALB was significantly less than in strain B6 ($p < 0.05$). Thus, several types of hippocampal CA1 LTP are intact in strains B6, A/J, B10, and SM, whereas strain BALB exhibits a selective deficit of L-LTP.

iv. Postsynaptic excitability in hippocampal area CA1 is intact in the strains, and elevated in A/J

Postsynaptic excitability can be gauged by measuring the relationship between presynaptic fibre volley amplitude and postsynaptic fEPSP slope. The presynaptic fibre volley amplitude reflects the number of axons recruited by electrical stimulation, and the postsynaptic fEPSP slope reveals synaptic strength (Johnston and Wu, 1995). I investigated the basal input/output (I/O) relationship in the five inbred strains by varying the stimulus intensity while recording fEPSPs in area CA1 of hippocampal slices. The resulting presynaptic and postsynaptic measurements were plotted and a linear regression

analysis (forced through origin) was done to determine the relationship between presynaptic activation and postsynaptic strength. The resulting plots, regression coefficients (slopes), and coefficients of determination (R^2) are shown in **Figure 5.4.** An analysis of covariance (ANCOVA) was conducted on the linear regression data for the five strains and it was significant: $F(4,425) = 7.143$, $p < 0.001$. Post-hoc testing showed that, in relation to B6, no strains had a reduced slope, but A/J exhibited an increased slope (**Figure 5.4f**, $p < 0.01$). Thus, CA1 pyramidal cells in strain A/J are more excitable than those in B6. To determine whether this resulted from altered presynaptic or postsynaptic function, I examined paired-pulse facilitation in the Schaeffer collateral-commissural pathway.

v. Paired-pulse facilitation is comparable between inbred strains

Paired-pulse facilitation (PPF) is an enhancement of synaptic transmission during the second of two closely-spaced stimuli. PPF results from residual calcium left over in presynaptic terminals following a first stimulus that precedes a closely-spaced second stimulus. This residual calcium adds to the presynaptic calcium influx from the second stimulus, thereby enhancing transmitter release (Katz and Miledi, 1968). Indirectly, PPF measures the ability of the presynaptic terminal to increase transmitter release in response to activity. Reduced PPF might indicate that presynaptic transmitter release is enhanced (this is because more transmitter is released in response to the first stimulus, leaving less available to be released by the second stimulus). Elevated transmitter release could result in a steeper I/O slope.

I measured PPF in the five inbred strains at three inter-pulse intervals: 50, 75, and 100ms **(Figure 5.5).** ANOVA analysis revealed no significant differences between

strains at 50ms: $F(4,68) = 1.511$, $p = 0.209$; 75ms: $F(4,68) = 1.948$, $p = 0.1124$; or 100ms: $F(4,68) = 1.195$, $p = 0.321$. Thus, it is likely that presynaptic transmitter release is comparable between strains, and the I/O enhancement in strain A/J does not result from altered presynaptic transmitter release.

vi. Synaptic plasticity in the medial perforant pathway is intact in the inbred strains

The medial perforant pathway (MPP) is a major input to the hippocampus that provides transmission of information from the entorhinal cortex. The MPP terminates in the dentate gyrus, and synapses onto dentate granule cells. Thus, the MPP has an integral role in providing input to the excitatory trisynaptic pathway of the hippocampus. LTP at this MPP-dentate gyrus synapse shares many properties with Schaeffer collateral-commissural pathway LTP, such as NMDA-receptor dependence and L-LTP that is protein synthesis-dependent (Hanse and Gustafsson, 1992; Colino and Malenka, 1993; Nguyen and Kandel, 1996; Wu et al., 2001). Because the MPP is important for hippocampal information processing, altered synaptic plasticity in this pathway might impair behavioural performance in tasks that rely on hippocampal processing. Thus, I examined MPP LTP in the five inbred strains.

In order to verify that I was recording from the MPP and not from the lateral perforant pathway, I examined paired-pulse depression (PPD) prior to LTP experiments (McNaughton, 1980). Paired pulses were applied to the MPP at a 50-ms inter-pulse interval. Only those responses that exhibited PPD were included in the analyses. **Figure 5.6a** shows average PPD values for each strain, prior to and 10min after LTP-inducing stimuli were applied. All five strains exhibited comparable levels of PPD prior to ($F(4,35) = 0.645$, $p = 0.634$) and after ($F(4, 34) = 2.280$, $p = 0.081$) the induction of LTP.

All strains showed a trend towards increased PPD after LTP induction, but only strains A/J ($t(13) = 3.741$, $p < 0.01$) and SM ($t(16) = 2.445$, $p < 0.01$) exhibited a significant increase in depression 10 min after LTP induction compared with baseline. Sample traces that illustrate PPD during baseline for each strain are shown in **Figure 5.6b**. I was able to induce robust LTP in the MPP using four trains (500ms duration) of 100-Hz stimulation at a 20-s inter-train interval. During tetanic stimulation I increased the pulse width to 0.1ms, and stimulus intensity was adjusted to 1.5x of test intensity. All five strains exhibited comparable LTP in the MPP 60min after induction (**Figure 5.6c-f**). ANOVA revealed no significant differences between strains: $F(4,37) = 0.3617$, $p = 0.834$. Thus, LTP is intact in the MPP of these inbred strains.

vii. Summary

Overall, my results show that several of the inbred strains tested here have multiple forms of memory impairment. Strains BALB, B10, and SM have impaired contextual and cued STM, and defective cued LTM. Thus, these three strains may have deficits in hippocampal and amygdalar functions. However, because contextual fear LTM was intact in these strains, it is likely that the functional impairments are primarily amygdalar. Also, I suggest that hippocampal LTP in area CA1 reflects hippocampus-dependent LTM processes more strongly than STM processes. Strain A/J exhibited intact contextual fear memory at both retention intervals, but impaired cued fear memory at both retention intervals. As such, strain A/J likely has impaired amygdalar, but intact hippocampal, memory function. Upon examination of amygdalar and hippocampal LTP, I found that strains A/J and BALB also have altered synaptic plasticity in the amygdala. Additionally, BALB exhibits impaired hippocampal L-LTP. No strains exhibited

impairments of LTP in the MPP. Thus, I have identified specific memory deficits in select inbred strains that, in some cases, correlate with impaired synaptic plasticity in brain regions that underlie these types of memory.

D. Discussion

The key findings of my study are that amygdalar LTP deficits correlate with impaired cued fear memory in selected inbred mouse strains, hippocampal area CA1 LTP correlates more strongly with LTM than STM, and MPP LTP is intact, even in strains that have impaired amygdalar LTP and defective fear memory (see **Table 5.1** for summary). I identified one inbred strain, A/J, that exhibits deficits in amygdalar, but not hippocampal, memory and defective synaptic plasticity. I suggest that some of the inbred strains studied here are not suitable for use as background strains for genetic modification aimed at cognitive and electrophysiological study of hippocampal and/or amygdalar function. Strain BALB/cByJ exhibited impairments of both hippocampal and amygdalar function and should not be used as a background strain if the aim is to determine whether a mutation impairs hippocampal or amygdalar function. Strain A/J also exhibited amygdalar impairments (LTP and cued fear memory), as did strains B10 and SM/J (cued fear memory). Thus, caution is needed in considering these strains for use in generating mutants for cognitive study. However, these four strains may be suitable genetic backgrounds if the aim is to detect improvements in hippocampal or amygdalar function following genetic manipulation.

Our STM and LTM data indicate that several strains exhibit multiple fear memory impairments. Ours is the first report of fear conditioning and electrophysiological data for strain SM, and A/J, BALB, and B10 have not previously undergone such conjoint

experimentation. My results indicate greater impairment in strains A/J, BALB, and B10 than previous studies have indicated (reviewed by Schimanski and Nguyen, 2004). A/J has previously been reported to exhibit high levels of contextual, and intermediate levels of cued, memory at 24hr post-training (Owen et al., 1997; Bolivar et al., 2001; Balogh and Wehner, 2003), in contrast to my data that indicate cued memory is substantially impaired in this strain. BALB previously displayed intermediate freezing levels for contextual, and high freezing levels for cued, fear memory tests at 24hr post-training (Owen et al., 1997; Bolivar et al., 2001; Balogh and Wehner, 2003). My data agree that BALB probably has intermediate contextual fear memory (although BALB was not significantly different from B6 on this measure in this study, BALB expressed considerably less freezing than B6), but indicate that BALB has impaired cued fear memory. Finally, my data agree with a previous study that showed that B10 exhibits a high capacity for contextual and cued fear memory at 24hr post-training (Owen et al., 1997).

One key methodological difference may explain why in my study strains A/J and BALB showed greater impairments of fear memory than in previous studies. All other studies utilized multiple pairings of CS and US during training, whereas I used only one pairing of CS and US. Thus, it may be that I conducted a more sensitive test of fear memory by using a less intensive training protocol. As such, my study may have unmasked subtle deficits in fear memory that were difficult to reveal with the stronger training used in previous studies.

Another factor that must be considered when testing inbred mouse strains is whether their sensory systems are intact. Several strains have mutations that affect

hearing, and others have impaired vision. For example, strains A/J (Owen et al., 1997) and BALB (Upchurch and Wehner, 1988) perform poorly on the visible platform task of the Morris water maze, a test that requires good visual acuity. However, good vision is not necessary for the behavioural tests that I conducted here. A different inbred mouse strain, C3H/HeJ, carries the retinal degeneration (*rd*) mutation that impairs vision (The Jackson Laboratory, 2002). However, experiments on a congenic strain, C3.BliA-$+^{Pde6b}$, showed that the *rd* mutation does not result in fear conditioning deficits in strain C3H (Bolivar et al., 2001). This congenic strain exhibited contextual fear memory comparable to C3H, but does not undergo retinal degeneration. Furthermore, Adams et al. (2002) suggest that visually-impaired mice are capable of learning a context-US association using non-visual cues (e.g., olfactory, auditory, or tactile cues). Thus, the memory impairments identified in A/J and BALB are probably not caused by visual deficits. In addition, strains A/J, BALB, and B6 carry the *ahl* hearing loss mutation (The Jackson Laboratory, 2004). This mutation results in progressive hearing loss beginning at 10 months of age in BALB and B6, and at 3-5 months of age in A/J. My mice were all less than 2 months of age when tested, so this mutation should not have affected my results. Also, it is likely that strains BALB and A/J heard the tone, as both strains expressed freezing to a 90-dB tone in another study of fear conditioning memory (Bolivar et al., 2001).

Sensitivity to footshock in the inbred strains should also be considered in interpreting fear conditioning data. Because all of the strains I tested exhibited at least one form of intact fear memory, I am confident that all strains sensed footshock. To further demonstrate that 0.7mA is of adequate intensity, I considered footshock

intensities used by other groups to elicit fear conditioning in these inbred strains. Owen et al. (1997) used a 0.35-mA footshock to train B6, A/J, BALB, and B10. All four strains exhibited evidence of fear memory in their study. Bolivar et al. (2001) used a 0.5-mA footshock to train B6, A/J, and BALB, all of which expressed fear responses. Because 0.35mA and 0.5mA intensities were adequate to induce fear memory in the inbred strains in these two studies, I conclude that 0.7mA, used here, should also be adequate.

Interestingly, strain SM exhibited several types of fear memory impairment (contextual and cued memory at 1hr, and cued memory at 24hr after training), but no LTP impairments. A possible reason that LTP and memory do not correlate in this instance might be that the types of LTP examined here, although extensive, do not necessarily mimic the actual physiological processes that underlie memory in mice. The examination of LTP *in vitro*, using artificial stimulation protocols, can identify strains of mice, or brain regions within these mice, that poorly express forms of synaptic plasticity that are believed to mediate learning and memory. However, these methods do not directly examine whether particular mice express adequate plasticity, at particular synapses, for learning and memory. As such, we may not be able to detect subtle deficits, at the level of small groups of synapses, that underlie learning and memory impairments. Additionally, other brain regions that were not examined for synaptic plasticity contribute to the encoding and expression of fear memory. I cannot rule out the possibility that dysfunction in other brain regions may be responsible for the memory deficits observed in strain SM.

In two inbred strains, A/J and BALB, I found that reduced amygdalar LTP correlated with impaired cued fear memory at both 1 and 24hrs after training. Thus, if

mechanisms of synaptic plasticity are altered in the amygdala, the ability to express amygdala-dependent memory should be compromised. However, these data might be more informative if we consider the amygdalar circuitry that underlies cued fear conditioning, and the specific pathway recorded in my study. I recorded LTP within the basolateral complex (BLA) of the amygdala by stimulating the lateral amygdaloid (LA) nucleus and recording from the basolateral amygdaloid (B) nucleus. It has been postulated that during fear conditioning, the BLA is a site for both formation, and storage, of CS-US associations (Maren, 2001). Information about the CS, and likely the US, can arrive in the LA via several different pathways (LeDoux, 2000). Subsequently, the LA projects directly to the central (CE) amygdaloid nuclei, and also indirectly via B (LeDoux, 2000). The CE nuclei are responsible for the primary output of the amygdala, and thus have a role in effecting the expression of fear responses (LeDoux, 2000). Thus, the projection from the LA to the B nuclei may be one site of memory storage within the amygdaloid complex. Alternately, information from the hippocampus concerning the context arrives at the B nucleus, which in turn projects to the CE nuclei for output (LeDoux, 2000). The circuitry indicates that LTP in the LA to B pathway is more likely to contribute to cued, than to contextual, fear memory.

Several strains displayed different performance for contextual STM versus LTM. These included strains BALB, B10, and SM, which exhibited contextual STM impairments, but intact LTM. Interestingly, my results indicate that LTP in area CA1 of the hippocampus correlates more strongly with contextual LTM than with STM. Strains BALB, B10, and SM exhibited nearly intact LTP in CA1 (except for 4-train LTP in BALB). Strains B6 and A/J both had intact contextual STM and LTM, and intact

hippocampal LTP. Thus, in all strains except BALB, hippocampal LTP correlates with hippocampus-dependent LTM. Schimanski and Nguyen (2005) also found that hippocampal LTP correlated with hippocampus-dependent LTM in three additional inbred mouse strains. I also note that, of the five strains tested here, BALB exhibited the lowest level of freezing during the contextual LTM test, along with a hippocampal SC LTP deficit. Thus, I suggest that hippocampal LTP in area CA1 correlates reasonably well with hippocampus-dependent memory in inbred mouse strains. LTP in the MPP was also intact in all strains studied here, which correlates with intact contextual LTM, but even in inbred strains that have impaired contextual LTM I have not discovered MPP LTP impairments (Schimanski and Nguyen, 2005). My current data reinforce the notion that MPP LTP does not correlate with contextual fear memory. I do not rule out the possibility that MPP LTP might still contribute to medial temporal lobe information processing that can influence behavioural expression of fear memory.

In strain BALB, I found both hippocampal CA1 and amygdalar LTP deficits. Hippocampal and amygdalar LTP share many mechanisms, and it is possible that similar genetic and molecular alterations in these two brain structures result in the LTP impairments identified in BALB. Molecular mechanisms common to amygdalar and hippocampal LTP include activation of voltage-gated calcium channels (VGCCs) and NMDA receptors, although amygdalar LTP may rely more on VGCCs than LTP in hippocampal area CA1 (reviewed by Chapman et al., 2003). PKA and extracellular-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) are required for L-LTP in both the hippocampus and the amygdala (reviewed by Schafe et al., 2001). Also, LTP maintenance in both structures likely involves the Ras/MAPK/CREB signaling pathway,

which may be involved in initiating protein synthesis (reviewed by Adams et al., 2000; Chapman et al., 2003). Any of these common molecular mechanisms may be altered in strain BALB. In contrast, strain A/J exhibited amygdalar, but not hippocampal, LTP impairments, indicating that genetic variations that affect the amygdala, but not the hippocampus, may cause reduced amygdalar LTP in this strain. These might include altered VGCC expression or subtle changes in various signalling pathways.

I have considered amygdalar LTP and hippocampal LTP individually, and have correlated these types of synaptic plasticity to my behavioural results. However, the relationship between the hippocampus and the amygdala has become the focus of much study, and hippocampo-amygdalar interactions should be considered in the interpretation of my data. It has been suggested that amygdalar innervation of the hippocampus can modulate memories under emotional conditions (Richter-Levin, 2004). Contextual fear conditioning can be considered an emotional event, and as such, its memory storage in the hippocampus may be modulated by the amygdala (Seidenbecher et al., 2003).

The contextual fear conditioning paradigm employed here may be subject to amygdalar modulation. Contextual fear conditioning relies on both amygdalar and hippocampal function, so it is likely that both brain structures were activated during training. Amygdalar activity would have been induced by inputs to the LA that convey information about the CS and the US. In fact, it has been shown that the amygdala contributes to contextual fear conditioning, and this emotional modulation might be one contribution of the amygdalar circuitry studied here. Furthermore, alterations of synaptic transmission within the amygdala might influence the effectiveness of amygdalar modulation of other brain structures and memory systems (Richter-Levin, 2004). It has

been shown that synaptic transmission in the amygdala is altered by cued fear conditioning (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997). Thus, we must consider the possibility that the training paradigm used may have introduced a sort of 'metaplasticity' of amygdalar modulation of hippocampal synaptic plasticity, learning, and memory.

The differential expression of contextual STM and LTM in BALB, B10, and SM supports the view that STM and LTM rely on different processes, although many molecular mechanisms have been shown to contribute to hippocampus-dependent STM and LTM (Izquierdo et al., 1999). Pharmacological manipulation during various stages of an inhibitory avoidance task, a type of conditioned fear, reveals that several molecules are required for intact STM and LTM in the rat hippocampus. These include the cAMP-dependent protein kinase (PKA) signalling pathway (incl. PKA, adenylyl cyclase, D1/D5 receptors), glutamate receptors, $GABA_A$ receptors, muscarinic cholinergic receptors, protein kinases C and G, and MAP kinase (Izquierdo et al., 1999; Izquierdo and McGaugh, 2000). However, manipulations of several molecules can disrupt STM but leave LTM intact. Specifically, post-training infusions of a dopamine D_1 receptor agonist, a 5-HT_{1A} agonist, and a mitogen activated protein kinase kinase (MAPKK) inhibitor into hippocampal area CA1 all impaired STM, but not LTM, on the inhibitory avoidance task (Izquierdo et al., 1999). Additionally, PKA inhibitors selectively blocked STM when infused at specific post-training intervals (22, 45, and 90 min). I speculate that, in the inbred strains that exhibit selective contextual STM impairments, one or more of these molecules that can selectively influence STM may be altered in expression.

I also found that strain BALB did not show a significant level of extinction of contextual LTM after six re-exposures to the context. Strains SM and A/J exhibited trends towards extinction, with significant levels of extinction on either a Student's t-test evaluation of freezing during trial 1 versus trial 6 (A/J), or a linear regression slope (taking into account all six trials) that was significantly different from zero (SM), but not both. In contrast, BALB froze to varying degrees over the six extinction trials, with no particular trend. This impaired extinction in BALB can be considered a learning impairment because extinction is a form of new learning (Pavlov, 1927; Bouton, 1993). Interestingly, extinction relies on many of the same mechanisms that LTP and LTM do, such as NMDA receptors (Lee and Kim, 1998; Santini et al., 2001; Szapiro et al., 2003; but see Berman and Dudai, 2001), the PKA, CAMKII, and MAPK signalling cascades (Szapiro et al., 2003) and protein synthesis (Flood et al., 1977; Berman and Dudai, 2001; Vianna et al., 2001; but see Fischer et al., 2004). Additionally, contextual fear extinction is hippocampus-dependent (Wilson et al., 1995; Frohardt et al., 2000). Thus, impairment of hippocampal L-LTP observed in BALB is correlated with this strain's lack of expression of fear extinction.

Overall, my data indicate that synaptic plasticity correlates with memory expression in brain regions essential for specific forms of learning and memory. Hippocampus-dependent LTM correlates better with hippocampal synaptic plasticity than STM does. In addition, LTP in hippocampal area CA1, but not the MPP, correlates with contextual fear LTM. Although I considered the relationships between synaptic plasticity and cognitive function in the hippocampus and amygdala, it is likely that the behavioural tests carried out here in fact rely on many brain regions other than those that I examined

for synaptic plasticity. As such, altered plasticity in regions such as the prefrontal, entorhinal, and perirhinal cortices, and other limbic regions, could contribute to the memory impairments that I observed. Nonetheless, my results provide evidence that hippocampal and/or amygdalar function is compromised in several inbred strains. When choosing background strains for cognitive studies that use genetic manipulation, the results of prior cognitive and physiological tests conducted on these strains should be examined. My data indicate that using strains A/J, BALB, B10, and SM as background strains may confound and complicate the ability to detect effects of genetic manipulation on particular cognitive and physiological measures. Furthermore, A/J, BALB, and SM are valuable mouse models of amnesia that may be further studied to determine the cellular and molecular mechanisms of memory dysfunction.

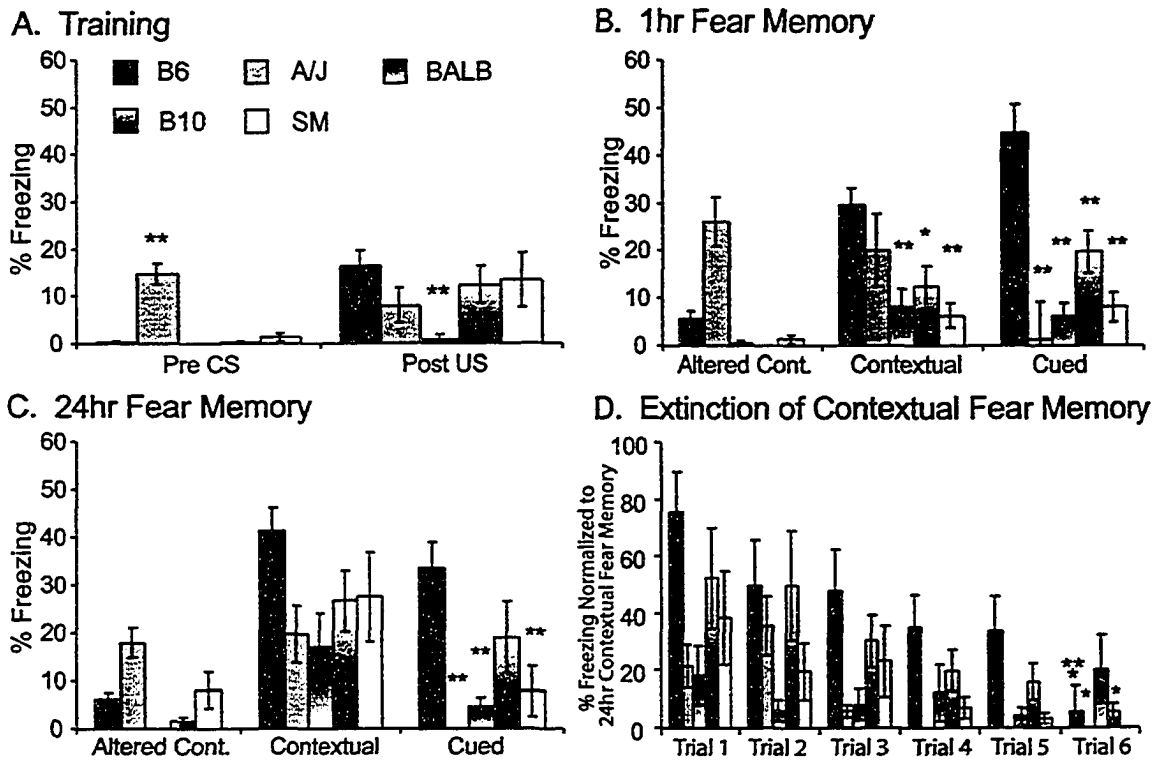


Figure 5.1: Several inbred mouse strains display impaired cued and contextual fear memory. A) Freezing during the 2-min period in the conditioning chamber prior to the CS-US pairing (*Pre CS*) and during the 30-sec period after the CS-US pairing (*Post US*). Strain A/J froze significantly more than strain B6 during Pre CS, and strain BALB froze significantly less than B6 during Post US: B6, $n = 20$; A/J, BALB, $n = 16$; B10, SM, $n = 15$. B) Freezing during the *contextual* and *cued* fear memory tests conducted 1hr after training, including during the 2min prior to CS application in the *altered context*. Because an ANOVA conducted on altered context freezing levels in the five strains was significant (see text), freezing values shown for contextual and cued memory are corrected (altered context values were subtracted from contextual and cued memory test values). Strains BALB, B10, and SM exhibited reduced contextual fear memory, and strains A/J, BALB, B10, and SM exhibited reduced cued fear memory 1hr after training: B6, $n = 10$; A/J, BALB, $n = 8$; B10, SM, $n = 7$. C) Freezing during the contextual and cued fear memory tests conducted 24hr after training. Because an ANOVA conducted on altered context freezing levels in the five strains was significant (see text), freezing values shown for contextual and cued memory are corrected (altered context values were subtracted from contextual and cued fear memory values). Contextual fear memory was intact in all five strains, and strains A/J, BALB, and SM exhibited reduced cued fear memory 24hr after training. B6, $n = 10$; A/J, BALB, B10, SM, $n = 8$. D) After the 24hr fear memory tests, mice underwent contextual fear extinction. The values shown are normalized to freezing levels for the 24hr contextual fear memory test. Strains B6, A/J, and B10 exhibited significantly less freezing during trial 6 compared with trial 1. B6, $n = 10$; A/J, BALB, B10, SM, $n = 8$. Asterisks indicate significant differences from B6 ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$). Bars indicate mean ± SEM.

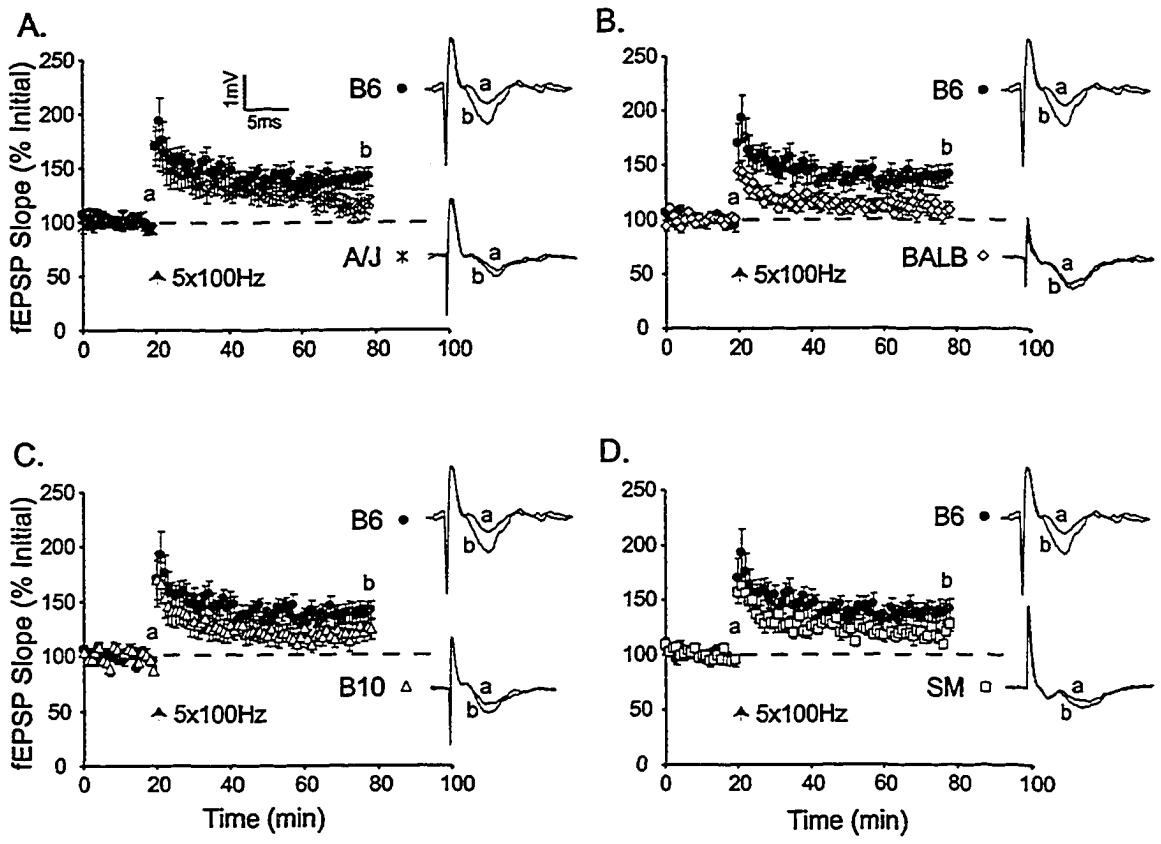
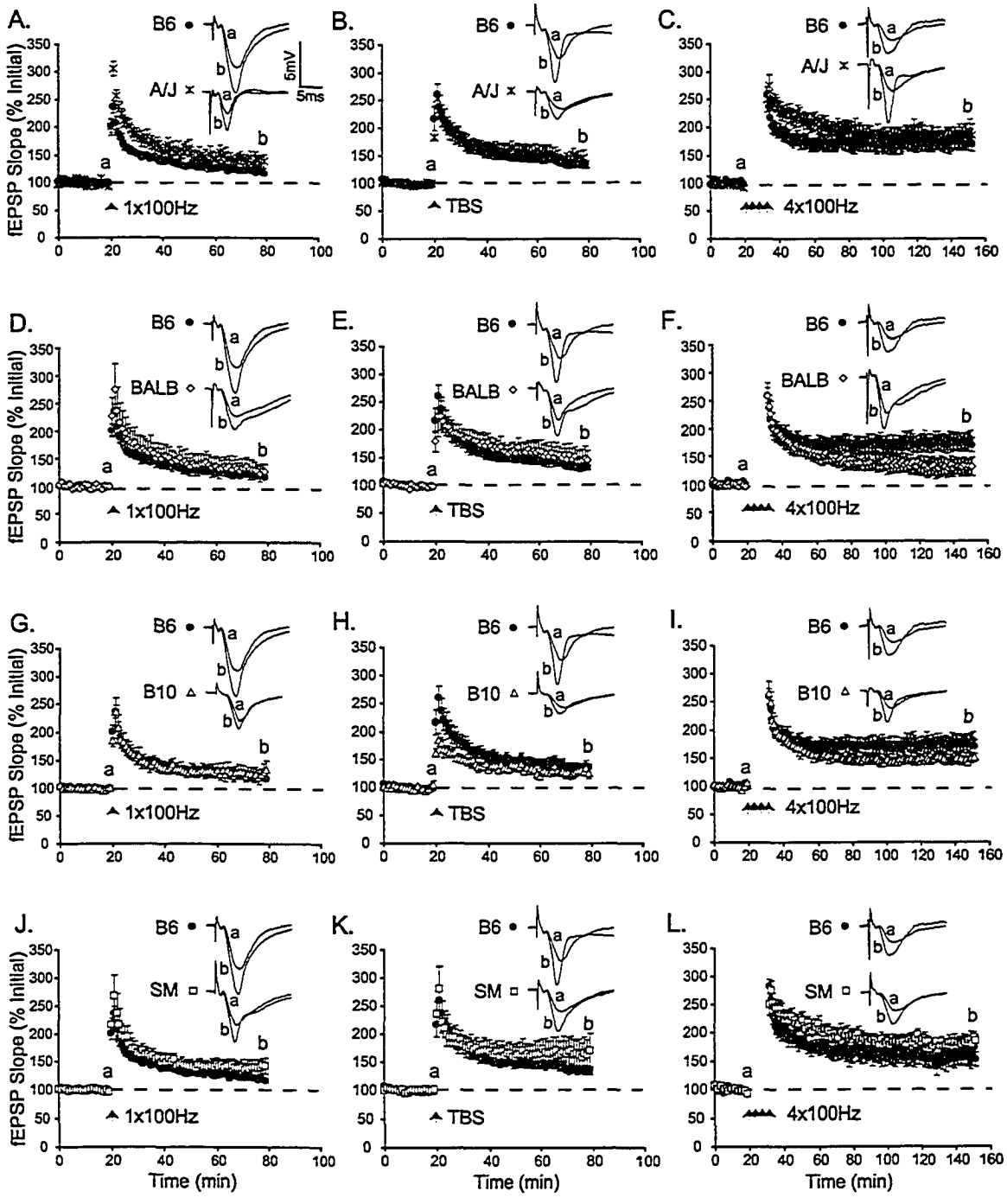


Figure 5.2: Amygdalar LTP is significantly reduced in strains A/J and BALB, both of which exhibit impaired cued fear memory. LTP was induced by applying 5 trains of 100Hz stimulation (1s duration) at a 10-s inter-train interval (*5x100Hz*). A,B) Strains A/J (n = 13) and BALB (n = 13) have significantly reduced amygdalar LTP 60min after tetanic stimulation, compared with B6 (n = 18). C,D) Amygdalar LTP is intact in strains B10 (n = 12) and SM (n = 14) 60min after tetanic stimulation. Sample traces for baseline values and for 60min after LTP induction are shown for each strain. Data points represent mean ± SEM.

Figure 5.3: Hippocampal SC LTP is intact in the five inbred strains, except for 4-train LTP in strain BALB. LTP was elicited in hippocampal area CA1 using three different stimulation protocols: one train of 100Hz with 1s duration (*1x100Hz*), theta-burst stimulation (*TBS*), and four trains of 100Hz with 1s duration and 5min inter-train interval (*4x100Hz*). A-C) Strain A/J exhibited intact SC LTP for all three induction protocols (*1x100Hz*, $n = 5$; *TBS*, $n = 7$; *4x100Hz*, $n = 5$) compared with B6 (*1x100Hz*, $n = 13$; *TBS*, $n = 8$; *4x100Hz*, $n = 8$). D-F) Compared with B6, strain BALB exhibited a selective reduction of 4-train LTP 120min after induction (*1x100Hz*, $n = 5$; *TBS*, $n = 7$; *4x100Hz*, $n = 8$). Strains B10 (G-I) and SM (J-L) did not exhibit any significant differences in LTP from B6 60min (*1x100Hz* and *TBS*) and 120min (*4x100Hz*) after tetanic stimulation (B10: *1x100Hz*, $n = 6$; *TBS*, $n = 8$; *4x100Hz*, $n = 6$; SM: *1x100Hz*, $n = 6$; *TBS*, $n = 6$; *4x100Hz*, $n = 6$). Sample traces were obtained during baseline and 60min (*1x100Hz* and *TBS*) or 120min (*4x100Hz*) after LTP induction. Data points represent mean ± SEM.



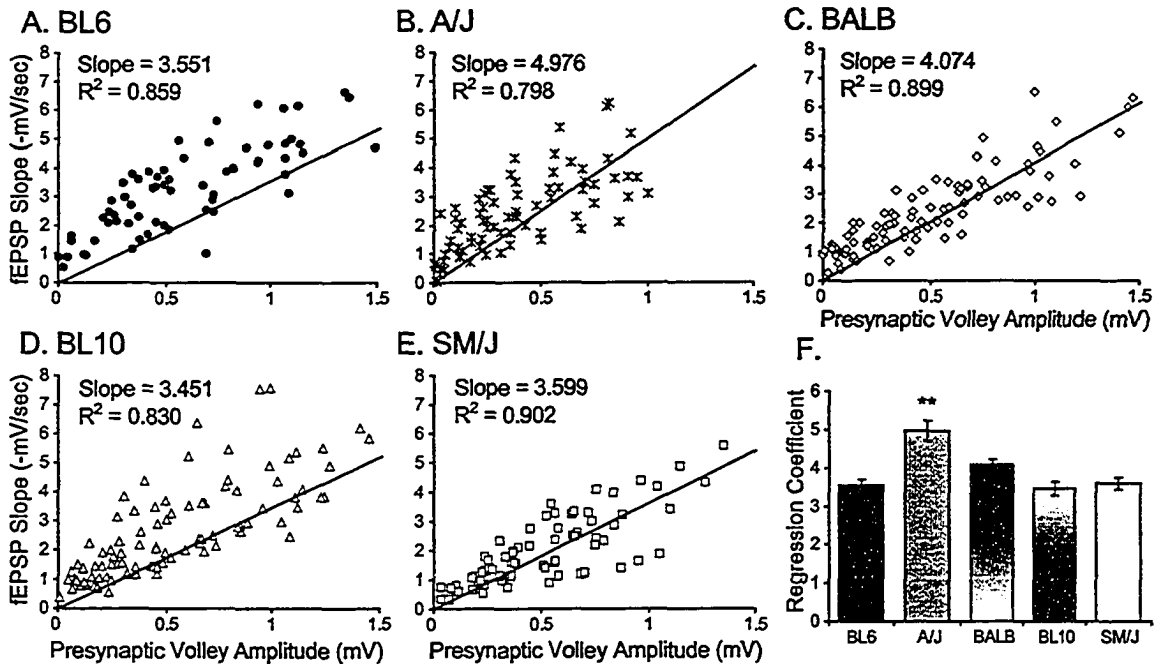


Figure 5.4: The input-output relation is intact in hippocampal area CA1 of all inbred strains, and is enhanced in strain A/J. The relation of the presynaptic fibre volley amplitude to the postsynaptic fEPSP slope was plotted for the five inbred strains (A-E). A linear regression analysis (forced through origin) was complete for each inbred strain, and the regression coefficients (*slope*) and coefficients of determination (R^2) are shown for each strain (B6, n = 80, 16 slices; A/J, n = 85, 17 slices; BALB, n = 90, 18 slices; B10, n = 100, 20 slices; SM, n = 80, 16 slices). F) Histogram of slopes ± 95% confidence intervals for the five strains. A/J exhibited an increased slope compared with strain B6 (**$p < 0.01$).

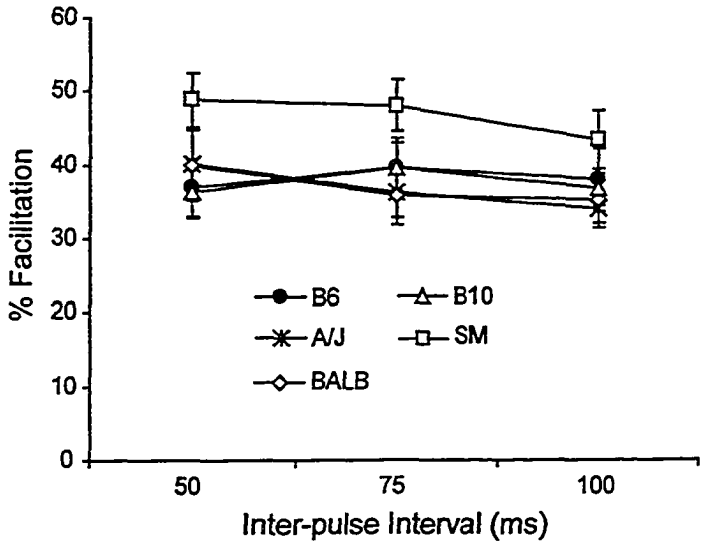


Figure 5.5: Paired-pulse facilitation is intact in hippocampal area CA1 of all five inbred strains. Paired pulses were delivered to the SC pathway at 50, 75, and 100ms inter-pulse intervals. The fEPSP slope of the second pulse was normalized to the first pulse, and reported as % facilitation. There were no significant differences between B6 and A/J, BALB, B10, or SM for the three inter-pulse intervals (B6, n = 14; A/J, n = 15; BALB, n = 14; B10, n = 15; SM, n = 15). Values shown are means ± SEM.

Figure 5.6: MPP LTP is intact in all five inbred strains. A) Selective stimulation of the MPP was verified by observing paired-pulse depression during baseline recordings (B6, n = 10; A/J, n = 8; BALB, n = 5; B10, n = 8; SM, n = 9). Paired-pulse depression was also observed 10min after LTP induction in the same pathway. There were no differences between strains for paired-pulse depression during baseline or during LTP, but strains A/J and SM exhibited significantly more depression after LTP induction than during baseline. B) Sample traces of paired fEPSPs recorded during baseline acquisition (50ms inter-pulse interval). Note paired-pulse depression of fEPSP slopes and sizes in the five strains. C-F) LTP in the MPP was induced using four trains of 100Hz stimulation, 500ms train duration, 20s inter-train interval, at 1.5X test intensity (*4x100Hz*). One hour after LTP induction, the average fEPSP slope for each strain did not differ significantly from that of B6 (B6, n = 10; A/J, n = 8; BALB, n = 7; B10, n = 8; SM, n = 9). Sample traces are shown during baseline and 60min after LTP induction. Asterisks indicate significant differences between paired-pulse depression values during baseline and potentiation within a strain (*$p < 0.05$, **$p < 0.01$). Data points represent mean ± SEM.

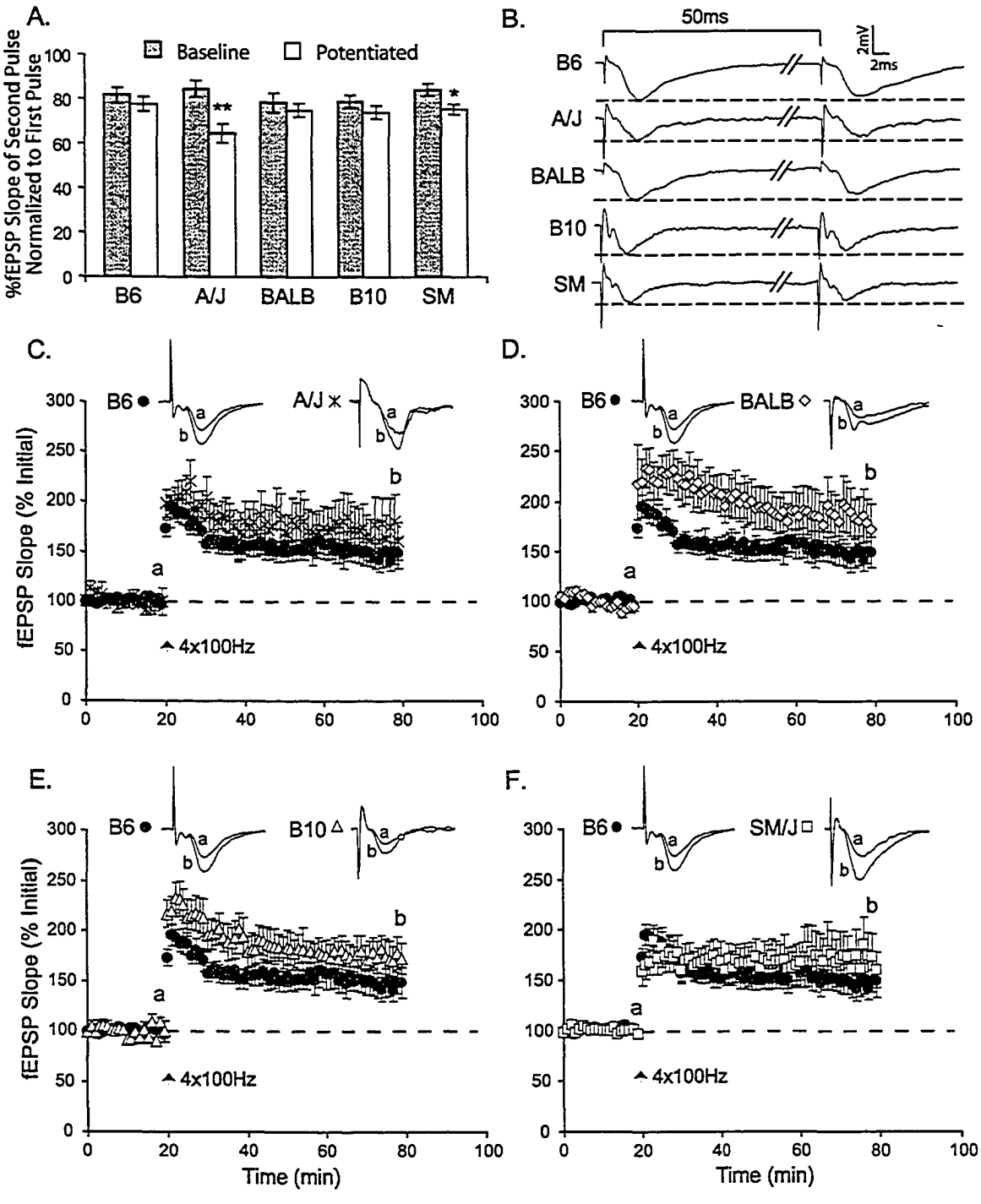


Table 5.1: Summary of Cued and Contextual Fear Memory, Contextual Fear Extinction, and Hippocampal and Amygdalar LTP in Five Inbred Mouse Strains

	Fear Conditioning			Hippocampal LTP		Amygdalar LTP
Strain	Contextual	Cued	Contextual Extinction	CA1	MPP	LA-B
B6	✓ STM ✓ LTM	✓ STM ✓ LTM	✓	✓	✓	✓
A/J	✓ STM ✓ LTM	↓ STM ↓ LTM	✓	✓	✓	↓
BALB	↓ STM ✓ LTM	↓ STM ↓ LTM	↓	✓ 1 train + TBS ↓ 4 train	✓	↓
B10	↓ STM ✓ LTM	↓ STM ✓ LTM	✓	✓	✓	✓
SM	↓ STM ✓ LTM	↓ STM ↓ LTM	✓	✓	✓	✓

✓, intact memory or LTP compared with strain B6; ↓, reduced memory or LTP compared with strain B6; STM, short-term memory (1hr post-training); LTM, long-term memory (24hr post-training); CA1, hippocampal area CA1; MPP, medial perforant pathway; LA, lateral amygdaloid nucleus; B, basolateral amygdaloid nucleus; TBS, theta-burst stimulation.

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CHAPTER 6:

Hippocampal LTP is Impaired in Inbred Mouse Strains with Reduced Brain Norepinephrine, but is Rescued by ß-adrenergic Activity

A. Introduction

The noradrenergic, serotonergic, and dopaminergic neuromodulatory systems can influence information processing in the forebrain (Buhot, 1997; Jay, 2003; McGaugh, 1989; McGaugh, 2000; McGaugh and Roozendaal, 2002; Meneses, 1999). For instance, activation of the β-adrenergic modulatory system can enhance memory, especially during increased emotional arousal (Cahill et al., 1994; McGaugh, 1989). Noradrenergic afferents extensively innervate the hippocampus (Loy et al., 1980; reviewed by Moore and Bloom, 1979). The activation of hippocampal β-adrenergic receptors is required for contextual fear and spatial memory (Ji et al., 2003a,b; Murchison et al., 2004), types of memory that require hippocampal information processing (reviewed by Eichenbaum and Cohen, 2001).

Activation of hippocampal β-adrenergic receptors can influence memory processing by enhancing hippocampal long-term potentiation (LTP). LTP is an activity-dependent increase in synaptic transmission (Bliss and Lømo, 1973; Lømo, 1966) that has been strongly correlated with some forms of hippocampus-dependent learning and memory (Barnes et al., 1994; Bourtchouladze et al., 1994; Doyere and Laroche, 1992; Morris et al., 1986). Interestingly, hippocampal β-adrenergic receptor activation can recruit signaling pathways that are important for LTP (Madison and Nicoll, 1986; Segal, 1982; Stanton and Sarvey, 1985). In hippocampal area CA1, application of isoproterenol (ISO), a β-adrenergic receptor agonist, does not elicit LTP on its own (Thomas et al., 1996). However, when ISO application is combined with sub-threshold stimulation for LTP induction, this stimulation becomes more effective in eliciting LTP (Gelinas and Nguyen, 2005; Katsuki et al., 1997; Thomas et al., 1996). Application of ISO during

high-frequency stimulation in hippocampal area CA3 can increase the magnitude of LTP (Hopkins and Johnston, 1984). Thus, β-adrenergic receptor activation within the hippocampus can lower the threshold for eliciting larger, longer-lasting increases in synaptic efficacy.

Neuromodulatory systems, and hippocampal β-adrenergic receptor activation, may vary across different genetic backgrounds. Using inbred mouse strains, which differ genetically from one another, we may examine the role of hippocampal β-adrenergic receptor activity in modulating synaptic plasticity in different genetic backgrounds. By definition, an inbred mouse strain is one that has been developed by mating siblings for 20 successive generations (Lyon and Searle, 1989). Within an inbred mouse strain, all mice are genetically identical, but between strains the genetic composition differs. There are several advantages of studying neurobiology in inbred mouse strains. Firstly, strains exhibit different phenotypes, and lend an opportunity for analysis of the neurophysiological and neurochemical mechanisms of complex traits. There is evidence that different strains express distinct genes and proteins in the CNS, or express the same genes and proteins at different levels within brain regions. For example, Zhao et al. (2001) found increased, lowered, or no expression of different genes in several subregions of the hippocampi of inbred strains, and protein kinase C expression differs in the hippocampi of strains DBA/2 and C57BL/6 (Bowers et al., 1995; Wehner et al., 1990). Also, there is evidence for differential expression of neurotransmitters and their receptors in the brains, or brain structures, of different strains (Gabellec et al., 1980; Marks et al., 1989; Sudak and Maas, 1964; Waller et al., 1983; Zilles et al., 2000). Secondly, inbred mouse strains provide an opportunity to test the general validity of a

finding across multiple genetic backgrounds. For example, application of a particular drug can promote memory in one strain but reduce it in another (Castellano et al., 1999).

Of particular interest are studies that have revealed that inbred strains vary in synaptic plasticity and memory phenotypes (reviewed by Schimanski and Nguyen, 2004). Many studies have examined hippocampus-dependent memory in inbred strains, and some strains have been identified as likely having impaired hippocampal memory function (e.g., CBA, DBA/2). Although fewer studies have focused on hippocampal synaptic plasticity, there is evidence that expression of hippocampal LTP varies across several different strains (Nguyen et al., 2000; also see review by Schimanski and Nguyen, 2004). The genetic differences between inbred mouse strains reflect natural genetic variation, and can be used to objectively identify the underlying cellular and molecular mechanisms of this variation.

It is unclear whether variations in the expression of noradrenergic neuromodulatory systems can significantly influence the expression of hippocampal LTP in inbred mice. To address this issue, a neurochemical analysis of the concentration of several monoaminergic transmitters and amino acids in the brains of eight different inbred mouse strains was carried out (data not shown). Concentrations of the key neuromodulators NE, dopamine, and serotonin were compared with long-term potentiation in these eight strains (data not shown), and a subset of four strains was chosen for further study. Strain B6 was chosen as the control for the study because it has been widely used to characterize LTP and its cellular and molecular mechanisms (see Schimanski and Nguyen, 2004). B6 generally exhibits intact learning, memory, and synaptic plasticity. Strains 129, BALB, and C3H were examined further because they

exhibited reduced concentrations of NE in the brain, and this correlated with an impairment of long-lasting hippocampal LTP. In these three mouse strains, I attempted to restore hippocampal LTP by activating β-adrenergic receptors with isoproterenol (a β-adrenergic receptor agonist) during LTP induction. The other four strains that were not further examined either did not exhibit deficient LTP, and thus posed no opportunity to test LTP rescue by application of isoproterenol, or exhibited reduced LTP but no reduction in brain norepinephrine. Preliminary experiments revealed that in strain B6, in which brain norephinephrine levels were intact, application of isoproterenol did not enhance the form of LTP studied here. As such, I chose to focus on inbred strains in which both LTP and brain norephinephrine levels were reduced. β_1-adrenergic receptor expression was also examined in hippocampi from the four mouse strains. The β_1 receptor subtype was studied because β_1-, but not β_2-, adrenergic receptors were found to be necessary for the enhancement of synaptic efficacy in hippocampal area CA1 in a study of mutant mice with different β-adrenergic receptor subtype deletions (Winder et al., 1999).

I found that reductions in brain NE correlate with reduced hippocampal LTP in these inbred strains, and that activation of β-adrenergic receptors can rescue hippocampal LTP in a variety of genetic backgrounds. Application of isoproterenol, a β-adrenergic receptor agonist, during LTP induction restored LTP maintenance in strains 129, BALB, and C3H to the levels seen in control strain B6. These four strains also displayed comparable expression of hippocampal β_1-adrenergic receptors (with the exception of strain BALB in which β_1-adrenergic receptor expression was enhanced). Thus, activation of hippocampal noradrenergic receptors can rescue hippocampal synaptic plasticity in

inbred mice with reduced brain NE. In light of the relationship between hippocampal LTP and memory, such enhancement of hippocampal LTP may lead to improved memory function across a variety of genetic backgrounds.

B. Methods

i. Subjects

I studied four inbred strains of mice: C57BL/6NCrlBR (B6), 129S1/SvImJ (129), BALB/cByJ (BALB), and C3H/HeJ (C3H). B6 mice were obtained from Charles River Canada (Saint-Constant, Quebec), and the other three strains were purchased from The Jackson Laboratory (Bar Harbor, ME) at 5-7 weeks of age (all female). Mice were killed for experiments at 8-13 wks of age.

ii. Tissue preparation

Brains from the same individual mice were used for both electrophysiological experiments and for either high-pressure liquid chromatography (HPLC) analysis or Western blot analysis. Brains were quickly removed, then placed into ice-cold artificial cerebrospinal fluid (ACSF). After a brief period of cooling (about 1 min), brains were dissected using the following procedure. First, brains were cut down the midline. From one brain hemisphere (randomized between right and left hemisphere) the hippocampus was dissected out and prepared for electrophysiological experiments. The other hemisphere was used either for HPLC analysis or Western blot analysis. To prepare tissue for HPLC analysis, the brain hemisphere was placed in a sealed cryogenic vial and quick-frozen in liquid nitrogen. Hemispheres were stored at -70°C until HPLC analysis. For Western blot analysis, hippocampi were dissected out of cooled fresh brains, and frozen and stored using the same protocol as for brain hemispheres.

iii. High-pressure liquid chromatography

The concentrations of biogenic amines and acid metabolites (norepinephrine, dopamine, serotonin, 3,4-dihydroxyphenylacetic acid, and 5-hydroxyindoleacetic acid) were measured using HPLC with electrochemical detection, as described by Baker et al. (1987). HPLC with fluorescence detection was used for analysis of the concentrations of amino acids (glutamate, γ-aminobutyric acid, and glycine); this procedure involves conversion of the amino acids to isoindole derivatives by reacting with o-phthaldialdehyde in the presence of mercaptoethanol (Parent et al., 2001).

iv. Electrophysiology: extracellular field recordings

Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded in stratum radiatum of area CA1. Tetanic stimulation of 4 trains of 100Hz (train duration 1s; inter-train interval 3s) was used to induce LTP. Isoproterenol (ISO: R(-)-isoproterenol(+)-bitartrate; Sigma, St. Louis, MO, USA), a ß-adrenergic receptor agonist, was prepared daily as a concentrated stock solution at 1mM in distilled water. It was then diluted to 1 μM in ACSF and bath applied. For experiments that utilized both ISO and HFS, ISO was applied for 10 min prior to tetanic stimulation, and for 5 min after. For control experiments, ISO was applied for 15 min with no tetanic stimulation. All experiments using ISO were performed under dimmed light conditions.

v. SDS PAGE and immunoblot

SDS PAGE and immunoblotting experiments were run blind. Tissues were weighed, diced into small pieces using a clean razor blade and thawed in ice-cold RIPA buffer containing protease inhibitors. Concentrations of the components (in mM) were: 50 Tris-HCl pH 7.4, 150 NaCl and 1 EDTA plus 1% NP40, 0.1% SDS, 2.5 mg/ml

sodium deoxycholate, 1 mM PMSF, 50 μg/ml leupeptin, 25 μg/ml aprotinin and 2 μg/ml pepstatin A. Tissues were homogenized in a dounce tissue grinder with 10 strokes of the loose pestle and 12 strokes of the tight pestle, and kept on ice for 30 min. Samples were transferred to microcentrifuge tubes and centrifuged at maximum speeds for 20 min at 4 °C. Aliquots of the supernatant were saved for protein determination using a Bio-Rad Protein Assay kit based on the Lowry method.

Samples containing exactly 40 μg of protein were boiled for 4 min in SDS sample buffer, separated on 10% SDS-PAGE gels and transferred onto nitrocellulose membranes using a semi-dry transfer apparatus. Membranes were blocked for 1 hr at room temperature in 5% milk powder in PBS with 0.3% TWEEN-20, and then incubated overnight with anti-β_1AR (1:1000, Calbiochem, EMD Biosciences, la Jolla, CA, USA) that specifically recognizes mouse β_1-adrenergic receptors (Hein and Kobilka, 1995; Tang et al., 1998; Calbiochem Technical Support Staff, personal communications). Membranes were then washed several times for 30 min in 0.1% TWEEN-20 TBS (Tris buffered saline) and incubated in HRP-conjugated goat-anti-Rabbit secondary (1:40,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 hr at room temperature on a shaker. Signals were detected with enhanced chemiluminescence (SuperSignal West Pico; Pierce, Rockford, IL, USA) and developed on X-ray film. Multiple exposures of the blots were performed in order to ensure development in the linear range of the film's sensitivity. Band intensities were quantified by densitometry using NIH imaging software.

vi. Data analysis

For HPLC analysis, average concentrations of amines or amino acids in brain tissue from each strain were calculated. One-way analysis of variance (ANOVA) was carried out, followed by Dunnett's multiple comparisons test to identify differences in concentrations between strains (Graphpad Instat software, San Diego, CA, USA). B6 was used as the control strain in Dunnett's multiple comparisons test. If standard deviations differed between groups, the Kruskal-Wallis statistic (non-parametric) was calculated, followed by Dunn's multiple comparisons test to identify differences between strains. For electrophysiological experiments, fEPSP slopes were averaged within each strain and the resulting means were compared across strains (Nguyen et al., 2000). fEPSP slopes were compared at 120 min after HFS (or 115 min after the end of ISO application for ISO controls). For immunoblot experiments, the Student's t-test was conducted on densitometry measurements of band intensity between strains. Measurements from strains 129, BALB, and C3H were compared with different sets of measurements from strain B6.

C. Results

i. Norepinephrine levels are reduced in the brains of some inbred mouse strains

HPLC analysis was used to quantify the levels of amines (**Figure 6.1**) and amino acids (**Figure 6.2**) in brain tissue from four inbred mouse strains. ANOVA and the Dunnett multiple comparisons test (or non-parametric ANOVA and the Dunn multiple comparisons test, as necessary) were used to compare concentrations of norepinephrine (NE) in the brains of the four strains. The ANOVA yielded a significant result: $F(3,56)$: 42.379, $p < 0.0001$. The Dunnett test revealed that NE levels were significantly reduced

in strains 129, BALB, and C3H compared with the control strain, B6 (all $p < 0.01$). Dopamine (DA) concentrations did not differ between strains: KW = 6. 839, $p > 0.07$. ANOVA revealed significant differences in 5-hydroxytryptamine (5-HT) levels between strains: KW = 15.764, $p < 0.001$. Compared with B6, 5-HT levels were greater in strain C3H ($p < 0.01$), but were not significantly different in strains 129 ($p > 0.05$) and BALB ($p > 0.05$). 3,4-Dihydroxyphenylacetic acid (DOPAC) is a product of DA catabolism; DOPAC levels were significantly different between strains: $F(3,56) = 25.006$, $p < 0.0001$. DOPAC concentrations were significantly elevated in strains 129, BALB, and C3H (all $p< 0.01$) compared with B6. Levels of 5-hydroxyindoleacetic acid (5HIAA), a product of 5-HT catabolism, differed between strains: KW = 39.973, $p < 0.0001$. 5HIAA concentrations were significantly increased in strains 129 ($p < 0.05$), BALB ($p < 0.05$), and C3H ($p < 0.001$) compared with B6.

Concentrations of the excitatory transmitter glutamate in brain tissue from the four strains did not differ: $F(3,56) = 1.858$, $p > 0.1$. However, concentrations of the inhibitory transmitter GABA differed between strains: KW = 10.385, $p < 0.02$. Multiple comparisons tests revealed that GABA levels were elevated in strains 129 ($p < 0.05$) and C3H ($p < 0.05$), but not in BALB ($p > 0.05$), compared with B6. Non-parametric ANOVA of glycine levels showed a significant effect (KW = 11.638, $p < 0.01$) but no strains differed significantly from B6.

ii. Hippocampal LTP maintenance is impaired in inbred strains with reduced norepinephrine, and can be rescued by β-adrenergic receptor activation during tetanic stimulation

The results of the neurochemical analyses, and the fact that pharmacological activation of β-adrenergic receptors can promote the long-lasting expression of

hippocampal LTP (Gelinas and Nguyen, 2005), prompted me to explore further the influence of reduced brain NE levels on hippocampal synaptic plasticity. In particular, I asked two key questions: Is maintenance of hippocampal LTP defective in strains with reduced levels of brain NE? If so, can pharmacological activation of hippocampal β-adrenergic receptors restore LTP to levels similar to those observed in strain B6 (which has significantly higher levels of brain NE than the other three strains tested here)? To address these questions, I measured LTP in the Schaeffer collateral pathway of hippocampal slices cut from these four strains.

I induced LTP with four trains of high-frequency stimulation (HFS; each 100-Hz train was 1s in duration, inter-train interval was 3s; **Figure 6.3**). ANOVA conducted on potentiation levels 120 min after LTP induction in the four strains showed a significant effect: $F(3,20) = 8.037$, $p = 0.001$. Post-hoc testing revealed that strains 129 ($p < 0.05$), BALB ($p < 0.01$), and C3H ($p < 0.05$) exhibited less potentiation than B6 120 min after LTP induction, indicating that LTP maintenance was impaired in these three strains.

Bath application of ISO, a ß-adrenergic receptor agonist, for 10 min prior to, and 5 min after, LTP induction increased LTP maintenance in strains 129, BALB, and C3H to the levels seen in B6 (**Figure 6.4**). ANOVA of 120 min potentiation levels in the four strains treated with ISO and given HFS, and also potentiation in B6 induced by HFS alone, did not show a significant effect: $KW = 0.637$, $p > 0.9$. As such, ISO application did not increase potentiation in strain B6, which already exhibited robust HFS LTP, but rescued LTP in strains 129, BALB, and C3H. ISO application alone (without HFS) did not cause lasting potentiation (**Figure 6.4**). At the concentration used here (1 μM), the facilitatory effect of ISO on LTP has been shown to rely on activation of β-adrenergic,

but not D1/D5 dopamine, receptors in area CA1 of mouse hippocampal slices (Gelinas and Nguyen, 2005).

In summary, these data show that reduced brain NE levels correlate with impaired maintenance of hippocampal LTP in the Schaeffer collateral pathway. Importantly, my data also demonstrate that activation of β-adrenergic receptors can rescue LTP in hippocampal slices derived from several mouse strains with reduced brain NE levels.

iii. β_1-adrenergic receptor expression is intact in the inbred strains

In addition to the observed reduction in brain NE levels in strains 129, BALB, and C3H, another possible molecular basis for the defective maintenance of hippocampal LTP in these three strains is reduced expression of β-adrenergic receptors in the hippocampus. Although I was able to rescue hippocampal LTP in these strains by activating β-adrenergic receptors, this does not preclude the possibility that mild reductions in β-adrenergic receptor expression would still permit hippocampal synapses to respond to applied receptor agonists such as ISO, but might contribute to reduced maintenance of LTP elicited by multiple trains of HFS. This hypothesis was tested by examining expression levels of β_1-adrenergic receptors in hippocampal tissue from the four inbred strains.

Immunoblot analysis of hippocampal tissue from the four inbred strains revealed differences in the expression of β_1-adrenergic receptors between strains (**Figure 6.5**). Student's t-test revealed that strains 129 ($p > 0.2$) and C3H ($p > 0.6$) exhibited similar levels of β_1-adrenergic receptor immunoreactivity to strain B6, but such immunoreactivity was increased in strain BALB compared with B6 ($p < 0.01$). These results indicate that strains B6, 129, and C3H express similar levels of hippocampal β_1-

adrenergic receptors, and hippocampal β_1-adrenergic receptor expression is enhanced in BALB. Thus, the impairment of hippocampal LTP in strains 129, C3H, and BALB cannot be explained by reduced levels of expression of hippocampal β_1-adrenergic receptors. Hippocampal β_1-adrenergic receptor expression may be enhanced in BALB in order to compensate for reduced levels of NE in the brain. Finally, the preserved levels of hippocampal β_1-adrenergic receptor expression in these mouse strains gives ISO adequate sites of action in the hippocampus in order to enable facilitation of LTP.

D. Discussion

Genetic differences between inbred mouse strains can contribute to varied concentrations of amines and amino acids in the brain. Here I focused on the study of hippocampal synaptic plasticity in inbred mouse strains that exhibited decreased levels of NE in the brain. Strains 129, BALB, and C3H exhibited reduced brain NE and impaired hippocampal LTP *in vitro* compared with strain B6. Activation of β-adrenergic receptors by pairing application of ISO with tetanic stimulation rescued hippocampal LTP in strains 129, BALB, and C3H to the levels seen in B6. Hippocampal β_1-adrenergic receptor expression was found to be intact in all strains, and enhanced in strain BALB. Thus, reduced brain levels of NE correlate with hippocampal LTP impairments, and β-adrenergic receptor activation in the hippocampus can rescue hippocampal LTP to levels seen in a "control" inbred strain. Interestingly, I was able to replicate these findings in several different genetic backgrounds, which suggests that the restorative effects of β-adrenergic receptor activation can be effective across a genetically diverse population. Finally, I note that the noradrenergic neuromodulatory system can enhance hippocampus-dependent memory processes (McGaugh and Roozendaal, 2002; Ji et al., 2003a,b;

Murchison et al., 2004). Because ISO can reverse impairments in hippocampal LTP in different genetic backgrounds and LTP may be a cellular mechanisms for some forms of memory (Roman et al., 1987; Doyere and Laroche, 1992; McKernan and Shinnick-Gallagher, 1997; Abel et al., 1997; Moser et al., 1998; see also review by Martin et al., 2000), I suggest that β-adrenergic receptor activation in the hippocampus may promote memory processes in different genetic backgrounds as well.

Previous data have shown that stimulation of β-adrenergic receptors by ISO can enhance maintenance of LTP induced by a single train of 100-Hz HFS (Gelinas and Nguyen, 2005). The present study extends these data by showing that LTP induced by multiple trains of 100-Hz HFS can also be enhanced by ISO, but only in hippocampal slices from inbred mouse strains with reduced levels of brain NE. This finding provides more evidence that the facilitatory effect of activating β-adrenergic receptors is not limited to just low-frequency synaptic stimulation, as shown by Thomas et al. (1996). Rather, this facilitatory effect can extend to higher frequencies of stimulation (e.g., Gelinas and Nguyen, 2005) and even to multi-train LTP induction protocols in particular circumstances.

In order for β-adrenergic receptor activation to rescue hippocampal LTP, there must be an adequate population of β-adrenergic receptors present in the hippocampus for isoproterenol to act upon. β_1-adrenergic receptor levels were preserved in strains 129 and C3H, and were elevated in strain BALB. Thus, these data suggest that ISO could activate β-adrenergic receptors in hippocampal slices in these strains in order to restore LTP maintenance. Interestingly, β_1-adrenergic receptor levels are higher in strain BALB. This might result from upregulation of receptor expression associated with decreased

levels of NE in the brain of BALB. Johnson et al. (1989) demonstrated an increase in β-adrenergic receptor binding in hippocampal areas CA1 and CA3 following destruction of catecholaminergic nerve terminals in the rat brain. Reduced levels of NE in the brains of BALB mice may similarly increase β-adrenergic receptor levels in the hippocampus.

Several studies have examined neural concentrations of catecholamines in the inbred mouse strains studied here. Although many earlier studies used methodologies other than HPLC analysis, most measurements of NE, DA, and 5-HT were similar in range to those determined here. NE, DA, and 5-HT levels in the B6 brain were slightly higher than those reported here (Dolfini et al., 1970; Kempf, 1974; Scudder et al., 1966; Yoshimoto and Komura, 1987). Kempf (1974) and Scudder et al. (1966) also measured 5-HT levels in the B6 brain, and found slightly higher levels than I report. Few studies directly compared neural levels of these catecholamines between the inbred mouse strains studied here. Yoshimoto and Komura (1987) found that strain C3H exhibited lower brain NE levels than B6, in agreement with my findings.

DA and NE are synthesized within a common pathway originating with the amino acid tyrosine (Nestler et al., 2001). Once synthesized, DA can act as a neurotransmitter, can be broken down into metabolites (e.g., DOPAC), or converted into NE by dopamine-ß-hydroxylase. DA levels in strains 129, BALB, and C3H were similar to those found in strain B6. However, NE levels were decreased, and DOPAC levels increased, in these three strains. The DOPAC measurements (Figure 1D) suggest that in strains 129, BALB, and C3H, DA is more intensely catabolized than in B6, possibly reducing the amount of DA that may be used for the synthesis of NE.

Reduced NE levels or ß-adrenergic receptor inhibition can result in LTP impairments in the hippocampus (Bramham et al., 1997; Munro et al., 2001; Yang et al., 2002). Conversely, increased NE levels or ß-adrenergic receptor activation enhance hippocampal LTP (Almaguer-Melian et al., 2005; Gelinas and Nguyen, 2005; Hopkins and Johnston, 1984; Katsuki et al., 1997; Walling et al., 2004; reviewed by Harley, 1991). In rats with pharmacological lesions of the endogenous noradrenergic system, hippocampal LTP in area CA1 of slices was impaired. This LTP could be rescued by activation of ß-adrenergic receptors with ISO, but not by phenylephrine, an α-adrenoceptor agonist (Yang et al., 2002). Activation of either adenylyl cyclase or cAMP-dependent protein kinase also rescued area CA1 LTP in this rat model (Yang et al., 2002), suggesting that the cAMP/PKA signaling pathway mediates the restorative effects of NE on hippocampal LTP.

Why does loss of NE in strains 129, BALB, and C3H impair LTP maintenance? The LTP induction protocol used here consisted of multiple trains of high-frequency stimulation, depends on transcription (Young and Nguyen, in press), and is similar to those known to elicit a long-lasting form of LTP ("late" LTP) that requires new protein synthesis (Frey et al., 1988; reviewed by Huang et al., 1996; Kandel, 2001) and transcription (Nguyen et al., 1994). Recruitment of the cAMP/PKA signaling cascade can promote protein synthesis-dependent forms of LTP (reviewed by Nguyen and Woo, 2003; Kandel, 2001). Interestingly, ß-adrenergic receptor activation can recruit PKA via G protein-induced cAMP synthesis (Dunwiddie et al., 1992; Madison and Nicoll, 1986; Thomas et al., 1996), potentially enabling downstream transcription factors and new protein synthesis. There is also evidence that ß-adrenergic receptor activation stimulates

mitogen-activated protein kinases (MAPKs), which can then modulate translation (Gelinas and Nguyen, 2005; Giovannini et al., 2001; Kelleher III et al., 2004; Winder et al., 1999). As such, reduced ß-adrenergic receptor activation, which may result from lack of NE in strains 129, BALB, and C3H, can influence signaling pathways involved in the maintenance of long-lasting LTP. Previous experiments have shown that the cAMP/PKA signaling pathway is recruited by pharmacologically increasing cAMP levels in area CA1 of hippocampal slices from these three inbred strains (129 and C3H: Schimanski and Nguyen 2005; BALB: L. Schimanski, unpublished observations). Thus, increased ß-adrenergic receptor activation by ISO in 129, BALB, and C3H hippocampi may recruit the cAMP/PKA cascade and promote transcription, new protein synthesis, and maintenance of LTP. In addition, this same treatment may enhance activity of MAPKs and promote maintenance of translation-dependent forms of LTP.

LTP induced by other stimulation protocols has been examined in the four inbred mouse strains studied here. Compared with B6, BALB and C3H exhibit impairments of LTP in area CA1 induced by one or four trains (5-min intertrain interval) of HFS (Schimanski and Nguyen, 2005, 2005a). No LTP impairments were found in either area CA1 of strain 129 or in the medial perforant pathway of strains 129, BALB, and C3H (Schimanski and Nguyen, 2005, 2005a). As such, I hypothesize that the LTP induction protocol used in the present study may induce a form of LTP that is susceptible to interference by low levels of NE, especially because it was the only form of LTP found to be impaired in strain 129 (cf. Schimanski and Nguyen, 2005). Different patterns of electrical stimulation can engage distinct signaling pathways and elicit different forms of LTP (Atkins et al., 2005; Cavus and Teyler, 1996; Huang et al., 1996; Woo et al., 2003).

My induction protocol, consisting of four trains of HFS with an inter-train interval of 3s, is unique in that it requires Ca^{2+} influx through L-type voltage-gated calcium channels (Young, 2005). Gray and Johnston (1987) reported that NE activated voltage-gated calcium channels in the hippocampal dentate gyrus. My induction protocol may elicit impaired LTP in these strains because NE-induced activation of voltage-gated calcium channels during tetanic stimulation is reduced. In addition, reduced NE levels may subdue activation of the cAMP/PKA signaling pathway. Application of NE during tetanic stimulation in strains 129, BALB, and C3H may rescue LTP maintenance by enhancing the activity of L-type calcium channels.

An interesting neurochemical feature of strain C3H is increased neural levels of GABA. This may reduce LTP in hippocampal area CA1 in this inbred mouse strain by increasing synaptic inhibition. Increased GABA in C3H correlates with the deficits in area CA1 LTP that Schimanski and Nguyen (2005) found in this strain. Increased GABAergic inhibition may also contribute to the decreased input-output relationship in area CA1 of C3H mice (Schimanski and Nguyen, 2005), a measure of postsynaptic excitability. Increased levels of GABA do not correlate with hippocampal LTP impairments or a reduced input-output relationship in strain 129 (cf. Schimanski and Nguyen, 2005). Other molecular mechanisms in the hippocampus of strain 129 might compensate for the influence of increased levels of GABA.

Hippocampal LTP plays roles in particular types of learning and memory (Barnes et al., 1994; Bourtchouladze et al., 1994; Doyere and Laroche, 1992; Morris et al., 1986). There is also evidence that NE and ß-adrenergic receptor activity regulate hippocampus-dependent memory (Ji et al., 2003a,b; Marino et al., 2005; Murchison et al., 2004;

Thomas and Palmiter, 1997). Do the strains identified here with impaired hippocampal LTP and reduced brain NE also have impaired hippocampus-dependent memory? Strain C3H exhibits poor or intermediate contextual fear memory, which depends on the hippocampus (as well as the amygdala; Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997; Schimanski and Nguyen, 2005). Strain BALB exhibits intermediate levels of contextual fear memory (Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997; Schimanski and Nguyen, 2005a), but contextual fear memory is intact in strain 129 (Bolivar et al., 2001; Cook et al., 2002; Schimanski and Nguyen, 2005). Thus, reduced brain levels of NE selectively impair contextual fear memory in inbred mouse strains (i.e., in C3H and BALB). These findings suggest that the memory-impairing effects of reductions of neural NE are dependent on the genetic background in which such reductions are expressed.

The primary finding of this study is that genetic variations among inbred mice can lead to reductions in brain levels of NE that correlate with impaired maintenance of hippocampal LTP. Interestingly, acute application of ISO, a ß-adrenergic receptor agonist, rescued hippocampal LTP in three genetically distinct inbred mouse strains that all exhibited reductions in brain NE. These three inbred mouse strains expressed similar, or enhanced, levels of hippocampal $ß_1$-adrenergic receptors compared with a control strain. I suggest that activation of ß-adrenergic receptors during synaptic activity is likely able to alter cellular processes in such a way as to facilitate LTP maintenance. Also, I propose that ß-adrenergic receptor activation may be an effective treatment for impairments in synaptic plasticity that occur in different genetic backgrounds. Because hippocampal synaptic plasticity is strongly correlated with particular types of memory

(Barnes et al., 1994; Bourtchouladze et al., 1994; Doyere and Laroche, 1992; Morris et al., 1986), I hypothesize that ß-adrenergic receptor activation may also be an effective treatment for memory impairments that occur in different genetic backgrounds.

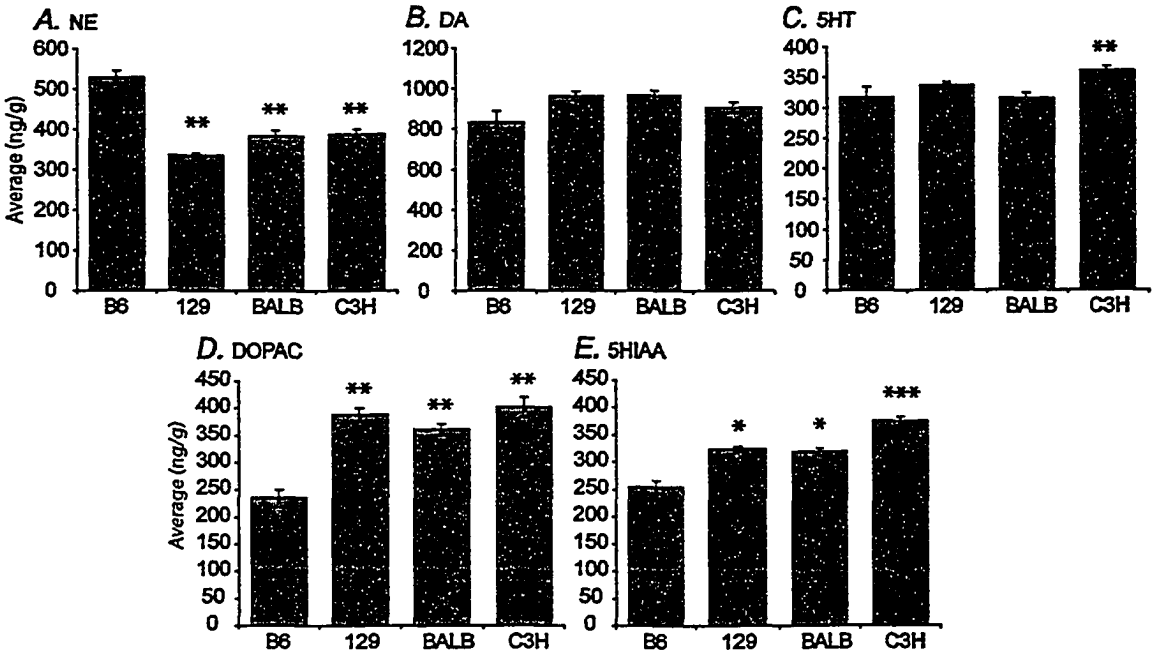

Figure 6.1: Brain levels of norepinephrine are lower in strains 129, BALB, and C3H than in B6. Concentrations of catecholamines and metabolites were measured in brain hemispheres from strains B6 (*n=13*), 129 (*n=13*), BALB (*n=17*), and C3H (*n=17*) by high-pressure liquid chromatography. A) Strains 129, BALB, and C3H exhibited lower concentrations of norepinephrine (NE) than B6. B) Levels of dopamine (DA) were not significantly different between strains. C) Serotonin (5HT) levels were increased in the brains of strain C3H compared with B6. D) 3,4-dihydroxyphenylacetic acid (DOPAC), a metabolite of DA, is present in higher concentrations in strains 129, BALB, and C3H compared with B6. E) 5-hydroxyindoleacetic acid (5HIAA), a metabolite of 5HT, shows increased levels in the brains of strains 129, BALB, and C3H compared with B6. Values shown are means ± SEM. For all figures in this chapter, asterisks indicate significant differences from B6 (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

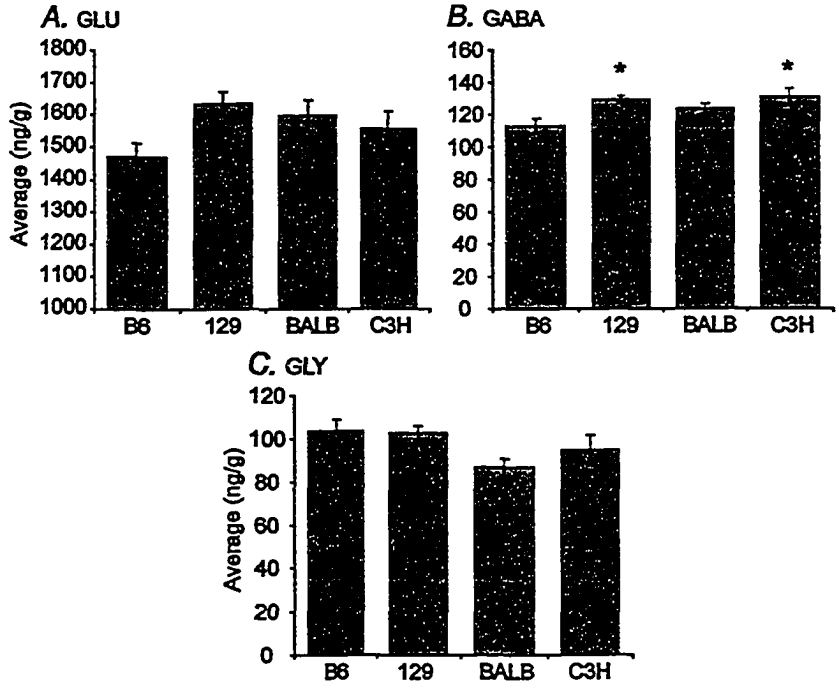

Figure 6.2: Brain levels of glutamate are similar between strains, but GABA concentrations are increased in strains 129 and C3H. Concentrations of amino acids were measured in brain hemispheres from strains B6 (*n=13*), 129 (*n=13*), BALB (*n=17*), and C3H (*n=17*). A) There were no significant inter-strain differences in glutamate concentrations. B) GABA levels were elevated in strains 129 and C3H compared with B6. C) Levels of glycine were not significantly different between strains. Values shown are means ± SEM.

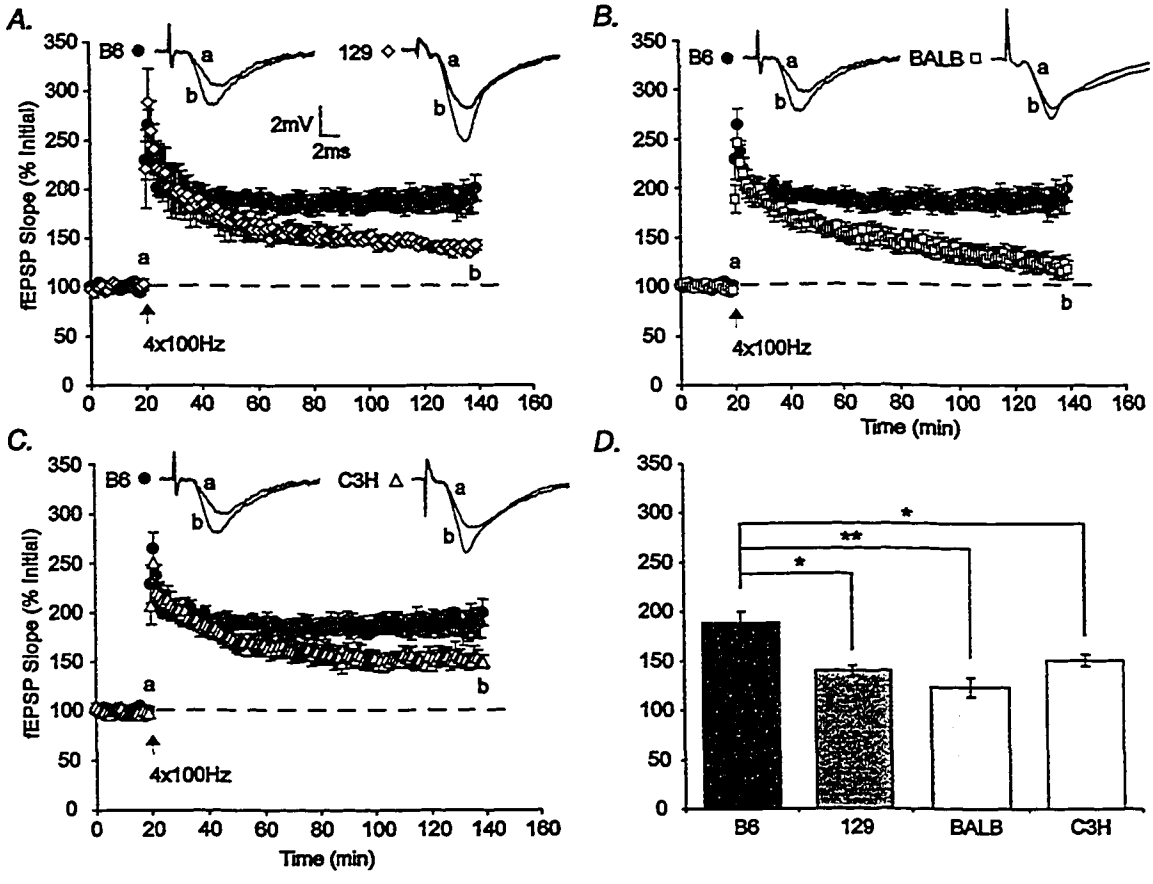

Figure 6.3: Impaired maintenance of area CA1 LTP in strains with reduced brain levels of norepinephrine. LTP was induced in area CA1 of hippocampal slices from the four inbred strains by four trains of high-frequency stimulation (*4x100Hz*: each train was 100Hz for 1s duration, and inter-train interval was 3s). A-C) Hippocampal LTP was measured in strains B6 ($n=8$), 129 (A: $n=4$), BALB (B: $n=5$), and C3H (C: $n=7$) for two hours after induction. Sample field EPSP traces are shown for time points "a" (before tetanization) and "b" (two hours after LTP induction). D) Comparison of average potentiation levels two hours after LTP induction in strains B6, 129, BALB, and C3H. LTP maintenance was reduced in strains 129, BALB, and C3H compared with B6. Data points represent mean ± SEM, and n's indicate number of slices.

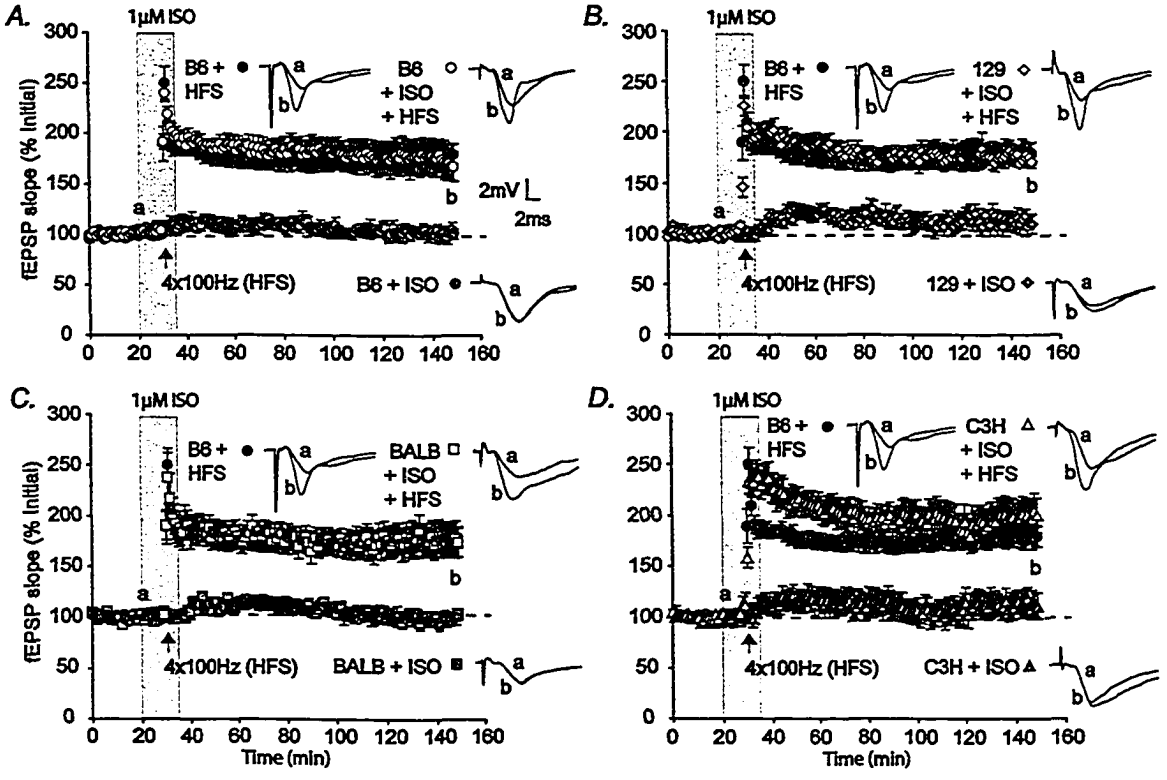

Figure 6.4: Activation of ß-adrenergic receptors during LTP induction rescues LTP maintenance in strains 129, BALB, and C3H to the levels seen in B6. Isoproterenol (ISO, 1μM) was bath applied (grey boxes) for 10min prior to, and 5min after, LTP induction by four trains of high-frequency stimulation (HFS). A) Activation of ß-adrenergic receptors during LTP induction in strain B6 does not influence LTP maintenance. In B6 slices, the level of potentiation two hours after induction without ISO (B6+HFS; *n=10*) was not significantly different from the level of potentiation two hours after induction by four trains of HFS plus ISO (B6+ISO+HFS: *n=6*). ISO application alone (B6+ISO: *n=5*) did not elicit lasting potentiation. B) Activation of ß-adrenergic receptors during LTP induction in strain 129 enhanced LTP maintenance to resemble that in strain B6. The level of potentiation two hours after induction with ISO in strain 129 (*n=8*) and the level of potentiation two hours after induction (without ISO) in strain B6 were not significantly different from one another. ISO application alone in strain 129 did not elicit lasting potentiation (*n=5*). C) Activation of ß-adrenergic receptors during LTP induction in strain BALB enhances LTP maintenance to resemble that in strain B6. The level of potentiation two hours after induction with ISO in strain BALB (*n=7*) and the level of potentiation two hours after induction (without ISO) in strain B6 were not significantly different from one another. ISO application alone in strain BALB does not result in lasting potentiation (*n=7*). D) Activation of ß-adrenergic receptors during LTP induction in strain C3H enhanced LTP maintenance to resemble that in strain B6. The level of potentiation two hours after induction with ISO in strain C3H (*n=11*) and the level of potentiation two hours after induction (without ISO) in strain B6 were not significantly different from one another. ISO application alone in strain C3H did not elicit lasting potentiation (*n=7*). Sample field EPSP traces are shown for time points "a" (before tetanization) and "b" (two hours after LTP induction).

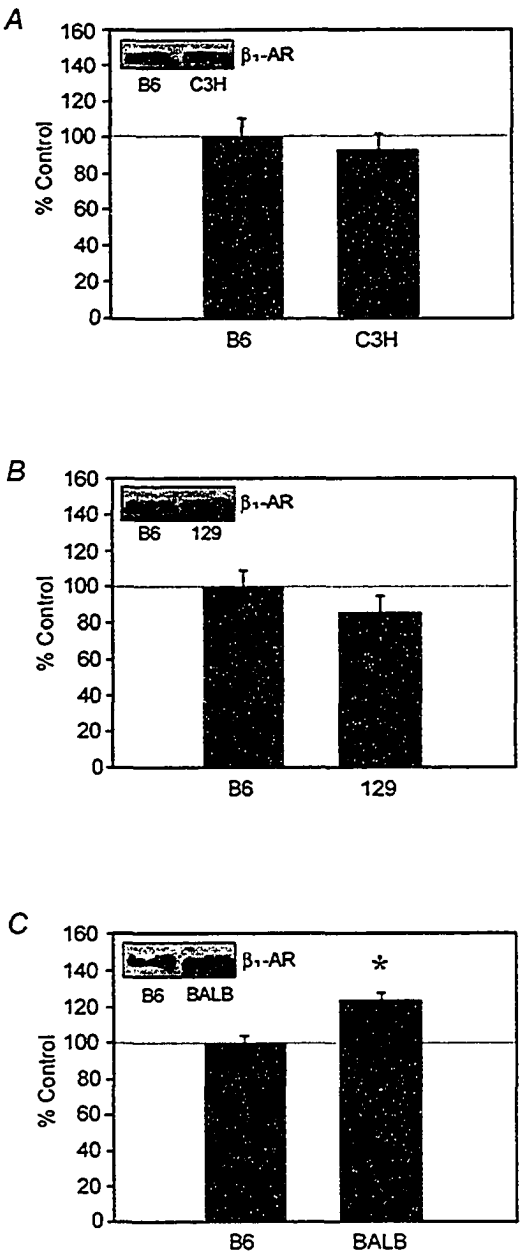

Figure 6.5: Quantification of ß1-adrenergic receptors (ß1-AR) in the hippocampi of several mouse strains. The B6 strain was used as a control for comparison with strains C3H (A), 129 (B), and BALB (C). The levels of $ß_1$-AR were not significantly different from C3H ($p = 0.61$) or 129 ($p = 0.29$), but were different from BALB ($p = 0.006$). The ordinate represents a percentage after normalizing the density readings to 100% for the B6 strain. Data represent mean ± SEM of the blot density readings. Inset shows example blots of $ß_1$-AR from hippocampi ($n = 4$-5).

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*CHAPTER 7:

General Discussion

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Schimanski LA, Nguyen PV (2004) Multidisciplinary approaches for investigating the mechanisms of hippocampus-dependent memory: a focus on inbred mouse strains. Neurosci Biobehav Rev 28: 463-483. Copyright © 2004, Elsevier Ltd.

The common element of the chapters of this thesis is that they all involve the study of synaptic plasticity and/or memory in inbred mouse strains. These inbred strains served as models in which the role of the hippocampal commissure in memory and synaptic plasticity was investigated (*Chapter 3*). The study of inbred strains here also revealed that synaptic plasticity and memory can be influenced by different genetic backgrounds (*Chapters 4* and *5*). Examining both LTP and memory in these strains provided an opportunity to correlate LTP with memory in different genetic backgrounds, generating support for the notion that LTP underlies particular forms of memory in a brain subregion specific manner. Finally, neurochemical measures, receptor expression, and LTP were examined conjointly in inbred mouse strains in order to investigate molecular mechanisms of genetic variation that influence synaptic plasticity (*Chapter 6*). A potential treatment for LTP impairment was derived from this study, and was tested in multiple inbred strains, providing the opportunity to test the broad validity of such treatment across variable genetic backgrounds. This chapter will summarize the key findings from my thesis research, relate these findings to the work of others, and propose further studies that stem from the results.

A. The Relationships Between Genetic Variation, Memory, and Synaptic Plasticity

My first thesis objective was to examine the role of the hippocampal commissure in hippocampal synaptic plasticity and hippocampus-dependent memory (*Chapter 3;* Schimanski et al., 2002). Cued and contextual fear memory were compared between two inbred mouse strains with different types of agenesis of the neural commissures. In collaboration with Dr. Doug Wahlsten (Dept. of Biological Sciences, Univ. of Windsor), it was shown that strain 9XCA/Wah exhibited a hippocampal commissure that was

reduced in size along with complete absence of the corpus callosum. The other strain, BALB/cWah1, served as a control because it exhibits a range in reductions of corpus callosum size but an intact hippocampal commissure, allowing the influence of absence of the hippocampal commissure to be isolated to strain 9XCA/Wah. No impairments of fear memory were found in strain 9XCA/Wah compared with BALB/cWah1. However, 9XCA/Wah failed to acquire contextual, but not cued, fear extinction as quickly as BALB/cWah1. Thus, mice with a reduced hippocampal commissure exhibit very selective hippocampus-dependent memory impairments. Although all forms of LTP measured in hippocampal area CA1 and the mossy fibre pathway of hippocampal slices from the two strains were intact, paired-pulse facilitation, a form of short-term synaptic plasticity, was reduced in area CA1 of 9XCA/Wah mice compared with BALB/cWah1 mice. Paired-pulse facilitation was rescued by decreasing extracellular [Ca^{2+}], suggesting that presynaptic calcium dynamics may be altered in this strain. In conclusion, this study revealed that interhemispheric connectivity is not required for acquisition or expression of cued and contextual fear conditioning, but an intact hippocampal commissure is crucial for the extinction of contextual fear. High levels of LTP expression in the Schaeffer collateral and mossy fibre pathways correlated with intact contextual and cued fear memory in these mice. Interestingly, impaired contextual memory extinction correlated with impaired short-term synaptic plasticity in mice without an intact hippocampal commissure. Thus, the hippocampal commissure is important for certain aspects of learning and memory, and for short-term synaptic plasticity in some hippocampal circuits.

My second thesis objective was to determine how genetic background influences memory and synaptic plasticity. It was already established that memory and synaptic plasticity could vary between inbred mouse strains, but there were nearly no studies (except for Nguyen et al., 2000a) that conjointly examined memory and synaptic plasticity in inbred mouse strains with the goal of identifying cellular correlates of memory impairment. There were no previous studies that conjointly examined amygdala-dependent memory and amygdalar LTP in inbred mouse strains. In two studies, I focused on memory mediated by, and LTP within, the hippocampus and amygdala of several inbred strains (*Chapters 4* and *5*; Schimanski and Nguyen, 2005a, 2005b).

In *Chapter 4*, four inbred mouse strains were studied: C57BL/6NCrlBR (B6), 129S1/SvImJ (129), C3H/HeJ (C3H), and DBA/2J (D2). Short-term (1 hr) and long-term (24 hr) cued and contextual fear memory was measured in these four strains. Then, LTP in hippocampal area CA1, the medial perforant pathway, and the basolateral amygdala, were examined. Short- and long-term contextual and cued fear memory, and hippocampal Schaeffer collateral and amygdalar LTP were intact in strains B6 and 129, but all were impaired in C3H and D2. Synaptic facilitation elicited by pharmacologically increasing levels of cAMP was similar in all strains, suggesting that the cAMP/PKA signaling pathway was intact, and could be recruited, in these mice. However, examination of the input/output relationship and paired-pulse facilitation in area CA1 of hippocampal slices from the four inbred strains revealed that CA1 neurons were less excitable in strain C3H compared with strain B6, perhaps contributing to the LTP impairments identified in area CA1 of strain C3H. There were no differences in medial

perforant pathway LTP expression between the four strains. Overall, these data indicate that LTP in hippocampal area CA1 correlates with hippocampus-dependent contextual fear memory, and amygdalar LTP correlates with amygdala-dependent cued fear memory in these inbred strains. LTP in the medial perforant pathway does not exhibit a correlational relationship with contextual fear memory, and there were no differences in the expression of short- versus long-term memory in these strains. This study demonstrated that mice with different genetic backgrounds express different levels of learning, memory, and synaptic plasticity. By examining these traits conjointly, it is possible to draw inferences about the roles of particular types of synaptic plasticity in memory function.

In *Chapter 5*, similar methodologies were used, but a different set of inbred mouse strains was examined: B6, A/J, BALB/cByJ (BALB), C57BL/10J (B10), and SM/J (SM). In addition to the methods used in *Chapter 4*, contextual fear extinction was also measured in this study. One hour after training, many strains exhibited contextual and cued fear memory deficits: all strains except for B6 and B10 showed impairments of both types of memory. Twenty-four hours after training, contextual fear memory was intact in all strains, but cued fear memory was impaired in strains A/J, BALB, and SM. In addition, contextual fear extinction was impaired in strain BALB. Interestingly, LTP in hippocampal area CA1 was intact in all strains (except for strain BALB, which exhibited a selective four-train LTP impairment that correlated with impaired contextual extinction in this strain). This indicates that hippocampal area CA1 LTP is more strongly correlated with long-term (24 hr) than with short-term (1 hr) contextual fear memory. Like in *Chapter 4*, LTP in the medial perforant pathway was similar in all strains,

supporting the notion that LTP in this pathway does not correlate with contextual fear memory expression. Furthermore, amygdalar LTP was reduced in strains A/J and BALB, in correlation with impaired cued fear memory in these strains. Thus, specific hippocampal and amygdalar LTP deficits were identified that correlate with fear memory impairments that likely result from altered information processing in these two brain regions.

My fourth, and last, objective was to determine whether inbred mouse strains also express differences in neuromodulatory transmitter levels in the brain. If such differences were found, their influences on hippocampal LTP would be studied (*Chapter 6*). In collaboration with Dr. Glen Baker (Dept. of Psychiatry, Univ. of Alberta), brain tissue levels of amines and amino acids were measured using high-pressure liquid chromatography in strains B6, 129, BALB, and C3H. Dopamine, serotonin, and glutamate concentrations were similar between the brains of all four strains, but norepinephrine (NE) levels were significantly lower in strains 129, BALB, and C3H than in B6. Hippocampal LTP and β_1-adrenergic receptor expression were examined in these inbred strains, and strain B6 was considered the control for this study. LTP was induced in area CA1 of hippocampal slices from the four strains by four trains of high-frequency stimulation of the Schaeffer collaterals. Two hours after induction, potentiation was significantly less in strains 129, BALB, and C3H than in B6. Bath application of 1μM isoproterenol, a ß-adrenergic receptor agonist, during induction rescued hippocampal LTP in strains 129, BALB, and C3H to levels seen in B6. Finally, Western blot analysis (done in collaboration with Dr. D Ali, Dept. of Biology, Univ. of Alberta) showed that hippocampal β_1-adrenergic receptor levels were similar in strains 129 and C3H, but were

elevated in BALB, compared with B6. Thus, reduced brain NE concentrations, but not hippocampal β_1-adrenergic receptor expression, correlate with impaired hippocampal LTP induced by multiple trains of high-frequency stimulation in strains 129, BALB, and C3H. Applying a ß-adrenergic agonist during induction rescues LTP to control levels in these strains. This study illustrates that genetic variation can result in impairments of hippocampal LTP that correlate with reduced brain levels of the neuromodulatory transmitter NE. However, acute activation of ß-adrenergic receptors during induction can restore LTP expression in multiple genetic backgrounds.

Overall, these four studies demonstrate that inbred mouse strains are useful models in which the mechanisms of LTP and memory may be studied. Collectively, these experiments have taken advantage of genetic variation between inbred mouse strains to elucidate important roles for particular brain structures and types of synaptic plasticity in memory, providing support for the hypothesis that synaptic plasticity underlies memory in the hippocampus and the amygdala.

B. Using Inbred Mouse Strains to Study the Link between Memory and Synaptic Plasticity in Particular Brain Structures and Subregions

Different memory systems can rely on distinct brain regions (e.g., McDonald and White, 1993). By examining genetically distinct inbred mouse strains, it is possible to conduct conjoint studies of synaptic plasticity and memory that reveal correlations between specific types of synaptic plasticity and memory. These correlations provide clues to the cellular and molecular mechanisms that underlie memory processes in particular brain subregions.

i. Hippocampal commissure

Congenital reductions in hippocampal commissure size occur naturally in several inbred mouse strains (Wahlsten, 1974,1989). Examining these mouse models provides a method by which the role of the hippocampal commissure in hippocampal synaptic plasticity and also memory function may be investigated. Because the commissural fibres exert excitatory influence primarily in the CA regions of the contralateral hippocampus (Buchhalter et al., 1990; Johnston and Amaral, 2004; Laurberg and Sorenson, 1981; Swanson et al., 1978; Van Groen and Wyss, 1990), they are able to influence hippocampal synaptic plasticity in these regions (*Chapter 3*; Hernandez et al., 1994; Kaibara and Leung, 1993; Markevich et al., 1997; Schimanski et al., 2002; Thiels et al., 1996; Young et al., 1998). The data presented in *Chapter 3* indicate that congenital, chronic loss of these fibres does not impair LTP in hippocampal area CA1 or in the mossy fibre pathway, but does result in decreased short-term synaptic plasticity in area CA1. Because reducing extracellular calcium levels rescues this short-term synaptic plasticity, it appears that the influence of calcium influx to the presynaptic terminals in area CA1 may be altered in mice lacking an intact hippocampal commissure. This effect does not appear to significantly alter excitatory synaptic transmission because the input-output relation is not altered in mice lacking an intact hippocampal commissure.

Interestingly, cued and contextual fear memory measured 24 hrs after training were intact in these mice (correlating with high levels of LTP in hippocampal area CA1 and the mossy fibre pathway), but contextual (and not cued) fear extinction was selectively impaired (*Chapter 3*). This finding suggests that there is a connection between hippocampal information processing that involves the hippocampal commissure,

short-term synaptic plasticity in area CA1, and the mechanisms that underlie contextual fear extinction in the mouse. Interestingly, contextual fear extinction has been shown to rely on the activity of many of the molecules and signaling pathways in the hippocampus that are also involved in hippocampal LTP and memory, including the PKA, MAPK, and CaMKII signaling pathways, and NMDARs (Szapiro et al., 2003). However, there is some evidence that contextual extinction does not require hippocampal protein synthesis (Lattal and Abel, 2001; but see Viannna et al., 2001). Although hippocampal LTP was not impaired when induced by tetanic stimulation *in vitro*, it is possible that the recruitment of these mechanisms of contextual extinction is altered in the behaving mouse that lacks an intact hippocampal commissure.

Also, brain regions other than the hippocampus contribute to contextual fear memory and extinction. It is possible that the absence of the hippocampal commissure, and resultant alterations in hippocampal short-term plasticity, affect the interactions of the hippocampus with other brain regions involved in extinction. For instance, the amygdala is required for contextual fear conditioning (Kim et al., 1993; Phillips and LeDoux, 1992), and there is some evidence that dopaminergic influence in the prefrontal cortex can influence contextual fear extinction (Fernandez Espejo, 2003). In the rat, neurons in the lateral nucleus of the amygdala also fire in a context-dependent manner during fear extinction; spike firing correlates with freezing behaviour (Hobin et al., 2003). Further experiments are required to determine whether the impaired contextual extinction observed in inbred mouse strains lacking an intact hippocampal commissure are due to altered hippocampal information processing that leads to abnormal interactions of the hippocampus with other brain regions involved in extinction.

Clinically, malformation of the hippocampal commissure is associated with mental disorders that can include reduced learning and memory function. Magnetic resonance imaging study of patients with fetal alcohol syndrome revealed that these patients exhibited agenesis of the hippocampal commissure (Bhatara et al., 2002). In rats, prenatal exposure to alcohol also leads to reductions in the size of the hippocampal commissure (Livy and Elberger, 2001). Prenatal alcohol exposure results in deficits in working memory, short-term memory, spatial learning, verbal learning and memory, and declarative learning in children and adolescents (Burden et al., 2005; Coles et al., 1991; Mattson et al., 1996; Olson et al., 1998; Streissguth et al., 1994; Uecker and Nadel, 1994; Willford et al., 2004; reviewed by Mattson and Riley, 1998). Similar symptoms of prenatal alcohol exposure are found in rats, including short-term and spatial memory impairments (Clements et al., 2005; Gianoulakis, 1990; Kim et al., 1997; Reyes et al., 1989; reviewed by Berman and Hannigan, 2000). Also, patients with Alzheimer's disease, and a transgenic mouse model of Alzheimer's disease, display reduced forebrain commissure sizes (Black et al., 2000; Gonzalez-Lima et al., 2001), correlating with the memory impairments characteristic of this disease (reviewed by Fleischman and Gabrieli, 1999; Walsh and Selkoe, 2004). It is important to note that patients with fetal alcohol syndrome and Alzheimer's disease exhibit other brain pathologies that contribute to memory impairment (reviewed by Roebuck et al., 1998; Walsh and Selkoe, 2004). Nonetheless, the clinical links between reduced hippocampal commissure size and memory impairment suggest that further study of mouse models of commissural agenesis will be valuable in understanding and developing treatments for human symptoms of this brain defect.

Overall, agenesis or loss of the hippocampal commissure results in subtle deficits in hippocampal information processing. This notion is exemplified by the impairment of hippocampal short-term synaptic plasticity, but not LTP, and contextual fear extinction, but not contextual fear memory, in mice with agenesis of the hippocampal commissure. These patterns are replicated in studies of loss of the hippocampal commissure in humans, in which memory impairment is restricted to verbal and visual recall (and not recognition) memory (Phelps et al., 1991), and possibly short-term memory (Zaidel, 1995). Further study of inbred mouse models of commissural agenesis will be useful in determining how subtle changes in hippocampal information processing result in specific memory impairments.

ii. Inbred mouse models of intact and impaired hippocampus-dependent memory

Because relatively few studies have compared hippocampal synaptic plasticity between inbred strains, our ability to correlate memory impairments with altered synaptic plasticity is currently limited. **Table 7.1** summarizes the relationships between memory performance and hippocampal LTP for all strains in which both have been measured (including the present thesis research). As is evident from **Table 7.1**, the correlations between memory and LTP are not perfectly consistent, but it appears that some strains that exhibit poor hippocampus-dependent memory also exhibit poor hippocampal LTP. More multidisciplinary studies will be necessary to adequately define the relationships between particular types of hippocampal LTP, learning, and memory in multiple inbred strains. For instance, multiple behavioural tests of hippocampus memory should be conducted in each strain; in addition to contextual fear conditioning and the Morris water maze (MWM), tests such as the radial arm maze and social transmission of food

preferences may be employed. Because each of these behavioural tests relies on distinct brain regions other than the hippocampus, conducting a variety of different behavioural tests will provide a much more reliable indication of whether it is hippocampal function, specifically, that is impaired in a particular mouse strain. Similarly, multiple forms of hippocampal LTP should be examined in each mouse strain. Different types of LTP can depend on distinct molecular mechanisms, and LTP in particular hippocampal subregions may contribute to memory in different ways (See *Chapter 1, Section E*). This section will examine relationships between hippocampal synaptic plasticity and memory in the small number of strains in which a variety of behavioral and electrophysiological studies have provided enough data to form testable hypotheses.

Because of its consistent expression of high levels of memory performance on the MWM and contextual fear conditioning task (see **Tables 1.2 and 1.3**), and also on other tests of hippocampus-dependent learning and memory such as the Barnes maze (Nguyen et al., 2000a) and the radial arm maze (Ammassari-Teule and Caprioli, 1985; Ammassari-Teule et al., 1993, 2001; Rossi-Arnaud et al., 1991; Roullet and Lassalle, 1992, 1995; Roullet et al., 1997; Schwegler et al., 1990; Zilles et al., 2000), the B6 strain is considered to be a mouse model of good hippocampus-dependent learning and memory. *In vivo*, robust LTP can be induced in the dentate gyrus of B6 mice by one or three trains of high-frequency stimulation (HFS), and by theta-burst stimulation (Jones et al., 2001; Matsuyama et al., 1997). Multiple types of LTP can be induced in area CA1 and the medial perforant pathway of B6 hippocampal slices (Nguyen et al., 2000a,b; Gerlai, 2002; Schimanski and Nguyen, 2005a,b). One train of HFS, four trains of HFS at different intertrain intervals, theta-burst stimulation, and application of forskolin (an

adenylyl cyclase activator) all induced LTP. Thus, the performance of C57BL/6 mice on hippocampus-dependent memory tasks correlates well with LTP induction and expression in area CA1 of hippocampal slices from this strain.

Another inbred strain that performs well on both contextual fear conditioning and the MWM is 129T2/SvEms (Cook et al., 2002; Nguyen et al., 2000a; Wolff et al., 2002). Nguyen et al. (2000a) found a slight impairment in this strain on the Barnes maze, but they proposed that this was due to lack of motivation. Thus, despite this subtle motivational factor concerning Barnes maze performance, 129T2/SvEms may be another model of good hippocampal learning and memory. Some forms of LTP are expressed well in area CA1 of 129T2/SvEms hippocampal slices, such as late-LTP induced by 4 trains of HFS at a compressed inter-train interval of 3 sec and LTP induced by forskolin application (Nguyen et al., 2000b). Paired-pulse facilitation (PPF) and LTD are also robustly expressed in 129T2/SvEms and C57BL/6. However, E-LTP induced by one train of HFS, theta-burst LTP, and L-LTP following spaced inter-train intervals of 20 sec and 5 min were all impaired in 129T2/SvEms (Nguyen et al., 2000a,b). Thus, there is a dissociation between some forms of hippocampal LTP and behavioral learning and memory as measured by contextual fear conditioning and the MWM in 129T2/SvEms. Similarly, Reisel et al. (2002) found hippocampal area CA1 LTP deficits in gene-targeted mice that lack the AMPA receptor subunit GluR1, but these mice exhibited intact spatial reference memory for the hidden platform MWM task.

DBA/2 performs poorly on contextual fear conditioning and displays mixed results in the MWM (see **Tables 1.2** and **1.3**), and is generally considered to display poor hippocampal learning and memory. As a result, DBA has been extensively studied in an

effort to elucidate molecular mechanisms for its impairment in hippocampal learning and memory. Several types of hippocampal synaptic plasticity are intact in DBA/2 compared with C57BL/6. For instance, in the dentate gyrus *in vivo*, PPF is intact, and three trains of HFS (Matsuyama et al., 1997) and theta-burst stimulation result in high levels of LTP (Jones et al., 2001). However, Bampton et al. (1999) found that multiple trains of HFS induced high levels of LTP initially, but this LTP then decayed faster than in other inbred strains. In area CA1 of hippocampal slices, E-LTP induced by one train of HFS was intact, as were LTD and L-LTP induced by four trains of HFS delivered at spaced (5 min) intervals (Nguyen et al., 2000a,b; Schimanski and Nguyen, 2005a). However, several types of hippocampal area CA1 synaptic plasticity were found to be impaired in DBA/2. Theta-burst stimulation, application of forskolin, and four trains of HFS delivered at more compressed intervals (20 sec and 3 sec) resulted in deficient LTP (Nguyen et al., 2000a,b; Schimanski and Nguyen, 2005a). Because PPF was impaired in CA1 of DBA/2 hippocampal slices, Nguyen et al. (2000a) hypothesized that presynaptic calcium dynamics, or synaptic vesicle proteins that mediate calcium-triggered transmitter release, may be altered in CA3 presynaptic nerve terminals. These could, in principle, account for some of the LTP deficits that were found in DBA/2, but more definitive experiments need to be done to address these issues. In contrast, Schimanski and Nguyen (2005a) found that PPF was not reduced in area CA1 of hippocampal slices from strain DBA; this inconsistency may be due to the use of male mice by Nguyen et al. (2000a) and female mice by Schimanski and Nguyen (2005a).

Several other strains are potential models of good or poor hippocampus-dependent memory. For instance, strain 129S1/SvImJ exhibited high levels of contextual

fear memory, and all forms of LTP measured in this mouse were intact (*Chapter 4*; **Table 1.2**; Bolivar et al., 2001; Cook et al., 2002; Schimanski and Nguyen, 2005a). Strains A/J, C57BL/10J, and SM/J also exhibited high levels of contextual fear memory and intact hippocampal LTP (*Chapter 5*; Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997; Schimanski and Nguyen, 2005b). Of these strains, only C57BL/10J has undergone further testing on the Morris water maze that confirmed the expression of hippocampus-dependent memory (Owen et al., 1997). These four strains should undergo further testing for types of hippocampus-dependent memory other than fear conditioning in order to bolster the conclusion that they display good hippocampal memory function. In contrast, in *Chapter 4*, strain C3H/HeJ was identified as having impaired contextual fear memory, and some forms of hippocampal LTP were also reduced in this strain (Schimanski and Nguyen, 2005a). C3H/HeJ has been tested on the Morris water maze, but because of its poor performance on the visible platform test (probably due to retinal degeneration; The Jackson Laboratory, 2002), testing was inconclusive concerning place learning (Owen et al., 1997; Upchurch and Wehner, 1988). Strain BALB/cByJ has exhibited varied memory performance and hippocampal LTP in several studies. In most studies it displays an intermediate level of contextual fear memory (Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997), and like in strain C3H/HeJ, testing on the Morris water maze was inconclusive due to poor visible platform performance (Upchurch and Wehner, 1988). As shown in *Chapter 5*, hippocampal LTP in BALB/cByJ is mostly intact, except for a selective reduction in L-LTP induced by 4 trains of 100-Hz stimulation, and contextual fear extinction is impaired (Schimanski and Nguyen, 2005b). Thus, further behavioural testing may confirm that strain C3H/HeJ is a model of poor

hippocampal function, and strain BALB/cByJ likely has mild deficits in hippocampal information processing.

Because few experiments have examined hippocampal synaptic plasticity and hippocampus-dependent learning and memory conjointly in inbred mice, the task of correlating these phenomena between individual studies can be difficult. Different laboratories conduct learning and memory testing using distinct methods, and they can often arrive at conflicting conclusions. This makes it difficult to arrive at a consensus on the learning and memory abilities of inbred strains, and also hampers the ability to relate these cognitive functions to cellular and molecular phenomena such as LTP. Furthermore, even when labs try their best to replicate data, their results may not be in agreement because of multiple factors that cannot be rigorously controlled (Crabbe et al., 1999). One solution might be to perform conjoint examinations of cognitive function and cellular/molecular properties within the same mouse, which may provide a more powerful means to correlate these measures. Another source of divergence is the use of different substrains and different mouse suppliers. Although all mice of a particular inbred strain were derived from the same original brother-sister matings, long-term maintenance of these inbred strains by different suppliers or under different conditions can allow genetic drift to occur within these isolated populations. Similarly, there is some evidence that strain differences might result not only from genetic differences but also from different types of maternal care (Francis et al., 2003). Furthermore, different types of LTP, or different brain regions (Ammassari-Teule et al., 2000), may support learning and memory in distinct inbred mouse strains. I must acknowledge that LTP measured *in vitro* or *in vivo*, induced by artificial electrical stimulation or by chemical

application, may not correlate perfectly with a particular mouse strain's ability to perform hippocampus-dependent contextual fear conditioning or spatial learning. However, there may be general properties of hippocampal LTP that correlate with learning and memory, such as LTP induction and persistence.

iii. Inbred mouse models of intact and impaired amygdala-dependent memory

My thesis research is the first work to conjointly examine fear memory and amygdalar LTP in inbred mouse strains. **Table 7.2** illustrates amygdala-dependent cued fear memory and *in vitro* LTP in the lateral-basolateral amygdala pathway in the inbred mouse strains studied to date. These data indicate that three inbred mouse strains exhibit high levels of cued fear memory that correlates with intact amygdalar LTP: 129S1/SvImJ, C57BL/6, and C57BL/10 (*Chapters 4* and *5*; see **Table 7.2** for references). Information processing in the amygdala appears to function well in these mice. On the other hand, four strains were identified that exhibit poor cued fear memory correlated with reduced amygdalar LTP: A/J, BALB/cByJ, C3H/HeJ, and DBA/2. It is important to note that several groups found that strains BALB/cByJ and C3H/HeJ in fact exhibit intermediate or high levels of cued fear memory, whereas these strains are reported to exhibit impairments of cued fear memory in *Chapters 4* and *5* (also see **Table 7.2**). However, these other groups generally used several training trials for fear conditioning, as opposed to the one training trial utilized in this thesis research. Thus, the experiments done in *Chapters 4* and *5* likely conducted a more sensitive test of cued fear memory than the other reports cited in **Table 7.2**, potentially accounting for the reported discrepancies in cued fear memory performance in strains BALB/cByJ and C3H. Thus, information

processing in the amygdala is likely at least slightly altered in strains BALB/cByJ and C3H, and is impaired in strains A/J and DBA/2.

Interestingly, two inbred mouse strains, FVB and SM/J, exhibited cued fear memory impairments, but intact LTP in amygdalar slices. Thus, the correlations observed between cued fear memory and LTP in the lateral-basolateral amygdalar pathway do not necessarily apply to all inbred mouse strains. There are several factors that may contribute to this apparent discrepancy. Firstly, like in the hippocampus, different forms of LTP in the amygdala can recruit different molecular mechanisms (Chapman et al., 2003; Schafe et al., 2001). Only one form of amygdalar LTP has been examined in these inbred mouse strains; perhaps if other induction protocols were used, different types of amygdalar LTP impairments would be found in strains FVB and SM/J. Secondly, cued fear conditioning depends not only on the amygdala, but also on other brain regions involved in sensing incoming stimuli and eliciting fear responses. It may be that amygdalar processing is indeed intact in strains FVB and SM/J, but other processes involved in perceiving fearful stimuli, or in expressing fear responses, are impaired. Lastly, I must acknowledge that the LTP examined in these studies is not necessarily physiological in nature. Although LTP and memory rely on many similar mechanisms (reviewed by Martin et al., 2000), the induction of LTP by mass stimulation of fibres in an *in vitro* slice preparation may not mimic the more subtle synaptic changes that may occur in the behaving animal. It is very likely that the particular subset of synapses that undergo LTP in the slice are not the same synapses that would undergo LTP during learning and memory in the intact animal. Thus, subtle impairments in LTP

induction and expression that may be responsible for reduced cued fear memory may not be observable in the preparation used here.

iv. Hippocampus-amygdala interactions: possible implications for memory and LTP

The relationship between the hippocampus and the amygdala has become the focus of much study, and hippocampo-amygdalar interactions should be considered in the interpretation of my data. Here, the focus will be on amygdalar influence on the hippocampus.

The amygdala projects heavily to the hippocampus. All nuclei of the BLA project to the entorhinal cortex, which provides input to the hippocampal dentate gyrus (DG) via the perforant pathways (Richter-Levin and Akirav, 2003). Stimulation of the BLA can evoke field potentials in the DG, and can enhance LTP in the DG. This response is mediated by ß-adrenergic and/or muscarinic cholinergic receptors (Frey et al., 2001). It has been suggested that amygdalar innervation of the hippocampus can modulate memories under emotional conditions (Richter-Levin, 2004). Contextual fear conditioning can be considered an emotional event, and as such, its memory storage in the hippocampus may be modulated by the amygdala (Seidenbecher et al., 2003).

For amygdalar modulation of hippocampal synaptic plasticity, activation of the amygdala and of hippocampal pathways must occur in close temporal proximity. Frey et al. (2001) suggest that amygdalar activation must occur within 30min of hippocampal activation (but not at the exact same time) to facilitate synaptic transmission in the DG. Alternately, Richter-Levin and Akirav (2003) reviewed related data and concluded that amygdala activation that occurs at the same time as hippocampus-dependent learning is facilitatory, whereas inhibition results when amygdala activation occurs more than 1-2hrs

after learning. Overall, these ideas indicate that if the amygdala is activated within 30-60min of hippocampus-dependent learning or hippocampal synaptic plasticity, it will facilitate hippocampal plasticity. Interestingly, this provides a time window during which emotions can affect the robustness of hippocampal synaptic plasticity and/or memory encoding. The contextual fear conditioning paradigm employed here may be subject to amygdalar modulation. Contextual fear conditioning relies on both amygdalar and hippocampal function (Kim et al., 1993; Phillips and LeDoux, 1992), so it is likely that both brain structures were activated during training. Amygdalar activity would have been induced by inputs to the LA that convey information about the CS and the US. In fact, emotional modulation might be one way that amygdalar circuitry contributes to contextual fear memory. Furthermore, alterations of synaptic transmission within the amygdala might influence the effectiveness of amygdalar modulation of other brain structures and memory systems (Richter-Levin, 2004). It has been shown that synaptic transmission in the amygdala is altered by cued fear conditioning (McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997). Thus, we must consider the possibility that the training paradigm used may have introduced a sort of 'metaplasticity' of amygdalar modulation of hippocampal synaptic plasticity, learning, and memory.

C. The role of norepinephrine and ß-adrenergic receptors in hippocampal LTP

Evaluating brain neurochemistry in conjunction with hippocampal LTP, pharmacological manipulation of LTP, and protein expression in hippocampi from inbred mouse strains gives the opportunity to investigate the specific mechanisms of variations in LTP. This approach also affords the opportunity to test the efficacy of pharmacological intervention across a range of genetic compositions. In *Chapter 6*, the

study of the role of norepinephrine (NE) in hippocampal LTP in four inbred mouse strains revealed some interesting interactions between genetic background, LTP, and ß-adrenergic receptor activity.

Human patients with a congenital absence of the enzyme dopamine ß-hydroxylase, which is required to produce NE from dopamine, don't exhibit any obvious mental impairments (Timmers et al., 2004). However, the noradrenergic neuromodulatory system has a role in facilitating multiple memory systems (Cahill et al., 1994; McGaugh, 2000), and NE and ß-adrenergic receptor activity can also influence LTP in hippocampal area CA1 (Gelinas and Nguyen, 2005; Thomas et al., 1996). In *Chapter 6*, it was demonstrated that mouse strains with reduced levels of brain NE also exhibit reductions in one form of long-lasting LTP in hippocampal area CA1. This is an interesting correlation because previous studies indicate that reduced levels of NE or application of ß-adrenergic receptor antagonists in area CA1 do not affect LTP induced by multiple trains of high-frequency stimulation (Murchison et al., 2004; Swanson-Park et al., 1999). Thus, one might not expect that reduced NE levels would influence these forms of LTP in the inbred strains studied here. However, acute activation of ß-adrenergic receptors during four-train (compressed 3 sec inter-train interval) tetanic stimulation elicited enhancement of potentiation levels in these mouse strains to match those seen in the control strain, B6. Together, these experiments provide evidence that a particular level of ß-adrenergic receptor activation might be required for the induction of this form of L-LTP in hippocampal area CA1. This is a particularly interesting notion for several reasons. Firstly, the cAMP/PKA and MAPK signaling pathways can be recruited by the induction of L-LTP, and are involved in the maintenance of L-LTP (Abel et al.,

1997; English and Sweatt, 1997; Matthies and Reymann, 1993; Roberson and Sweatt, 1996; Rosenblum et al., 2002). ß-adrenergic receptor activation is also known to recruit these two signaling pathways (Brown et al., 2000; Gelinas and Nguyen, 2005; Giovannini et al., 2001; Thomas et al., 1996; Winder et al., 1999). It may be that the activation of ß-adrenergic receptors by NE works synergistically with tetanic stimulation to recruit the cAMP/PKA and MAPK signaling pathways and elicit the cellular and molecular processes required for L-LTP. In this case, the pharmacological activation of ß-adrenergic receptors during tetanic stimulation in an animal with adequate neural NE levels may not have an influence on LTP in area CA1 because endogenous NE levels are sufficient to mediate this synergistic effect, and occlude any possible further effects of greater NE concentrations.

Secondly, experiments have shown that activating ß-adrenergic receptors during one train of high-frequency stimulation in area CA1 can elicit translational processes in dendrites that have a role in the maintenance of a long-lasting form of LTP (Gelinas and Nguyen, 2005). This poses some interesting questions: what is the role of dendritic translation in the type of LTP studied in *Chapter 6*? Does dendritic translation contribute to the LTP induced by four trains of high-frequency stimulation (3 sec inter-train interval) in the control strain, B6, which has higher neural NE concentrations than strains 129, BALB, and C3H? Or, does dendritic translation have a special role in rescuing LTP when ß-adrenergic receptors are activated during LTP induction in strains 129, BALB, and C3H? We do know that the LTP induced by four trains of high-frequency stimulation (without isoproterenol) in *Chapter 6* relies on transcription (Young and

Nguyen, in press), but its dependence on translation (dendritic or somatic) has not been examined.

Thirdly, Western blotting experiments showed that β_1-adrenergic receptors were expressed in strains with low brain NE concentrations at levels at least comparable to those in the control strain B6. Strain BALB exhibited a significant increase in β_1-adrenergic receptor expression compared with B6. Interestingly, a study of rats with pharmacological lesions of the NE neuromodulatory system, and reduced brain NE, revealed that reduced neocortical NE levels were correlated with an increase in neocortical β_1-adrenergic receptor binding (Dooley et al., 1987). Such compensatory increases in β_1-adrenergic receptor expression also appear to occur in the mouse hippocampus (strain BALB; *Chapter 6*), but are regulated by genetic background because they do not occur in all inbred strains.

Lastly, the noradrenergic neuromodulatory system is generally considered to modulate memory in response to emotional stimuli, and also has a role in controlling attention and arousal (Cahill et al., 1994; Coull, 1998; McGaugh, 2000; Robbins, 1997; Robbins et al., 1998). It might be expected that strains BALB, C3H, and 129 should exhibit altered emotionality, stress responses, and attention/arousal compared with B6. Interestingly, strains BALB and 129 are thought to be more emotionally reactive and/or anxious than strain B6 (e.g., Bouwknecht and Paylor, 2002; Ducottet and Belzung, 2004; Kalueff and Tuohimaa, 2004; Tannenbaum and Anisman, 2003), and this may be related to changes in neural norepinephrine utilization and levels (Shanks et al., 1991, 1994). Such strain differences in emotionality and arousal may influence learning and memory (Cahill and McGaugh, 1998). Furthermore, considering the role of the noradrenergic

modulatory system and arousal in post-traumatic stress disorder (O'Donnell et al., 2004; Southwick et al., 1999), these strains may be valuable animal models in which mechanisms and pharmacological treatments for post-traumatic stress disorder might be studied.

Given the potential role of LTP in memory (Martin et al., 2000; Morris et al., 2003), the experiments discussed in *Chapter 6* lay out an interesting framework in which NE modulation might influence synaptic plasticity in hippocampal area CA1 and hippocampus-dependent memory in different inbred mouse strains. Compared with strain B6, strains 129, BALB, and C3H exhibit lower concentrations of NE in the brain, and noradrenergic neuromodulation may be reduced in these mice. One potential consequence of this, that has been tested, is that the expression of emotional memory may be reduced in these strains. Schimanski and Nguyen (2005a,b) tested cued fear memory in these strains, and found that cued fear memory is reduced in strains BALB and C3H, but not 129, compared with B6. Thus, reduced brain NE correlates with reduced emotional memory and reduced hippocampal LTP in area CA1 of strains BALB and C3H, but not in 129; perhaps mechanisms are in place to compensate behaviorally for reduced neural noradrenergic influence in strain 129. Here we have an example of one factor, reduced neural NE levels, having different consequences depending on genetic background.

D. Conclusion and Future Directions

This thesis has focused on using inbred mice, which exhibit genetic variation between strains, to explore the roles of brain structures and molecules in hippocampal synaptic plasticity, and the role of synaptic plasticity in different neural subregions in

specific forms of memory. These mice exhibited widely varying phenotypes that allowed for interesting correlations to be revealed. The core findings from this thesis work were that absence of the hippocampal commissure results in subtle changes in hippocampal information processing, LTP in the hippocampus and amygdala correlates with the expression of different forms of long-term fear memory, and reductions in neural norepinephrine levels impair normally long-lasting forms of LTP. The findings of *Chapters 3-5* concerning the relationships between hippocampal and amygdalar LTP and fear memory would be bolstered by carrying out further tests of hippocampus- and amygdala-dependent memory in the inbred mouse strains. Although fear conditioning is a valuable paradigm with which brain region-dependent memory may be gauged, further behavioural testing would help to rule out the possibility that the memory impairments characterized in this thesis research were due to dysfunction of other brain regions that contribute to fear conditioning rather than the hippocampus and/or amygdala.

Another interesting finding that warrants further experimentation is the lack of correlation between LTP in the medial perforant pathway and hippocampus-dependent memory in inbred mouse strains. In eight different inbred strains, no deficits in LTP in the medial perforant pathway were found. Like with amygdalar LTP, only one induction protocol was tested in the medial perforant pathway, opening up the possibility that if other types of LTP were examined in that pathway, perhaps deficits would be found. Nonetheless, the fact that this form of LTP was intact in eight different strains suggests that there may be something qualitatively different about medial perforant pathway LTP compared with that in the Schaeffer collateral pathway. Perhaps once induced, LTP in the medial perforant pathway is more resilient than area CA1 LTP. Such resiliency could

be advantageous, considering that much of the informational input to the hippocampus enters via this pathway, and loss of information at this stage would greatly hamper processing by subsequent synapses in the trisynaptic pathway. Further experiments should be done using different LTP induction protocols to provide more evidence that LTP in the medial perforant pathway is not as vulnerable to interference by genetic variation as that in the Schaeffer collateral pathway.

In *Chapter 5*, evidence for parallel memory phases was found in several inbred mouse strains. Compared with B6, strains BALB, B10, and SM/J exhibited significant impairments of contextual fear memory at one hour, but not 24 hours, after training. This work nicely reflects the evidence for parallel memory phases in single-gene learning mutants of *Drosophila melanogaster* (Dubnau and Tully, 1998). From the work of Izquierdo and colleagues (reviewed by Izquierdo and McGaugh, 2000), we can hypothesize about a few of the molecular mechanisms of hippocampus-dependent memory that might be impaired in strains BALB, B10, and SM/J. The activation of dopamine D_1 receptors and 5-HT_{1A} receptors, and the inhibition of mitogen-activated protein kinase kinase (MAPKK) after training all led to impaired short-term, but not long-term inhibitory avoidance memory in rats (Izquierdo et al., 1999; Izquierdo and McGaugh, 2000). Also, PKA inhibitors could block short- but not long-term memory if infused at particular time points after training (Izquierdo et al., 1999; Izquierdo and McGaugh, 2000). It might be interesting to test the activity of these receptors and kinases in the hippocampi of strains BALB, B10, and SM/J in relation to contextual fear memory. Perhaps inhibition of D_1 or 5-HT_{1A} receptors, or activation of MAPKK or PKA after contextual fear conditioning would rescue fear memory in these strains. Alternately,

Western blot analysis might be employed to examine expression of these receptors and kinases in hippocampi from these strains.

Lastly, although I have focused here on the hypotheses we can make about memory and synaptic plasticity in the inbred mouse strains studied during the course of my thesis research, the information presented in this thesis is valuable in another very important way. Genetically-modified mice, such as transgenic or knockout mice, are created on backgrounds of inbred, or F1 hybrids of two inbred strains of mice. As such, it is very important for investigators to be aware of relevant phenotypes of their background strains. If an investigator aims to enhance or impair memory or LTP by genetically modifying inbred mice, background strains should be chosen upon which enhancements or impairments will be detectable. If a poor-learner inbred mouse strain such as DBA were chosen as the background strain when the expectation is that the imposed mutation will impair memory, a falsely negative finding may be obtained. Thus, the findings of this thesis are applicable not only to those interested in the effects of genetic variation on LTP and memory, but also to those that will use these inbred strains as tools for genetic modification in their own research. However, this body of work is most valuable in showcasing the study of the relationship between specific types of LTP and memory in a novel way – by comparing phenotypes across different genetic compositions.

Table 7.1: Memory Performance Correlates with Hippocampal LTP in Some Inbred Strains

Inbred Strain	*CFC*	*MWM Probe Trial*	*Hippo. LTP*	*Type of LTP*	*References*
129 Ola		↑	↑	MPP, in vivo	(Bampton et al., 1999; Royle et al., 1999)
129S1/SvImJ	↑		↑	Area CA1, hippo. Slices	(Schimanski and Nguyen, 2005a)
129S6/SvEv	↑	↑	↓	Area CA1, hippo. Slices	(Balogh and Wehner, 2003; Cook et al., 2002; Gerlai, 2002; Holmes et al., 2002; Owen et al., 1997)
129T2/SvEms	↑	↑	↓	Area CA1, hippo. Slices	(Cook et al., 2002; Nguyen et al., 2000a,b; Wolff et al., 2002)
9XCA/Wah	↑		↑	Area CA1, hippo. Slices	(Schimanski et al., 2002)
A/J	↑		↑	Area CA1, hippo. Slices	(Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997; Schimanski and Nguyen, 2005b)
BALB/cByJ	↑		↓	Area CA1, hippo. Slices	(Schimanski and Nguyen, 2005b)
BALB/cWah1	↑		↑	Area CA1, hippo. Slices	(Schimanski et al., 2002)
C3H/HeJ	↓		↓	Area CA1, hippo. Slices	(Schimanski and Nguyen, 2005a)
C57BL/6	↑	↑	↑	Area CA1, hippo. Slices	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Chen et al., 1996; Contet et al., 2001; Cook et al., 2002; Fitch et al., 2002; Francis et al., 1995; Francis et al., 2003; Fox et al., 1999; Gerlai, 1998; Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Leitinger et al.., 1994; Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a,b; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Radulovic et al., 1998; Royle et al., 1999; Schimanski and Nguyen, 2005a,b; Stiedl et al., 1999; Upchurch and Wehner, 1988; Upchurch and Wehner, 1989; Valentinuzzi et al., 1998; Voikar et al., 2001; Wolff et al., 2002; Yoshida et al., 2001; Young et al., 2000)

Table 7.1: Memory Performance Correlates with Hippocampal LTP in Some Inbred Strains (Ctd.)

Inbred Strain	*CFC*	*MWM Probe Trial*	*Hippo. LTP*	*Type of LTP*	*References*
C57BL/ 10J	↑		↑	Area CA1, hippo. slices	(Schimanski and Nguyen, 2005b)
CBA	↓	↓	↓	Area CA1, hippo. slices	(Balogh and Wehner, 2003; Bolivar et al., 2001; Nguyen et al., 2000a,b; Nie and Abel., 2001)
DBA/2	↓	↓	↓	Area CA1, hippo. slices	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Fitch et al., 2002; Francis et al., 1995; Francis et al., 2003; Gerlai, 1998; Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a,b; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Schimanski and Nguyen, 2005a; Stiedl et al., 1999; Upchurch and Wehner, 1988; Upchurch and Wehner, 1989; Valentinuzzi et al., 1998; Young et al., 2000)
FVB/N	↓	↓	↑	MPP, in vivo	(Bampton et al., 1999; Bolivar et al., 2001; Fox et al., 1999; Owen et al., 1997; Royle et al., 1999; Voikar et al., 2001)
SM/J	↑		↑	Area CA1, hippo. slices	(Schimanski and Nguyen, 2005b)

↑ indicates high levels of memory performance or LTP; ↓ indicates impairments of memory performance or LTP. In vivo LTP was induced by high frequency stimulation to the medial perforant pathway (MPP). In most cases, area CA1 hippocampal slice LTP was induced by four trains of high frequency stimulation. CFC, contextual fear conditioning; MWM, Morris water maze. Adapted from Schimanski and Nguyen (2004).

Table 7.2: Memory Performance Correlates with Amygdalar LTP in Some Inbred Strains

Inbred Strain	*Cued Fear Conditioning*	*Amygdalar LTP*	*References*
129S1/SvImJ	↑	↑	(Bolivar et al., 2001; Cook et al., 2002; Schimanski and Nguyen, 2005a)
A/J	↓	↓	(Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997; Schimanski and Nguyen, 2005b)
BALB/cByJ	↓	↓	(Schimanski and Nguyen, 2005b; but see Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997)
C3H/HeJ	↓	↓	(Schimanski and Nguyen, 2005a; but see Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Owen et al., 1997)
C57BL/6	↑	↑	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Chen et al., 1996; Cook et al., 2002; Fitch et al., 2002; Francis et al., 2003; Gerlai, 1998; Holmes et al., 2002 (trace fear conditioning); Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Radulovic et al., 1998; Schimanski and Nguyen, 2005a; Stiedl et al., 1999; Valentinuzzi et al., 1998; Young et al., 2000)
C57BL/10	↑	↑	(Owen et al., 1997; Schimanski and Nguyen, 2005b)
DBA/2	↓	↓	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Fitch et al., 2002; Francis et al., 2003; Gerlai, 1998; Holmes et al., 2002 (trace fear conditioning); Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Schimanski and Nguyen, 2005a; Stiedl et al., 1999; Valentinuzzi et al., 1998; Young et al., 2000)
FVB	↓	↑	(DeBock et al., 2003; Bolivar et al., 2001; Owen et al., 1997)
SM/J	↓	↑	(Schimanski and Nguyen, 2005b)

↑ indicates high levels of memory performance or LTP; ↓ indicates impairments of memory performance or LTP. Amygdalar LTP was induced by five trains of high frequency stimulation in the lateral-basolateral amygdala pathway.

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*APPENDIX

Multidisciplinary approaches for investigating the mechanisms of hippocampus-dependent memory: a focus on inbred mouse strains

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A. Introduction

A challenging goal of neuroscience research is to elucidate the cellular and molecular mechanisms of learning and memory. The advent of murine transgenic and gene-targeting techniques has provided behavioral geneticists and neurophysiologists with invaluable experimental technology for clarifying the functions of particular genes, molecules, and signalling pathways in certain forms of learning and memory (Chen and Tonegawa, 1997; Martin et al., 2000; Micheau and Riedel, 1999; Picciotto and Wickman, 1998; Wehner et al., 1996). Within specific brain regions, such as the hippocampus, single genes may be artificially overexpressed (Mayford et al., 1996; see also Costantini and Lacy, 1981; Jaenisch and Mintz, 1974; Palmiter et al., 1982, for the seminal reports of the development of transgenic techniques), or their expression may be reduced or eliminated by targeted mutagenesis (e.g., Bradley, 1993; Capecchi, 1989; Soriano, 1995; Thomas and Capecchi, 1986). The physiological mechanisms of learning and memory are not fully understood, but there is good evidence that activity-dependent modifications of synaptic strength ("synaptic plasticity") are critical for specific types of learning and memory (Barnes et al., 1994; Brun et al., 2001; Doyere and Laroche, 1992; Ishihara et al., 1997; Manahan-Vaughan and Braunewell, 1999; Maren, 1999; McKernan and Shinnick-Gallagher, 1997; Moser et al., 1998; Okada et al., 2003; Roman et al., 1987; Schimanski et al., 2002; Skelton et al., 1987). Hippocampal long-term potentiation (LTP) is an activity-induced enhancement of synaptic transmission that has attracted much attention as a candidate cellular mechanism for some forms of mammalian learning and memory, but its exact roles in learning and memory are unresolved and controversial (Bliss and Collingridge, 1993; Micheau and Riedel, 1999; Shors and Matzel, 1997).

Genetically-modified mice have provided abundant insights on the specific signalling molecules responsible for the expression of LTP, learning, and memory (Mayford et al., 1995; Picciotto and Wickman, 1998). A three-pronged approach to the mechanistic analysis of learning and memory, involving molecular biological, electrophysiological, and behavioral techniques, has commonly been used to probe incisively the cellular and molecular mechanisms of mammalian learning and memory (see Figure A1; Chen and Tonegawa, 1997; Grant et al., 1992; Picciotto and Wickman, 1998; Tsien et al., 1996). However, genetic manipulation comes with a few caveats and challenges (see Gerlai, 1996, 2001; Wehner and Silva, 1996; Wolfer et al., 2002). For example, deletion or overexpression of a gene can lead to compensatory up- or down-regulation of other genes that can mask the effect of the genetic manipulation. Furthermore, these compensatory mechanisms can vary according to the animal's genetic background. Mutant mice are frequently generated using inbred strains or F1 hybrids of two inbred strains as backgrounds. Inbred strains are created by at least 20 consecutive generations of matings between siblings, resulting in genetically identical mice within a strain. Inbred strains differ from one another in genetic makeup, and they can display marked variations in neurochemistry (Ingram and Corfman, 1980), synaptic plasticity, learning, and memory (e.g., Nguyen et al., 2000a,b). Thus, directed mutation of one gene might elicit distinct compensatory mechanisms in different inbred strains, depending on the presence of other genes and signalling molecules that modify the function of the mutated gene and its encoded protein. In short, there is always potential for specific interactions between a certain gene and a particular genetic background.

Because inbred strains vary in performance on behavioral tests and display differing levels of hippocampal synaptic plasticity, the inbred strains used for genetic manipulation will influence the phenotypes of mutant mice. If an inbred strain that performs poorly on the MWM were used to generate mutant mice to test the hypothesis that knockout of a protein will impair spatial memory performance, subsequent testing of mutant mice on the MWM will produce ambiguous results. Similarly, enhancement of memory by genetic manipulations that use inbred strains that display robust memory function may lead to falsely negative findings. Thus, it is important to choose inbred strains with known behavioral and physiological phenotypes that are suited to an experiment's goal. Crawley (2000) has suggested that most studies requiring production of genetically altered mice would benefit from selecting an inbred mouse strain in which bidirectional changes in behavioral performance can be readily detected. For example, if a mutation is expected to increase memory performance, a strain with innately poor behavioral memory performance might be selected to provide a good chance of detecting modest improvements of memory performance.

The complications that arise in choosing an inbred strain background for generating mutant mice can also be viewed as an opportunity to shed new light on the neural mechanisms of learning and memory. The inherent genetic variability of different inbred strains can be studied to reveal novel mechanisms of learning and memory. Using this forward genetics approach, inbred strains may be phenotyped for particular types of learning and memory deficits, and then the molecular, cellular, and genetic correlates of these deficits may be identified. This contrasts with the reverse genetics used in the study

of genetically modified mice, in which a gene of interest is mutated, and alterations in physiology and behavior arising from the mutation are assayed (Crawley, 2000).

Forward genetics using inbred strains offers several advantages. First, the genetic variability between strains is of natural origin. The allelic differences between strains reflect those that occur via breeding within a population, and they result in detectable learning and memory phenotypes across strains. Although targeted mutagenesis can create alleles that are identical to disease alleles, directed mutagenesis is an artificial genetic manipulation that can give rise to memory impairments that might not fully replicate the molecular characteristics and etiologies of memory deficits that exist in nature. Alternatively, the more complex phenotypes found in inbred strains may more closely mimic naturally occurring memory impairments. A second advantage is that, by isolating particular types of memory deficits in individual strains, mouse models of specific memory impairments may be revealed. These mouse models can then be examined for alterations in neurophysiology and/or synaptic plasticity compared with a mouse strain with intact memory. For example, Schimanski et al. (2002) compared two inbred strains of mice, BALB/cWah1 and 9XCA/Wah, for brain anatomy, hippocampal synaptic plasticity, and cued and contextual fear conditioning and extinction (see **Figure A2**). In comparison to BALB/cWah1, 9XCA/Wah was found to have deficient contextual fear extinction that was correlated with a reduction of the hippocampal commissure and deficient paired-pulse facilitation in hippocampal region CA1. This study shows that incorporating a multi-faceted approach to examining inbred strain differences can lead to significant advances in determining the roles of particular brain regions (and types of synaptic plasticity) in specific aspects of learning and memory.

Finally, inbred mouse models of memory impairment can be used to identify ways to enhance learning and memory through experiential or pharmacological intervention.

Two key studies exemplify the use of inbred strains to develop manipulations that improve learning and memory. First, the 129X1/SvJ inbred mouse strain performs poorly on the hidden platform version of the MWM but performs well on the visible platform version of the MWM (Leil et al., 2002; Owen et al., 1997), and thus may be a good model for studying the mechanisms that underlie defective spatial learning and impaired spatial memory. Kempermann et al. (1998) housed 129X1/SvJ mice in an enriched environment (a large cage with toys, running wheels, and tunnels) for 40 days, and then tested them on the hidden platform MWM. 129X1/SvJ mice that were in the enriched environment performed better on the MWM than control mice that were housed in standard laboratory caging. This improved performance correlated with an increased number of proliferating neurons in the hippocampal dentate gyrus of this mouse strain. Similarly, Fordyce and Wehner (1993) determined that eight weeks of physical activity (moderate treadmill running) increased spatial learning performance in DBA/2, which correlated with changes in hippocampal protein kinase C (PKC) activity. Secondly, Wehner et al. (1990) determined that DBA/2 inbred mice perform poorly on the Morris water maze, which correlates with reduced hippocampal PKC activity. Fordyce et al. (1995) and Smith and Wehner (2002) treated DBA/2 mice with oxiracetam and aniracetam, respectively, prior to behavioural testing. These cognition-enhancing drugs significantly improved spatial learning on the Morris water maze (Fordyce et al., 1995), and improved memory for contextual fear conditioning (Fordyce et al., 1995; Smith and Wehner, 2002). Interestingly, these treatments also elicited significant increases in

activated membrane-bound ?-PKC. From a broader perpective, studies such as these emphasize that the behavioral phenotypes of inbred mice can be used to both elucidate mechanisms of memory dysfunction and to develop behavioral or pharmacological strategies to reduce memory dysfunction by targetting cellular or molecular substrates believed to be important for normal memory processing.

The use of inbred strains to identify cellular and molecular contributions to memory impairment has some disadvantages. Because the genetic differences between strains are not artificially imposed and controlled by the experimenter, it can be difficult to discern exactly which genes are different from strain to strain. Furthermore, because multiple genes are different between strains and many cognitive traits are polygenic, it can be difficult to determine which genes are responsible for altered cognitive phenotypes. These difficulties can be partially resolved by incorporating sophisticated, sometimes complex, genetic analyses (e.g., quantitative trait loci, or QTL, analysis; microarray techniques). These techniques will be discussed in more depth in subsequent sections of this review.

There is abundant data on the behavioral performance of inbred mice on tests of hippocampal learning and memory. In contrast, much less information is available concerning the synaptic and molecular mechanisms underlying strain-dependent differences in hippocampal learning and memory. For new investigators planning to enter this promising field, gaining familiarity with the cumulative literature on hippocampal memory function in general, and strain-dependent differences in hippocampal learning and memory in particular, can be a daunting task. For those more familiar with this field, it is important to take note of current advances that elucidate key behavioral and

cellular/molecular distinctions in inbred mouse strains. This may guide research that aims to reveal the causes of strain-dependent variations in hippocampal learning and memory. With these two target audiences in mind, we here summarize and discuss selected techniques and data from studies that have examined hippocampus-dependent learning, memory, and hippocampal synaptic plasticity in several inbred mouse strains. We identify inbred mouse models of hippocampus-dependent learning and memory deficits and relate these deficits to hippocampal synaptic plasticity and neurochemical differences between strains. Finally, we suggest further experiments utilizing inbred strains that may shed light on the causes of memory impairment.

B. Cued and Contextual Fear Conditioning: General Methodology

Fear conditioning tasks measure an animal's ability to learn and remember an association between an auditory tone and footshock (cued fear), or between an environment and footshock (contextual fear). These tests take advantage of the natural tendency of mice to freeze in response to fearful stimuli (Crawley, 2000). Both tasks are sensitive to lesions of the amygdala, but contextual fear conditioning is also disrupted by hippocampus lesions (Holland and Bouton, 1999; Kim et al., 1992; Phillips and LeDoux, 1992). Thus, by testing both cued and contextual fear conditioning in a population of mice, deficits in amygdalar and hippocampal function may be studied.

The general procedures for cued and contextual fear conditioning are illustrated in **Figure A3.** The tests require two environments. One, which is used for training and contextual testing, has an electrifiable floor usually made of metallic rods. The walls and ceiling may be made of metal or plastic. Most of these conditioning boxes (available commercially) have a rectangular base and can vary in size. The second environment

should be quite different from the first. It may be of different shape (e.g., triangular base), constructed of different material (e.g., smooth floor rather than metal rods, or walls of different texture or color) and scented differently (e.g., with orange extract or lemon cleaning solution). In addition to the conditioning boxes, a shock delivery system is required, along with a sound source. White noise, or tones of 80-85 dB intensity, are often used as the conditioning stimulus.

During *training*, the mouse acclimates to the electrifiable chamber for 2 min. This is followed by a 30 sec presentation of the conditioned stimulus (CS), a tone or white noise, which co-terminates with a 2 sec footshock of 0.35-1.5 mA intensity (unconditioned stimulus, US). A single pairing of CS and US is adequate to induce strong learning of an association between CS and US in some inbred strains (e.g., Nguyen et al., 2000a; Paylor et al., 1994; Valentinuzzi et al., 1998), but some labs have used up to 6 pairings of CS and US in training their mice (e.g., Chaudhury and Colwell, 2002). After the final CS-US pairing, the mouse is left in the training chamber for 30 sec longer, and then removed to the home cage. Throughout training, the mouse is observed for freezing behavior. This may be done either by an experimenter with a stopwatch and pad of paper, or by an automated system (Contarino et al., 2002; Valentinuzzi et al., 1998).

After 24 hrs, or another time period, the mouse is tested for *contextual fear conditioning*. It is placed in the training chamber for 5 min with no tone or shock presentation, and it is observed for freezing behavior. An hour later, the mouse may be tested for freezing in an *altered context*, and then for *cued fear conditioning*. For these tests, the mouse is placed in a second, different chamber and observed for freezing for 2-

3 min (altered context). Then, the CS is presented for 3 min, during which freezing behavior is also observed (cued fear conditioning). The best indices of contextual and cued fear are freezing during the contextual and cued test, respectively, in relation to freezing in the altered context. This accounts for such factors as general activity level, stress, and handling. Although we have focussed on these forms of contextual and cued fear conditioning, other hippocampus-dependent forms of fear conditioning (such as contextual discrimination (Frankland et al., 1998) and trace fear conditioning (Huerta et al., 2000; McEchron et al., 1998) are effective for measuring hippocampus function.

C. Survey of Fear Conditioning in Inbred Mice

Table A1 summarizes cued and contextual fear conditioning results in a number of inbred strains. Because very few studies examined sex differences within or between strains, this variable was not included in the table. A strain was rated to have 'good' performance if it performed comparably to another strain that displayed good freezing behavior (i.e., C57BL/6). A strain was rated as 'intermediate' if some memory was evident but was not expressed as strongly as in a 'good' performing strain, or if memory was only evident under restricted conditions. 'Poor' ratings were given to those strains that failed to show any evidence of having fearful memories.

About half of inbred strains tested exhibited good memory for both contextual and cued fear conditioning at 24 hrs after training (see **Table A1** for references). All "129" substrains that were tested, and strains C57BL/6, C57BL/10, LP, and SJL performed well. These strains can be postulated to have intact hippocampal and amygdalar function underlying contextual and cued fear memory.

Several strains exhibited poorer contextual than cued fear memory. These strains, including AKR, BALB/cByJ, C3H/He, DBA, and FVB/N, probably have some impairment in hippocampal function. Because some cued memory is still intact in these strains, amygdala function is likely intact. Thus, the hippocampal contribution to contextual fear memory, but not the amygdalar contribution, is probably diminished in these strains. These five strains are potential models for the study of hippocampal memory deficits, and further work is needed to pinpoint the physiological mechanisms that underlie these behavioral memory deficits.

A number of inbred strains are homozygous for the age-related hearing loss mutation *ahl*. Hearing loss might affect the ability of these strains to perform in the cued fear conditioning paradigm. Strains BALB/cByJ, BuB/Bn, and C57BL/6 exhibit hearing loss beginning at about 10 months of age (The Jackson Laboratory, 2003). However, BALB/cByJ and C57BL/6 exhibited intact cued memory, and most groups have used mice younger than 10 months. Thus, hearing loss probably did not influence the outcomes of cued conditioning experiments for these three strains. A/J and DBA/2J exhibit earlier onset hearing loss (around 3 months) as a result of *ahl* (The Jackson Laboratory, 2003), and both were rated 'intermediate' on the cued fear conditioning test. Thus, expression of cued memory may be compromised in these two strains because of hearing loss.

Some of the strains tested for contextual and cued fear conditioning also carry the retinal degeneration allele, $Pde6b^{rd1}$,and could have vision deficits (The Jackson Laboratory, 2002). However, Bolivar et al. (2001) determined that this mutation does not result in fear conditioning deficits in C3H/He mice. Vision is likely not essential for

strains to perform well on the fear conditioning tests. It should also be noted that strain A/J has been described to exhibit generalized freezing (Owen et al., 1997), and this must be taken into consideration when conducting fear conditioning in this strain.

D. Morris Water Maze: General Methodology

The Morris water maze (MWM) requires animals to navigate within a circular pool, using spatial cues, in search of a hidden escape platform (Morris, 1984). This task relies on the motivation of animals to escape water and climb onto the hidden platform as quickly as possible; escaping the water serves as a positive reinforcement for this task (Crawley, 2000). The spatial learning involved in successfully completing the MWM is hippocampus-dependent; hippocampal lesions impair acquisition of the task in rats (Eichenbaum et al., 1990; Morris et al., 1982) and mice (Logue et al., 1997).

An overview of the procedures used in the MWM is illustrated in **Figure A4**. The pool is circular and can vary in diameter from about 60-150 cm. It is filled with water made opaque by the addition of skim milk powder or non-toxic white paint. The hidden platform is usually made of clear or white Plexiglas, and is anchored to the bottom of the pool. Water covers the platform to a depth of 1-2 cm. A video camera may be mounted overhead and connected to an automated video tracking system. Extramaze visual cues are mounted around the pool at fixed positions. They can include geometric patterns or shapes on nearby walls, equipment and/or furniture within the room, curtains or walls of different colours, cages of animals waiting to be tested, or the experimenter. All of these cues must be maintained consistently throughout training and testing.

The training and testing protocols used in the MWM vary widely between labs. An overview of the most common approach is provided here. *Pretraining* usually

precedes training by a day, or alternately, is done immediately before the first training trial. Pretraining involves first placing the mouse on the hidden platform. The mouse will usually jump off the platform into the water and swim (Crawley, 2000), and if not, the mouse is removed from the platform and placed gently in the water. The mouse is allowed to swim for 15-30 sec, and then it is guided back to the platform and allowed to climb onto the platform. One to three pretraining trials are adequate for most strains of mice to learn to climb onto the platform to escape the water, but in some cases, more trials might be necessary (Crawley, 2000). The platform may be moved from trial to trial during pretraining to ensure that the mice do not habituate to a particular platform position. Alternately, pretraining may be done in another pool in a different room.

Following pretraining, the mouse is given a series of *training trials*. It is placed in the pool facing a wall such that its heading is not biased when it starts to swim. It is given about 60 sec to swim around the pool and find the hidden platform. If it does not find the platform within that time it is placed on the platform. After the mouse reaches the platform it is kept there for 15-60 sec. The starting position is randomised across trials so that the mouse must rely on spatial cues, and not on static sequences of movement, to find the platform. The training trials are either spaced, with few trials per day over a period of 1-2 weeks, or they are massed, with many trials per day for 2-4 days. Examples might be having one trial per day for 14 days (spaced) or 8 trials per day, in two blocks of 4 trials, for 3 days (massed). Most frequently, within a block of trials a mouse is given a few minutes between trials to rest, and a longer period between blocks to dry off. Over the series of training trials, successful mice will decrease the time and path length that they swim in order to reach the hidden platform.

A *probe trial* is given after the last training trial. The hidden platform is removed from the pool and the mouse swims for one minute. The path that the mouse swims is tracked and analysed for the proportion of swim time and/or path length spent in each quadrant of the pool, swim speed, and the number of times the path crosses the former location of the hidden platform. The Gallagher Proximity Measure may also be computed (Gallagher et al., 1993). A mouse that has learned and remembered the location of the hidden platform will spend significantly more time swimming in the quadrant that contained the hidden platform, and it will cross the former location of the platform significantly more times than it crosses corresponding locations in other quadrants.

Many experimenters also conduct a *visible platform task* in which the motor ability and visual acuity of the mouse (both important in MWM performance) are tested. This task is similar to the training trials of the MWM, but the platform is made visible to the mouse. It may be elevated such that it stands just above the water level, or a flag may be attached to the platform to indicate its position.

E. Survey of MWM Data in Inbred Mice

Table A2 summarizes MWM learning and probe trial results for a variety of inbred strains. Ratings were assigned to inbred strains using the same criteria as for fear conditioning.

In most cases, within a strain, mice performed similarly on the learning and probe trials – that is, if a strain was capable of learning during the training trials, it also performed well on the probe trial. Many strains performed well on the MWM learning and probe trials (see **Table A2** for references). C57BL/6 performed well on both,

justifying its use as a strain commonly used to generate mutant mice, and as a "standard" performance strain for multi-strain comparisons of spatial learning and memory ability. C57BL/10J also performed well, as did most of the "129" substrains, with the exception of 129X1/SvJ.

Many of the strains that showed poor performance on the MWM also performed poorly on the visible platform task. This indicates that they are likely impaired in visual perception of spatial cues. Thus, the poor performance of strains A, BALB/cByJ, BuB/BnJ, C3H, CBA/J, FVB/N, and SJL on the MWM does not reflect deficits in learning abilities, because these strains did not successfully complete the visible platform task. Several of these strains are albino (A, BALB/cByJ, BuB/BnJ, FVB/N, SJL) and as a result may have poor vision. In addition, BuB/BnJ, C3H, CBA/J, FVB/N, and SJL are homozygous for the retinal degeneration allele *Pde6b*^{rd1} (The Jackson Laboratory, 2002), which compromises vision. 129X1/SvJ and BALB/c have not been tested on the visible platform task, but they are also albino, which may contribute to their poor and intermediate performance, respectively, on the MWM. The strains DBA/2, DBA/2J-I, and LP have no known visual impairments, and yet they still performed poorly on at least one aspect of MWM testing (see also Experiment 2B in Paylor et al., 1993). These three strains most likely have impaired spatial learning and memory as a result of altered hippocampal function. However, an important note is that some groups have shown that DBA/2 mice can display good probe trial performance (see **Table A2** for references). This disagreement between groups may be explained by small differences in methodology, genetic drift between different mouse suppliers, or variations in rearing and in housing environments. Crabbe et al. (1999) have shown that even when separate

groups go to great lengths to replicate methods, behavioral tests done in different locations can yield significantly distinct results.

In summary, several strains have been tested on both the MWM and contextual fear conditioning. Inbred strains that performed well on both the MWM and contextual fear conditioning include 129S6/SvEv, 129T2/SvEmsJ, C57BL/6, and C57BL/10. Of the strains examined in this review, these strains are the most likely to have intact hippocampal function. In contrast, BALB/c showed intermediate performance on both tests. CBA, DBA/2 (**see Figure A5a,b**), and FVB/N performed poorly on both tests and most likely have impaired hippocampal function. However, CBA and FVB/N may have performed poorly on the MWM due to deficits in visual acuity.

Other strains showed divergence in performance on these two tests of hippocampus-dependent learning and memory (i.e., 129X1/SvJ, A, BALB/cByJ, C3H, LP, and SJL). Although the MWM task and contextual fear conditioning have both been shown to depend on intact hippocampal function, they may require distinct hippocampal processes that engage different hippocampal circuits or recruit certain types of synaptic plasticity (e.g., Bannerman et al., 1999; Ferbinteanu and McDonald, 2001; Hock Jr and Bunsey, 1998; Maruki et al., 2001; Moser et al., 1993). Both tasks also depend on brain regions outside of the hippocampus. If extra-hippocampal brain structures are dysfunctional in a particular strain, it may show a memory impairment when compared with another strain that has equivalent hippocampal but normal extra-hippocampal function. Thus, it is advisable to conduct several behavioral tests of hippocampal function that vary in their documented dependence on extra-hippocampal brain structures.

F. Survey of Hippocampal Synaptic Transmission in Inbred Mouse Strains

Hippocampal synaptic plasticity in mice has often been examined along with behavioral performance (see reviews Chen and Tonegawa, 1997; Martin et al., 2000). The common goal of these studies is to demonstrate a causal relationship between certain types of hippocampal synaptic modification, such as long-term potentiation (LTP) or long-term depression (LTD), and specific forms of hippocampus-dependent learning and memory, such as spatial or contextual learning. LTP and LTD represent activity-dependent enhancement and reduction, respectively, of excitatory synaptic transmission, and they have been suggested to play key roles as regulators of information processing in the mammalian brain (Bear and Abraham, 1996; Bliss and Collingridge, 1993; Martin et al., 2000). The issue of whether LTP, or other forms of synaptic plasticity, is causally linked to particular types of learning and memory is hotly debated (for discussion, see Martin et al., 2000). There is evidence that LTP can critically influence the expression of some types of learning and memory (e.g., spatial learning and memory: Brun et al., 2001; Moser et al., 1998).

There is little detailed information on the comparative electrophysiology of hippocampal neurons in inbred strains of mice. Below, we review the few studies that have started to investigate this important aspect of the comparative physiology of inbred strains.

Table A3 summarizes studies that have examined hippocampal synaptic transmission in inbred mice. Fewer inbred strains have been studied for hippocampal synaptic transmission than for expression of hippocampus-dependent learning and memory. Several strains exhibit impairments in particular forms of synaptic plasticity,

including 129/SvEv, 129T2/SvEmsJ, CBA/J, and DBA/2. Deficits in LTP were detectable using both *ex vivo* (hippocampal slice) and *in vivo* preparations (see ***Notes*, Table A3**).

The most interesting finding in **Table A3** is that LTP deficits can vary greatly between inbred strains. It does not appear that an inbred strain has 'good' LTP or 'poor' LTP *per se*, with the exception of C57BL/6, which has been chosen to represent a "standard" for robust LTP. Rather, most strains show selective deficits in a subset of LTP variants. Furthermore, the composition of the deficient subset is different between strains.

LTP induced by different types of tetanic stimulation can rely on different cellular and molecular mechanisms for induction and maintenance. For example, in area CA1 of hippocampal slices, one train of high-frequency stimulation (HFS) yields LTP that decays to baseline within 1-2 hours ("early" LTP, or "E-LTP") and is dependent on the activation of particular kinases (such as PKC and Ca^{2+}/calmodulin-dependent protein kinase II and NMDA receptors (Collingridge et al., 1983; Malinow et al., 1989; Silva et al., 1992). E-LTP does not require protein synthesis or activation of PKA (Collingridge et al., 1983; Malinow et al., 1989). However, if three or more trains of HFS are delivered to CA1 within a particular time frame, "late", long-lasting LTP (L-LTP) can be induced and it can last for several hours in slices (Andersen et al., 1977). The maintenance of L-LTP in CA1 requires protein synthesis and the activation of other molecules, including PKA and CRE-mediated gene expression (Abel et al., 1997; Bourtchouladze et al., 1994; Frey et al., 1988, 1993; Huang and Kandel, 1994; Impey et al., 1996; Matthies and Reymann, 1993; Nguyen et al., 1994; but see Matsushita et al., 2001; Schulz et al., 1999).

Thus, it is plausible that if any of these signalling pathways are expressed differentially in particular inbred strains, then specific forms of LTP may be altered in these strains. Indeed, the finding that LTP deficits can vary across inbred strains indicates that the different genetic makeup of the strains affects the availability and/or function of the cellular and molecular mechanisms, or the neural anatomy and structure, that underlie different types of hippocampal LTP. For example, Nguyen et al. (2000b) demonstrated that induction and maintenance of CA1 LTP were increased in 129T2/SvEmsJ hippocampal slices following stimulation by 4 trains of HFS at 3 sec intervals as compared to stimulation by 4 trains of HFS at 20 sec intervals. Thus, the same total number of stimuli applied on a slightly different time scale yielded LTP with differing magnitudes in this strain. In contrast, in the other three strains tested in that study (C57BL/6J, CBA/J, and DBA/2J), changing the inter-train interval did not affect the magnitude of LTP. The authors concluded that the hippocampal neurons of some mouse strains might be optimally tuned to particular temporal patterns of synaptic activity due to variations in genetic background. It is unclear which specific properties of hippocampal neurons in strain 129T2/SvEmsJ differ from those of the other strains examined by Nguyen et al. (2000b). The authors examined several biophysical and cellular electrophysiological properties of CA1 pyramidal neurons (including spike-frequency accommodation, membrane input resistance, and number of spikes elicited by current injection) in these strains and found no differences between strains. Thus, the frequency-dependent deficit in LTP observed in this study cannot be readily explained by inter-strain variations in the biophysical and spike-firing properties of hippocampal neurons.

Rather, this L-LTP deficit is potentially the result of biochemical alterations in 129T2/SvEmsJ (e.g., modified spatial localization of kinases such as PKA).

G. Inbred Mouse Models of Intact and Impaired Hippocampus-Dependent Memory

Does LTP underlie hippocampal learning and memory? One way to address this important question is to examine synaptic plasticity in inbred strains that display good or poor hippocampal learning and memory. Because relatively few studies have compared hippocampal synaptic plasticity across inbred strains, our ability to correlate memory impairments with altered synaptic plasticity is currently limited. In **Table A4**, we have summarized the relationships between memory performance and hippocampal LTP for all strains in which both have been measured. As is evident from **Table A4**, the correlations between memory and LTP are not perfectly consistent, but it appears that some strains that exhibit poor hippocampus-dependent memory also exhibit poor hippocampal LTP. More multidisciplinary studies will be necessary to adequately define the relationships between particular types of hippocampal LTP, learning, and memory in multiple inbred strains. The next section will examine such relationships in the small number of strains in which a variety of behavioral and electrophysiological studies have provided enough data to form testable hypotheses.

Because of its consistently good performance on the MWM and contextual fear conditioning (see **Tables A1 and A2**), and also on other tests of hippocampus-dependent learning and memory such as the Barnes maze (Nguyen et al., 2000a) and the radial arm maze (Ammassari-Teule and Caprioli, 1985; Ammassari-Teule et al., 1993,2001; Rossi-Arnaud et al., 1991; Roullet and Lassalle, 1992, 1995; Roullet et al., 1997; Schwegler et al., 1992; Zilles et al., 2000), the C57BL/6 strain is considered to be a mouse model of

good hippocampus-dependent learning and memory. In vivo, robust LTP can be induced in the dentate gyrus of C57BL/6 mice by one or three trains of HFS, and by theta-burst stimulation (Jones et al., 2001; Matsuyama et al., 1997). Multiple types of LTP can be induced in CA1 of C57BL/6 hippocampal slices (Gerlai, 2002; Nguyen et al., 2000a,b). One train of HFS, four trains of HFS at different intertrain intervals, theta-burst stimulation, and application of forskolin (an adenylyl cyclase activator) all induced LTP. Thus, the performance of C57BL/6 mice on hippocampus-dependent memory tasks correlates well with LTP induction and expression in area CA1 of hippocampal slices from this strain.

Another inbred strain that performs well on both contextual fear conditioning and the MWM is 129T2/SvEms (Cook et al., 2002; Nguyen et al., 2000a; Wolff et al., 2002). Nguyen et al. (2000a) found a slight impairment in this strain on the Barnes maze, but they proposed that this was due to lack of motivation. Thus, despite this subtle motivational factor concerning Barnes maze performance, 129T2/SvEms may be another model of good hippocampal learning and memory. Some forms of LTP are expressed well in CA1 in 129T2/SvEms hippocampal slices, such as late-LTP induced by 4 trains of HFS at a compressed inter-train interval of 3 sec and LTP induced by forskolin application (Nguyen et al., 2000b). Paired-pulse facilitation (PPF) and LTD (long-term depression) are also robustly expressed in 129T2/SvEms and C57BL/6. However, E-LTP induced by one train of HFS, theta-burst LTP, and L-LTP following spaced inter-train intervals of 20 sec and 5 min were all impaired in 129T2/SvEms (Nguyen et al., 2000a,b). Thus, there is a dissociation between some forms of hippocampal LTP and behavioral learning and memory as measured by contextual fear conditioning and the

MWM in 129T2/SvEms. Similarly, Reisel et al. (2002) found hippocampal area CA1 LTP deficits in gene-targeted mice that lack the AMPA receptor subunit GluR1, but these mice exhibited intact spatial reference memory for the hidden platform MWM task.

DBA/2 performs poorly on contextual fear conditioning and displays mixed results in the MWM (see **Tables A1** and A2), and is generally considered to display poor hippocampal learning and memory. As a result, DBA has been extensively studied in an effort to elucidate molecular mechanisms for its impairment in hippocampal learning and memory. Several types of hippocampal synaptic plasticity are intact in DBA/2, as compared to C57BL/6. For instance, in the dentate gyrus *in vivo*, PPF is intact, and three trains of HFS (Matsuyama et al., 1997) and theta-burst stimulation result in good LTP (Jones et al., 2001). However, Bampton et al. (1999) found that multiple trains of HFS induced LTP that was initially good but then decayed faster than in other inbred strains. In area CA1 of hippocampal slices, early LTP induced by one train of HFS was intact, as were LTD and L-LTP induced by four trains of HFS delivered at spaced (5 min) intervals (Nguyen et al., 2000a,b). However, several types of hippocampal area CA1 synaptic plasticity were found to be impaired in DBA/2, including PPF. Theta-burst stimulation, application of forskolin, and four trains of HFS delivered at more compressed intervals (20 sec and 3 sec) resulted in deficient LTP. Because PPF was impaired in CA1 of DBA/2 hippocampal slices, Nguyen et al. (2000a) hypothesized that presynaptic calcium dynamics, or synaptic vesicle proteins that mediate calcium-triggered transmitter release, may be altered in CA3 presynaptic nerve terminals. These could, in principle, account for some of the LTP deficits that were found in DBA/2, but more definitive experiments need to be done to address these issues.

Because few experiments have conjointly examined hippocampal synaptic plasticity and hippocampus-dependent learning and memory in inbred mice, the task of correlating these phenomena can be difficult. Different laboratories conduct learning and memory testing using distinct methods, and they can often arrive at conflicting conclusions. This makes it difficult to arrive at a consensus on the learning and memory abilities of inbred strains, and also hampers the ability to relate these cognitive functions to cellular and molecular phenomena such as LTP. Furthermore, even when labs try their best to replicate data, their results may not be in agreement because of multiple factors that cannot be rigorously controlled (Crabbe et al., 1999). One solution might be to perform conjoint examinations of cognitive function and cellular/molecular properties within the same mouse, which may provide a more powerful means to correlate these measures. Another source of divergence is the use of different substrains and different mouse suppliers. Although all mice of a particular inbred strain were derived from the same original brother-sister matings, long-term maintenance of these inbred strains by different suppliers or under different conditions can allow genetic drift to occur within these isolated populations. Similarly, there is some evidence that strain differences might result not only from genetic differences but also from different types of maternal care (Francis et al., 2003). Furthermore, different types of LTP, or different brain regions (Ammassari-Teule et al., 2000), may support learning and memory in distinct inbred mouse strains. We must acknowledge that LTP measured in vitro or in vivo, induced by artificial electrical stimulation or by chemical application, may not correlate perfectly with a particular mouse strain's ability to perform hippocampus-dependent contextual fear conditioning or spatial learning. However, there may be general properties of

hippocampal LTP that correlate with learning and memory, such as LTP induction and persistence.

H. Potential Molecular Substrates for Memory Impairments in Specific Strains

Cellular and molecular differences between inbred strains likely contribute to the modifications of learning and memory that have been observed. For instance, several neurochemical differences between DBA/2 and C57BL/6 have been reported in the literature. Compared to C57BL/6 mice, DBA/2 mice have reduced hippocampal PKC activity (**see Figure A5 and Table A5**; Bowers et al., 1995; Wehner et al., 1990; Paylor et al., 1996). PKC is required for induction of LTP (Malinow et al., 1989), and it has a role in hippocampal learning and memory (Abeliovich et al., 1993; Olds and Alkon, 1991; Van der Zee et al., 1992). Paylor et al. (1996) found that hippocampal γ-PKC expression is significantly reduced in DBA/2 mice as compared to C57BL/6 mice beginning at an age of 24 days. Remarkably, also at 24 days of age, C57BL/6 mice begin to outperform DBA/2 mice on spatial learning measured using the Morris water maze (Paylor et al., 1996). In addition, Young et al. (2000) found that GAP-43, a substrate for PKC, is less phosphorylated in the hippocampus following contextual fear conditioning training in DBA/2 than in C57BL/6 mice, which is consistent with the finding that hippocampal PKC activity is reduced in DBA/2 mice. McNamara et al. (2003) also found that hippocampal MARCKS (myristoylated alanine-rich C kinase substrate) mRNA and protein expression (primarily in the cytosolic fraction) is greater in DBA/2 than in C57BL/6 mice. This difference might result from altered transcription and/or from different PKC levels between the two strains. PKC downregulates and destabilizes MARCKS mRNA and protein, independent of transcription (Brooks et al., 1992). Thus,

the higher level of PKC in the C57BL/6 hippocampus may inhibit MARCKS levels, resulting in the strain difference between C57BL/6 and DBA/2. This interaction between PKC and MARCKS in DBA/2 mice may also underlie their deficits in hippocampal synaptic plasticity (**Table A3**). Fordyce et al. (1994) determined that basal expression of *Zif/268*, an immediate-early gene that is upregulated during particular types of associative learning, and by LTP-inducing tetanic stimuli (Davis et al., 2003), is lower in DBA/2 than in C57BL/6. Finally, Zilles et al. (2000) found that the density of hippocampal muscimol binding sites ($GABA_A$ receptors) in CA1 and the dentate gyrus was reduced in DBA/2 mice, which correlated with poor spatial learning in the radial arm maze. Reduced density of AMPA receptors and kainate binding sites in the DBA/2 hippocampus also correlated with poor spatial learning in the radial arm maze (Zilles et al., 2000).

Some groups have asked whether the hippocampus is as important for contextual fear conditioning in the DBA/2 mouse as it is in C57BL/6. Logue et al. (1997) determined that in DBA/2 mice, hippocampal lesions (induced by injection of a kainic acid and colchicine cocktail) do not significantly reduce contextual fear responses as compared to sham controls. The authors concluded that DBA/2 mice probably do not use their hippocampi to the same degree as C57BL/6 mice during the contextual fear task. To test this hypothesis, Ammassari-Teule et al. (2000) gave bilateral ibotenic acid lesions to three brain areas in C57BL/6 and DBA/2 mice, the dorsal hippocampus, basolateral amygdala, and shell of the nucleus accumbens, before fear conditioning training. They found that all three lesions reduced freezing to the context in C57BL/6 mice. However, sham DBA/2 mice exhibited a low level of freezing to the context that was reduced only

by basolateral amygdala and nucleus accumbens shell lesions, and not by dorsal hippocampal lesions. The authors concluded that the hippocampus is likely contributing minimally, if at all, to contextual fear conditioning in DBA/2 mice. They also suggested that the small contextual fear conditioning response obtained in DBA/2 mice might result from the association of one particular cue with the US rather than an association of the training environment with the US. This is a reasonable suggestion for two reasons. First, DBA/2 mice exhibit good cued fear conditioning, which is sometimes more robust than that in C57BL/6 mice, indicating that DBA/2 mice do have the capacity to learn an association between CS and US. Second, recall that contextual fear conditioning is dependent on both the hippocampus and the amygdala. However, the amygdala facilitates cued fear associations without hippocampal involvement (Kim et al., 1993). Because lesions of the amygdala impaired the contextual fear conditioning response, the contextual fear response measured in DBA/2 might be due just to associations with individual cues and not with the context. Alternatively, Balogh et al. (2002) found that when DBA/2 mice are tested for contextual fear conditioning at different time points after training, different levels of impairment are evident. Specifically, DBA/2 mice have contextual fear impairments soon after training and later than 24 hours after training. No deficits were apparent from one to three hours after training. They suggest that some form of hippocampus-to-neocortical 'dialogue' is impaired in DBA/2 mice, preventing consolidation of long-term memory for the contextual fear association. All of these studies indicate major impairments in hippocampal function in DBA/2 mice, which are likely also compromising the ability of the DBA/2 hippocampus to integrate its function with other interconnected brain regions.

I. Future Directions: Probing for Mechanisms of Memory Impairment

Some inbred mouse strains have impairments in particular types of hippocampus-dependent memory. To date, most of these mouse models of hippocampal amnesia have not been studied in depth, and they remain an untapped resource for elucidating novel mechanisms of memory impairment. In this section, we discuss some ways by which these mechanisms might be elucidated.

Spatial memory impairments are of particular interest because the hippocampus is involved in forming spatial representations and it contributes to the formation of spatial memories. Specifically, within the hippocampus, *place cells* can fire preferentially when a rodent is in particular spatial locations. These place fields can form within minutes and are long-lasting (reviewed in Eichenbaum and Cohen, 2001; Shapiro, 2001). Furthermore, manipulations that interfere with the induction of LTP and with spatial learning also destabilize these place fields (e.g., PKA mutant mice: Rotenberg et al., 2000; CREB and CaMKII mutant mice: Cho et al., 1998; Rotenberg et al., 1996; NMDA receptor mutants: McHugh et al., 1996; and NMDA receptor blockade: Kentros et al., 1998). In fact, place fields have been postulated to play an integral role in spatial memory formation (Moser and Paulson, 2001; Shapiro, 2001; Wilson and Tonegawa, 1997). For example, place fields are less stable and are less likely to be retrieved in aged rats as compared to younger adult rats, and these attributes correlate with diminished performance in aged rats on tasks that rely on the retrieval of spatial representations (reviewed by Rosenzweig and Barnes, 2003). Place field instability in an inbred strain with impaired spatial memory would identify the hippocampus as a prime locus of cognitive dysfunction in inbred strains and would strengthen the correlation between

hippocampal dysfunction and poor spatial memory performance. Furthermore, such a finding would underscore hippocampal circuitry as a target for directed anatomical and functional analyses of spatial memory impairments.

Another method that may be employed to pinpoint the neural locus of dysfunction in amnesic mouse strains is Fos staining. This may help to determine which brain regions are involved in executing behavioral tasks. For instance, inbred strains might be given a single training trial for contextual fear conditioning, and after an interval during which long-term memory could be established (e.g., 90 minutes), brain tissue could be fixed and then immunohistochemically stained for Fos-protein, the product of the immediate-early gene *c-fos* (Herdegen and Leah, 1998). Areas that stain positively are considered to be activated during learning of the behavioral task carried out before tissue staining (note that Fos can also be activated by other aspects of the training experience, such as exposure to the context, or handling). Fos staining might be a valuable tool for discerning whether particular mouse strains are using their hippocampi (or other brain structures) to the same degree as other strains in carrying out specific behavioral tasks known to require hippocampal function. For example, this method could be used to test Ammassari-Teule et al.'s (2000) hypothesis that DBA/2 mice do not engage hippocampal function during contextual fear conditioning.

In addition, a specific type of functional magnetic resonance imaging (fMRI) has been developed that enables the analysis of resting activity levels in particular brain regions of the mouse (Small et al., 2000). Because the method relies on a measure of resting oxygenation (rather than temporal changes in oxygenation), the spatial resolution of this modified fMRI (submillimeter) can be greater than that of other currently used

functional imaging techniques. Small et al. (2000) demonstrated this increased resolution by measuring signal intensity in the hippocampal CA1 region in R(AB) transgenic and wild-type mice. R(AB) mice have a 50% reduction of basal hippocampal PKA activity, and they display both deficient hippocampal late-LTP and impaired hippocampus-dependent long-term memory (Abel et al., 1997). R(AB) mice display reduced fMRI signals in hippocampal CA1 as compared to wild-type littermates (Small et al., 2000). This reduced signal was not the result of reduced cell numbers or gross structural changes in CA1 neurons. To pinpoint specific brain regions with altered activation characteristics, this type of fMRI analysis may be applied to inbred mouse strains known to have memory deficits. Importantly, if regions are identified to have altered activity levels via fMRI analysis, follow-up experiments would be necessary to determine the causes of differences in activation characteristics. These might include histological analysis, such as that employed by Small et al. (2000), and electrophysiological or molecular biological techniques that could shed light on cellular signalling abnormalities in the region of interest.

Anatomical analysis can give insight into several factors that contribute to the functions of particular brain regions. The Mouse Brain Library (http://mbl.org) is an online resource that contains brain atlases of several inbred mouse strains. In addition, Nervenet (http://nervenet.org) contains several databases of anatomical information that include information from many inbred and recombinant inbred mouse strains. Inbred strains with impaired cognitive function suspected to be related to dysfunction of a particular brain region could be probed for characteristics such as gross volume, density and composition of cells, and capacity for structural plasticity in the brain region of

interest. For example, an examination of neurogenesis using BrdU immunohistochemistry in the hippocampal dentate gyrus of four inbred strains revealed significant inter-strain differences in cell proliferation, survival, and differentiation, as well as in total cell counts and volumetric measurements of the dentate gyrus (Kempermann et al., 1997). Shors et al. (2001,2002) determined that reduction of hippocampal neurogenesis can impair certain types of hippocampus-dependent memory, and Drapeau et al. (2003) showed that within a population of aged rats, higher rates of hippocampal neuronal proliferation correlated with greater preservation of spatial learning and memory performance. Thus, it might be relevant to examine neurogenesis in inbred strains with impaired hippocampal function. Another point of interest is the connectivity of the brain region of interest – fibre tracts may be examined using Timm's stain or retrograde labelling from target structures. A number of inbred strains have already been identified as exhibiting agenesis of the forebrain commissures (e.g., Livy and Wahlsten, 1997; Wahlsten, 1982; Wahlsten et al., 2003). Further study revealed specific memory impairments in a strain that lacks intact hippocampal commissures [0]. Finally, dendritic, and dendritic spine, morphology may be assessed with Golgi staining, electron microscopy, or intracellular injection of fluorescent dyes. Because dendritic morphology is important for the reception and integration of synaptic signals, these morphological measures might provide insight into the capacity of different inbred strains for structural plasticity and neuronal signal processing either at a basal level, or following behavioral, chemical, or electrical challenge.

Specific methods that may be useful in determining the genetic and molecular causes of memory impairment in inbred mouse strains include measurements of

neurotransmitter or enzyme concentrations in the whole brain or in particular brain regions, electrophysiological measurement of synaptic transmission/plasticity, and genetic analyses such as gene chip DNA arrays and quantitative trait loci (QTL) analysis for behavioural phenotypes.

High-performance liquid chromatography (HPLC) is used to quantify the concentration of neurotransmitters, and/or their metabolites, in samples of brain tissue. Particular brain regions, such as the hippocampus, may be dissected from inbred mouse strains and assayed for specific neurochemical constituents. The most interesting application of HPLC analysis might be to elucidate whether altered capacity for neuromodulation results in impaired memory in inbred strains. Significantly altered levels of neuromodulatory transmitters, such as dopamine, serotonin, acetylcholine, or norepinephrine, in the hippocampi of inbred mice might influence synaptic plasticity (Hopkins and Johnston, 1988; Jay, 2003; Ji et al., 2001; Schmitz et al., 1998; Segal and Auerbach, 1997) and lead to memory impairments.

Concentrations of enzymes, including protein kinases, might also be measured in brain regions of interest in inbred strains. Enzymes and transcription factors known to be involved in hippocampal synaptic plasticity, such as CaMKII, PKA, MAPK, or CREB (see reviews by Ahn et al., 2000; Kandel, 2001; Lisman et al., 2002; Malenka and Nicoll, 1999; Nguyen and Woo, 2003), may be assayed in brain tissue using Western blotting to determine basal expression levels. This approach may reveal strain differences in the capacity of these molecules to be activated by experience. Furthermore, the brain region might be challenged by behavioral experience, application of specific neuromodulatory receptor agonists to *in vitro* slices, or electrical stimulation. Activation of these

signalling molecules could then be assayed by Western blotting (perhaps using phospho-specific antibodies) to determine the expression levels of specific signalling cascades suspected to underlie memory. One important caveat is that expression differences might only be found in particular subcellular compartments. Wehner et al. (1990) and Bowers et al. (1995) measured PKC expression in DBA/2 and C57BL/6 hippocampi and found significantly reduced PKC activity in the membrane, but not in the cytosolic, fraction of DBA/2 hippocampal tissue (see **Figure A5**). Thus, the suspected roles and locations of these enzymes should be considered when planning experiments.

Results from neurotransmitter and enzyme quantification studies might guide electrophysiological investigations that probe the physiological underpinnings of memory impairment. A small number of studies have already examined LTP and LTD in a few inbred strains (see **Table A3**), but unambiguous correlations between various long-term forms of synaptic plasticity and the expression of different types of learning and memory in inbred strains are scarce. A large-scale study in which hippocampus-dependent memory and hippocampal synaptic plasticity are examined conjointly within the same mice of many inbred strains would be valuable in determining whether there are definitive links between hippocampal synaptic modification and memory. Also, if signalling cascade abnormalities are identified in specific strains by molecular biological methods, characterization of LTP/LTD in these strains may shed light on the synaptic functions mediated by these signalling cascades. The most convincing evidence for the molecular causality of memory impairment in an inbred strain would be a rescue of memory function by reversal of this molecular alteration.

Another aspect of synaptic plasticity that would be interesting to examine in inbred mouse strains is *metaplasticity*, or the modifiability of synaptic plasticity (reviewed by Abraham and Bear, 1996; Abraham and Tate, 1997; Bear, 2003). In other words, synaptic activity at a particular synapse can influence the induction of further synaptic changes later on. For example, low-frequency stimulation (LFS) that is subthreshold for inducing synaptic plasticity can impair subsequent LTP induction (Christie and Abraham, 1992; Fujii et al., 1996; Huang et al., 1992) or it can erase previously established LTP (Barrionuevo et al., 1980; Fujii et al., 1991; Staubli and Lynch, 1990). Metaplasticity may function to keep synaptic strength within a range that is amenable to further modification, and to facilitate temporal integration of synaptic signals that occur within minutes, rather than seconds, of each other (Abraham, 1999). Conjoint examination of behavioural performance in tasks that require integration of information across multiple sessions (e.g., fear extinction) and electrophysiological analysis of synaptic metaplasticity in inbred strains might reveal correlations that will shed light on the roles of synaptic metaplasticity in behavioral flexibility and memory function.

Other types of analysis may help determine whether correlations between synaptic plasticity and hippocampal learning/memory reflect *causal* relationships. One type of analysis involves quantitative genetics (Wehner et al., 2001). This requires examining a particular trait in a mouse strain, quantifying the population variation for this trait, and then examining the many genetic influences that are believed to underlie such variation. Wehner et al. (2001) state that quantitative genetic studies can elucidate genes that regulate differences between individuals, determine whether common genes are involved

in multiple processes, and whether non-allelic genes interact. A quantitative genetic study might start by examining a number of different inbred strains for a particular phenotype. Strains that perform most differently (e.g., good and poor learners) may be crossed to make recombinant inbred strains that can be analysed for quantitative trait loci (QTL) (the WebQTL service at http://webqtl.roswellpark.org provides for fast QTL mapping of CNS traits for major recombinant inbred sets). QTL analysis involves phenotyping a trait in a large number of individuals within a segregating population (such as F2 crosses) or in several recombinant inbred strains. The associations between particular genetic markers and the quantified phenotype are determined in the population. The genomic locations of associated genetic markers indicate regions in which genes responsible for expression of the phenotype reside (Nguyen and Gerlai, 2002). This region is a QTL, defined by Wehner et al. (2001) to be "a chromosomal region that contains a gene, or genes, that regulates a portion of the genetic variation for a particular phenotype." At the first stage of analysis, this chromosomal region can be quite large, containing hundreds of genes. Using a number of methods, the details of which are beyond the scope of this article, this region can be narrowed down such that the particular gene(s) responsible for phenotypic differences can be determined (e.g., see Darvasi, 1998; Mott et al., 2000).

Some studies have searched for QTLs associated with variation in mouse performance on the MWM and in contextual fear conditioning. Wehner et al. (1990) examined 11 recombinant inbred strains created by crossing C57BL/6 and DBA/2. They determined that MWM performance relies on numerous genes, and that it significantly correlates with hippocampal PKC activity levels. More specifically, Steinberger et al.

(2003) used F2 mice from crosses between C57BL/6 and DBA/2 to determine that two QTLs influence performance on the MWM. These QTLs were localized to chromosomes 4 and 12. Furthermore, Milhaud et al. (2002) found that in C57BL/6 and DBA/2 recombinant inbred strains, a QTL on chromosome 1 influenced latency to find the hidden platform during training trials, and a QTL on chromosome 5 influenced spatial preference during the probe trial. Wehner et al. (1997) examined C57BL/6 and DBA/2 F2 intercross mice for QTLs associated with contextual fear conditioning. QTLs were found on portions of chromosomes 1 and 3. Caldarone et al. (1997) examined a backcross population of C3H/He and C57BL/6 in the same way, and also found QTLs on chromosomes 1 and 3. This result suggests that some QTLs for contextual fear conditioning may be consistent across inbred strains (Wehner et al., 2001). Radcliffe et al. (2000) confirmed that a QTL for contextual fear conditioning is located on chromosome 3 by performing a short-term phenotypic selection for contextual fear conditioning and mapping QTLs. QTLs on chromosomes 2 and 16 were found as well.

Another potentially useful application of QTL analysis might be to determine whether QTLs for hippocampal LTP and for hippocampus-dependent learning and memory overlap (Nguyen and Gerlai, 2002). If this were the case, a causal relationship between LTP and memory might be strengthened. QTL analysis has provided some insight into which loci of the mouse genome may contribute to particular learning and memory phenotypes. The identification of trait-related genes within the QTLs should be the next area of focus.

In addition to QTL analysis, other types of genetic analysis have been applied to inbred strains, such as gene expression analysis using microarray techniques. These

techniques can be used to simultaneously screen the mRNA expression levels of several thousands of genes. Such gene expression profiling may identify genes that contribute to cognition by comparing behavioral phenotypes to gene expression levels across groups that exhibit different levels of cognitive performance. Several studies have already applied DNA arrays to two or more inbred mouse strains (Carter et al., 2001; Leil et al., 2002; Sandberg et al., 2000). Klose et al. (2002) also analyzed expression of nearly 9 000 proteins in two inbred mouse strains. These studies have found differences in gene expression between distinct inbred strains. For example, Sandberg et al. (2000) profiled gene expression in strains C57BL/6 and 129/SvEv, and found that about 1% of genes are differentially expressed between these two strains in at least one region of the brain. Zhao et al. (2001) compared gene expression between subregions of the mouse hippocampus, and they found both enrichment and absence of expression of certain genes in CA1, CA3, and the dentate gyrus. Thus, the potential exists to identify genes that are enriched or absent in brain regions suspected to be responsible for memory impairment in inbred strains. However, researchers utilizing this gene chip methodology should keep in mind that false positives can be prevalent. This drawback can be remedied by conducting quantitative RT-PCR and *in situ* hybridization to confirm increased or decreased gene expression (i.e., mRNA expression patterns) in particular brain regions (Barlow and Lockhart, 2002; Henry et al., 2003).

Together, QTL analysis and gene expression profiling may enable the determination of specific genetic correlates of memory impairment. Sandberg et al. (2000) suggest that gene expression profiling might 'augment' QTL analysis, because genes that are differentially expressed between strains can be localized to chromosomal

regions that are thought to contain genes important to strain differences in cognitive phenotypes.

The identification of inbred mouse models of learning and memory is an important prerequisite to using inbred strains to establish the causes of normal, and impaired, learning and memory. Presently, a few inbred strains that possess specific memory impairments have been identified. These strains should be phenotyped further using several different tests of hippocampal learning and memory (e.g., radial arm maze, object recognition, social transmission of food preference) to confirm that their memory deficits are indeed end-products of hippocampal dysfunction *per se*. Then, these strains can be studied in depth to probe the genetic, cellular, and molecular mechanisms of these impairments. Murine "physiome" databases may be generated, and used in conjunction with the results obtained from the Mouse Genome Project to provide insight into the molecular mechanisms of memory function. Specific mechanistic hypotheses, based on available data from studies performed on mutant mice and on other animal species, can then be formulated to guide the multi-faceted experimentation needed to yield mechanistic insights. Even more exciting is the prospect of testing putative treatments for memory deficits in many of these inbred strains. This might include attempting to rescue memory deficits by genetic modification. To date, pharmacological and experiential approaches have been successful in DBA/2 and 129X1/SvJ mice respectively, and there is great potential for further progress.

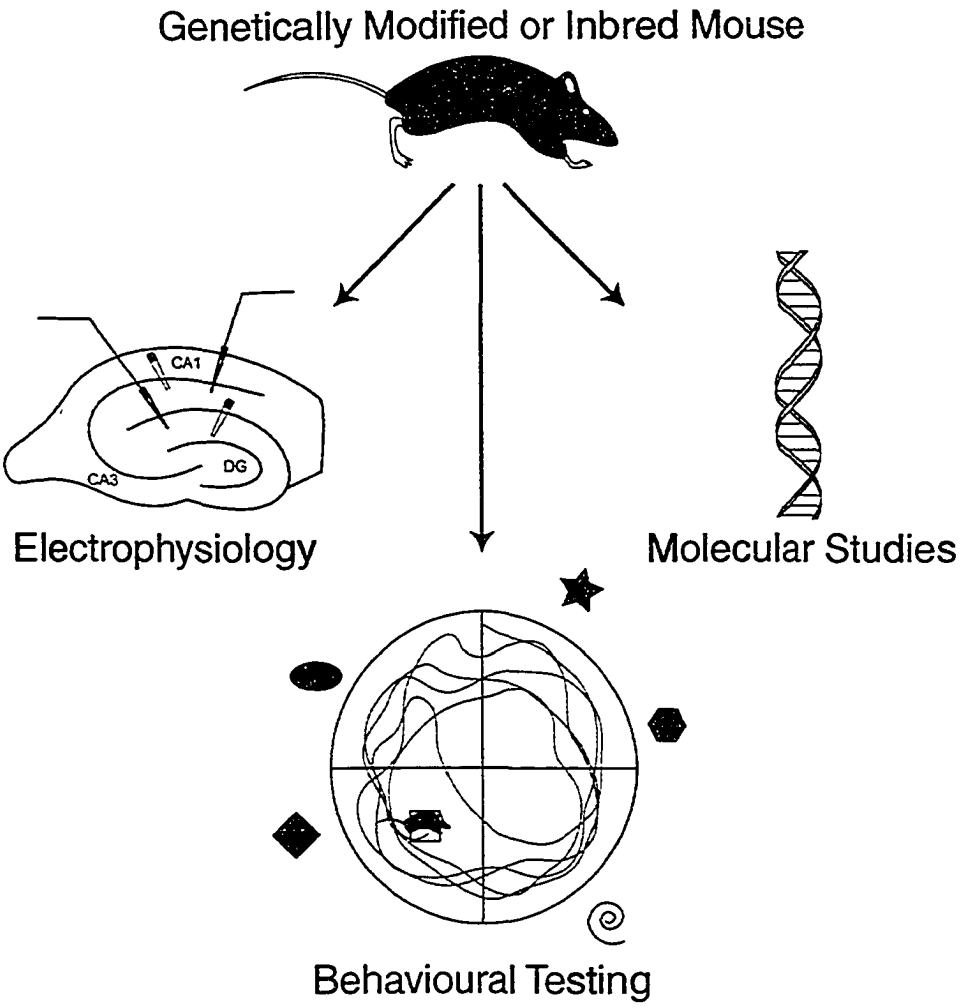
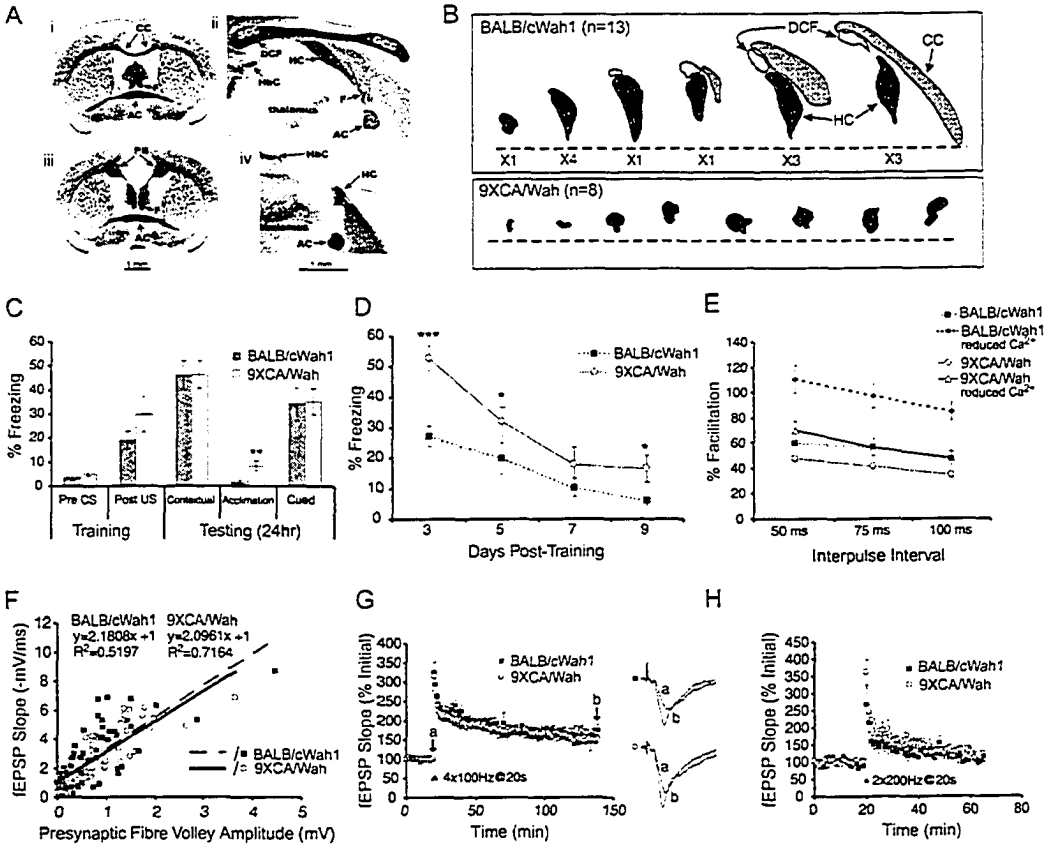


Figure A1: A three-pronged approach to studying the neurobiology of learning and memory in mice. Genetically modified or inbred mice can serve as models of learning and memory impairments that can be examined for underlying neurophysiological mechanisms. Electrophysiological, behavioral, and molecular characteristics may be determined and compared to shed light on the neurophysiological causes of cognitive deficits.

Figure A2: Case Study: Role of the hippocampal commissures in contextual fear extinction and hippocampal synaptic plasticity determined by comparison of inbred strains (adapted from Schimanski et al., 2002). A) Brain morphology of normal and acallosal mice. i, Coronal section, normal brain: the corpus callosum spans the cerebral hemispheres. ii, Sagittal section, normal brain: the corpus callosum extends rostrocaudally along the midline of the brain. iii, Coronal section, acallosal brain: there is no corpus callosum, and fibers have turned to form the Probst bundle. iv, Sagittal section, acallosal brain: there is no corpus callosum, and the hippocampal commissure is reduced in size. *A*C, Anterior commissure; *DC*F, dorsal commissure of the fornix; F, columns of the fornix; *GC*C, genu of the CC; *Hb*C, habenular commissure; *P*B, Probst bundle; *SC*C, splenium of the CC. B) Diagrams of the CC, HC, and dorsal commissure of the fornix *(DC*F) in BALB/cWah1 and 9XCA/Wah mice. In the 9XCA/Wah strain, every animal showed total absence of the CC and dorsal commissure of the fornix, as well as severe reduction of the HC. In BALB/cWah1, several patterns were seen, and the frequency of mice with each type is indicated by *X_*. For example, *X4* means that four mice had a normal HC but no dorsal commissure of the fornix. Only one BALB/cWah1 mouse had an HC as small as the largest HC among 9XCA/ Wah mice. C) A reduced HC impairs contextual fear extinction but does not affect cued and contextual fear conditioning. BALB/cWah1 *(n* =12) and 9XCA/Wah *(n* =12) performed normally on tests of 24 hr contextual and cued memory. Both strains displayed significantly different levels of freezing for "acclimation" during a 2 min test interval in a novel context, before presentation of the CS for the cued memory test. Thus, acclimation freezing values were subtracted for each mouse (both strains) to calculate percentage of freezing for the cued memory test. Cued memory was not significantly different between strains. D) Both strains received 5 min contextual extinction trials 3, 5, 7, and 9 d after training. 9XCA *(n*=7) exhibited delayed contextual extinction compared with BALB/cWah1 *(n*= 7), and they still showed less extinction during the final trial on day 9. *Asterisks* indicate significance values derived from Student's *t* test (**p* < 0.05; ***p* < 0.01; ****p* < 0.001). CS, Tone; US, foot shock. E) Paired-pulse facilitation is decreased in mice with a reduced HC and is rescued by reduced extracellular [Ca^{2+}]. Plot of percentage of facilitation [ratio of fEPSP slopes: ((pulse 2/pulse 1) - 1) X 100] versus interpulse interval for strains BALB/cWah1 and 9XCA/Wah. In standard ACSF, 9XCA/Wah exhibited significantly less facilitation than BALB/cWah1. When measured under conditions of reduced extracellular [Ca^{2+}], facilitation in 9XCA/Wah was not significantly different from that seen in BALB/cWah1 slices in standard ACSF. Reduced extracellular [Ca^{2+}] also significantly increased facilitation in BALB/cWah1. F) Input–output curve slopes (fEPSP slope vs. presynaptic fiber volley amplitude) are not significantly different between BALB/cWah1 *(n* = 11 slices, 5 data points per slice) and 9XCA/ Wah *(n* = 17 slices, 5 data points per slice). G, H) Hippocampal LTP in two pathways is normal in mice with a reduced HC. G) LTP was elicited by four bursts of 100 Hz (4X*100Hz@20 sec*) in BALB/cWah1 *(n* = 5 slices) and 9XCA/ Wah *(n* = 11 slices). H) E-LTP in the mossy fiber pathway, elicited by twin bursts of 200 Hz *(2X200Hz@20 sec*) in BALB/cWah1 *(n* = 9 slices) and 9XCA/Wah *(n* = 17 slices), was not significantly different between strains.



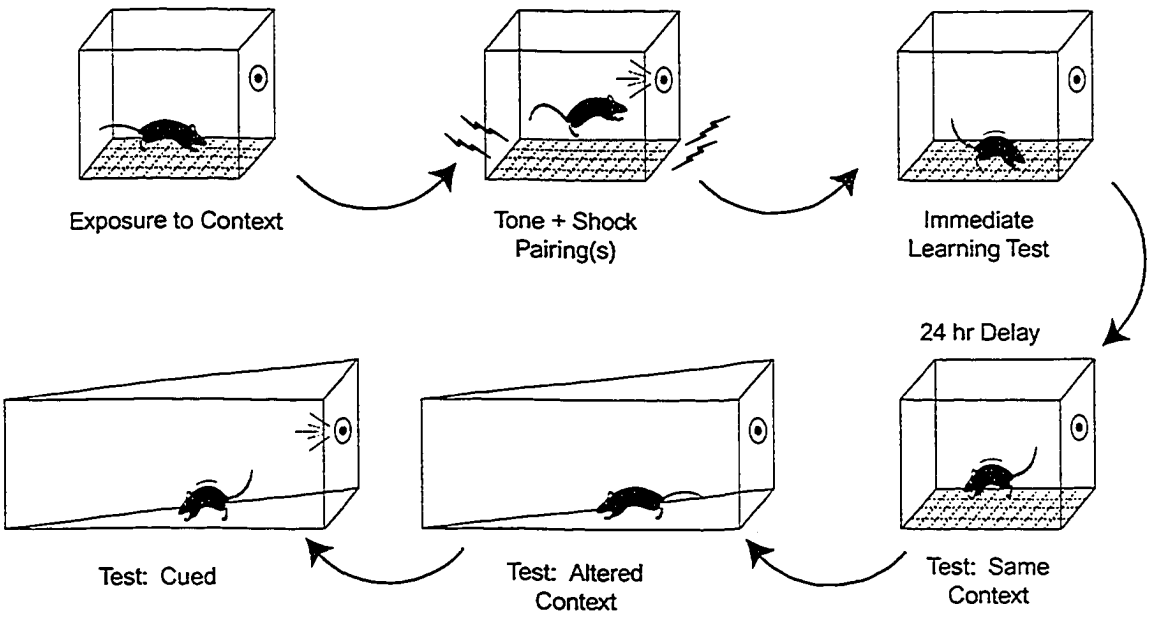
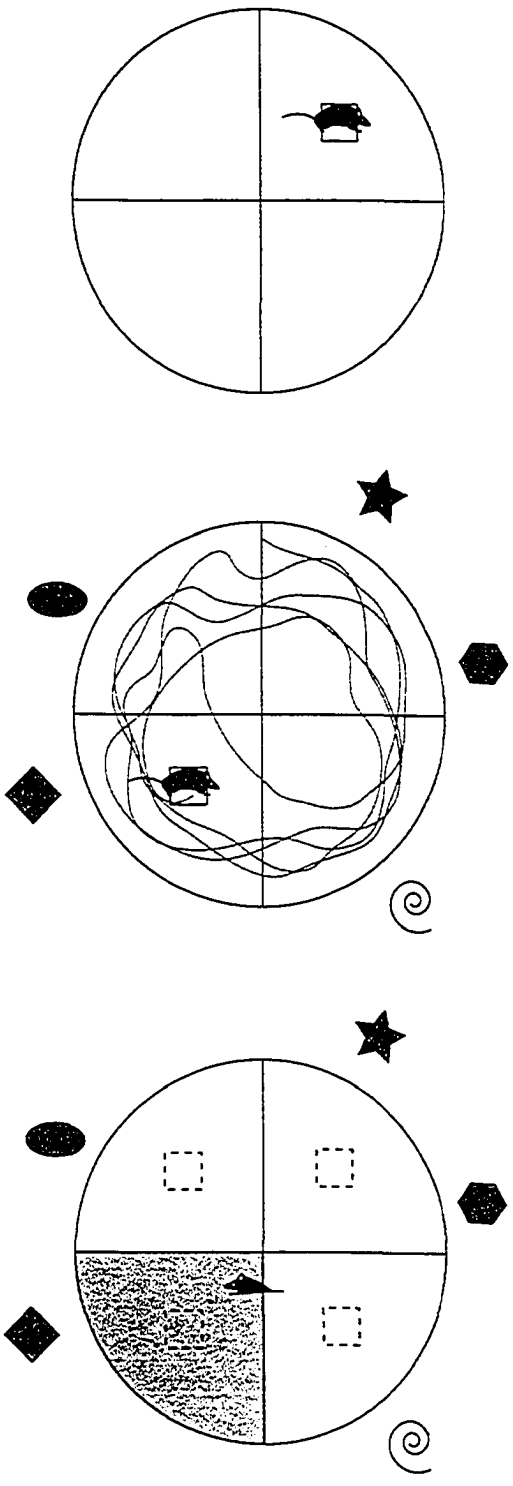


Figure A3: Contextual and cued fear conditioning methodology. See text for further details. Mice are first placed in the training context and allowed to explore for about 2 min. Then, they are presented with one to three pairings of an auditory cue (CS) and a footshock (US). The mice are left in the training context and their freezing behavior is quantified for 30 sec after the last CS-US pairing (this is a measure of immediate learning for the association of the US with the context). 24 hours later, mice tested for contextual, altered context, and cued fear memory by quantifying freezing behavior in three different conditions. First, mice are returned to the training context for 5 min and tested for contextual fear memory. Next, mice are placed in a different environment for 2-3 min and tested for fear of a new environment. The last test is for cued fear memory, in which mice remain in the new environment while the CS is presented again.



Pretraining - mouse learns to climb onto hidden platform. The position of the platform can be varied to avoid habituation to one location.

Learning trials - mouse searches for hidden platform using visual cues, then climbs onto it. Location of platform remains constant, start position of mouse varies from trial to trial.

Probe trial for memory of platform location - hidden platform is removed. Mouse swims freely for one minute.

Figure A4: Morris water maze methodology. See text for further details.

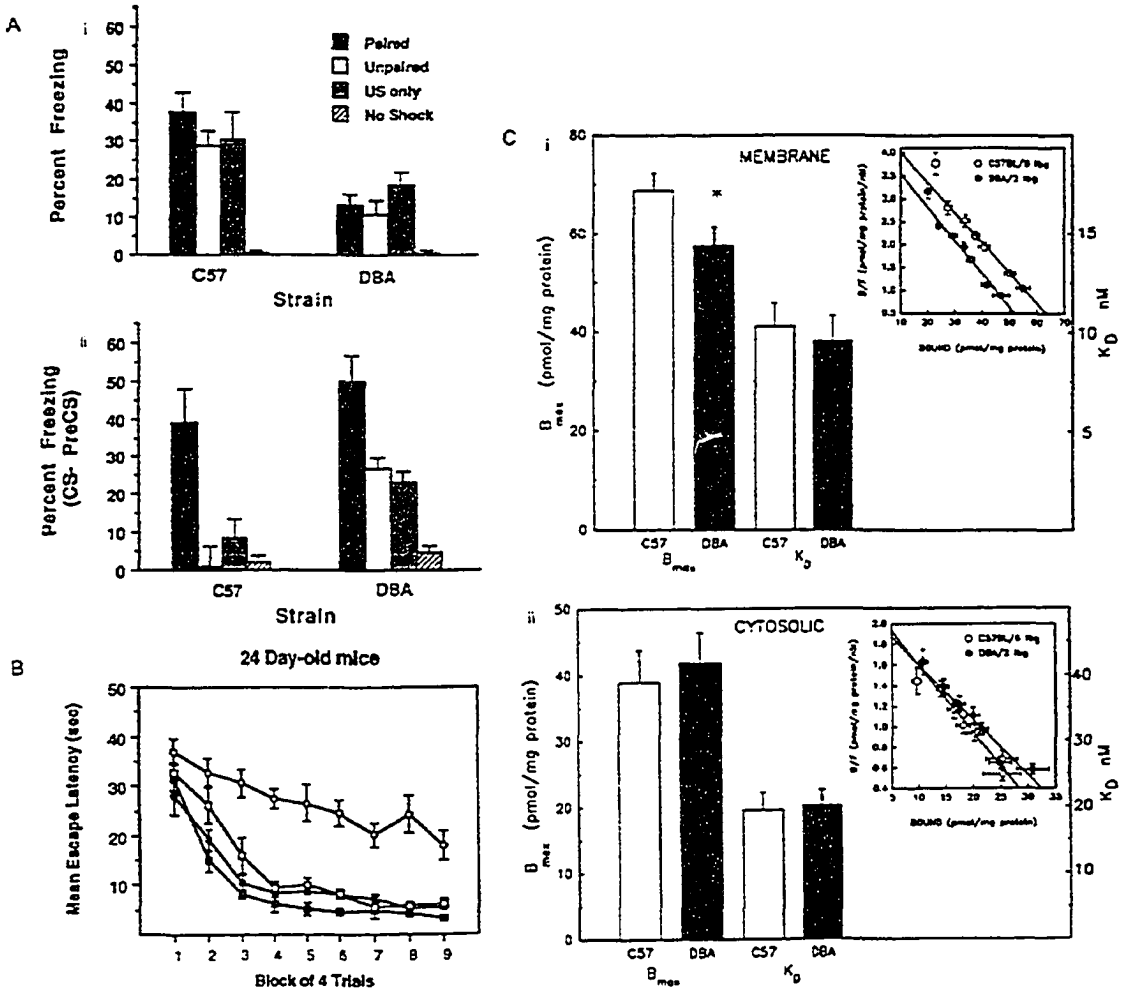


Figure A5: Learning and memory impairments and PKC expression in DBA/2 mice. A) Mean percentage freezing observed during the i) context test and ii) auditory cue test of Experiment 1 in (Paylor et al., 1994). Bars represent the SEM. C57BL/6 and DBA/2 mouse strains were tested. CS = conditioned stimulus, US = unconditioned stimulus. B) Mean escape latencies for C57BL/6 and DBA/2 mice trained to locate a hidden or visible platform (from Paylor et al., 1996). Subjects started training at 24 days of age (C57BL/6 $n = 8$ for the hidden-platform task [filled circle] and 5 for the visible-platform task [filled square]; DBA/2 $n = 12$ for the hidden-platform task [open circle] and 7 for the visible task [open square]). Bars indicate SEM. C) Hippocampal [^{3}H]PDBu binding (from Bowers et al., 1995). Cytosolic and membrane fractions were analyzed. Data from each animal were subjected to Scatchard analysis, and the means ± SEM for the derived K_D and B_{max} values are presented as bar graphs. The inset shows results of Scatchard analyses calculated using the mean values at each ligand concentration for all animals of each genotype. Each point represents the mean ± SEM for each ligand concentration ($n =$ 7 for each genotype); $^*p < 0.05$.

Table A1: Cued and Contextual Fear Conditioning in Inbred Mouse Strains

Inbred Strain	*Contextual Fear Conditioning*	*Cued Fear Conditioning*	*Notes*	*Reference(s)*
129S1/SvImJ	Good	Good		(Bolivar et al., 2001; Cook et al., 2002)
129S6/SvEv	Good	Good	Cook et al. (2002) show differences in cued fear conditioning performance across 129 substrains, but all performed at a high level.	(Balogh and Wehner, 2003; Cook et al., 2002; Holmes et al., 2002; Owen et al., 1997)
129T2/SvEmsJ	Good	Good		(Cook et al., 2002; Nguyen et al., 2000a)
129X1/SvJ	Good	Good		(Cook et al., 2002; Owen et al., 1997)
A	Good	Intermediate	Generalized freezing may elevate performance.	(Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997)
AKR	Poor	Good		(Balogh and Wehner, 2003)
BALB/c	Intermediate			(Chen et al., 1996; Radulovic et al., 1998)
BALB/cByJ	Intermediate	Good		(Balogh and Wehner, 2003; Bolivar et al., 2001; Owen et al., 1997)
BuB/Bn	Poor	Poor	High freezing to altered context.	(Owen et al., 1997)
C3H/He	Intermediate	Good	Required more training trials than C57BL/6 to show good contextual fear conditioning (Chaudhury and Colwell, 2002).	(Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Owen et al., 1997)
C57BL/6	Good	Good	Substrain C57BL/6N shows generalized freezing to new contexts after training. Less generalized freezing in C57BL/6J (Radulovic et al., 1998; Stiedl et al., 1999).	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Chen et al., 1996; Cook et al., 2002; Fitch et al., 2002; Francis et al., 2003; Gerlai, 1998; Holmes et al., 2002 (trace fear conditioning); Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Radulovic et al., 1998; Stiedl et al., 1999; Valentinuzzi et al., 1998; Young et al., 2000)

Table A1: Cued and Contextual Fear Conditioning in Inbred Mouse Strains (Ctd.)

Inbred Strain	*Contextual Fear Conditioning*	*Cued Fear Conditioning*	*Notes*	*Reference(s)*
C57BL/10	Good	Good	20% baseline freezing.	(Owen et al., 1997)
CBA	Poor	Poor	Performance increases with more training trials (Bolivar et al., 2001).	(Balogh and Wehner, 2003; Bolivar et al., 2001; Nguyen et al., 2000a; Nie and Abel, 2001)
DBA/2	Poor	Intermediate	Performance increases with more training trials.	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Fitch et al., 2002; Francis et al., 2003; Gerlai, 1998; Holmes et al., 2002 (trace fear conditioning); Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Stiedl et al., 1999; Valentinuzzi et al., 1998; Young et al., 2000)
FVB/N	Poor	Intermediate	Performance increases with more training trials (Bolivar et al., 2001).	(Bolivar et al., 2001; Owen et al., 1997)
LP	Good	Good		(Balogh and Wehner, 2003; Owen et al., 1997)
SJL	Good	Good		(Balogh and Wehner, 2003; Owen et al., 1997)

Table A2: Hidden Platform Morris Water Maze Performance in Inbred Mouse Strains

Inbred Strain	*MWM Learning*	*MWM Probe Trial (Memory)*	*Notes*	*Reference(s)*
129S2/Sv	Good	Good		(Contet et al., 2001; Voikar et al., 2001)
129S6/SvEv	Good	Good		(Gerlai, 2002; Holmes et al., 2002; Owen et al., 1997)
129T2/SvEms	Good	Good	Slow swimmers (Fox et al., 1999; Wolff et al., 2002).	(Nguyen et al., 2000a; Wolff et al., 2002)
129X1/SvJ	Poor	Poor		(Leil et al., 2002; Owen et al., 1997)
129/Ola	Good	Good		(Royle et al., 1999)
A	Poor	Poor	Poor visible platform task – could be vision problem.	(Owen et al., 1997)
BALB/c	Intermediate	Intermediate	Might lack motivation (Yoshida et al., 2001). Better performance with more pretraining (Royle et al., 1999).	(Francis et al., 2003; Royle et al., 1999; Yoshida et al., 2001)
BALB/cByJ	Poor	Poor	8-20% of mice acquire and perform well (Francis et al., 1995). Poor visible platform performance (Upchurch and Wehner, 1988).	(Francis et al., 1995; Owen et al., 1997; Upchurch and Wehner, 1988)
BuB/BnJ	Poor	Poor	Poor visible platform – vision deficit likely.	(Owen et al., 1997)
C3H	Poor	Poor	Poor visible platform (Owen et al., 1997; Upchurch and Wehner, 1988).	(Gutekunst et al., 1993; Owen et al., 1997; Upchurch and Wehner, 1988)
C57BL/6	Good	Good		(Contet et al., 2001; Fox et al., 1999; Francis et al., 1995; Francis et al., 2003; Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Leitinger et al., 1994; Logue et al., 1997; Nguyen et al., 2000a; Owen et al., 1997; Royle et al., 1999; Upchurch and Wehner, 1988; Upchurch and Wehner, 1989; Voikar et al., 2001; Wolff et al., 2002; Yoshida et al., 2001)
C57BL/10J	Good	Good		(Owen et al., 1997)

Table A2: Hidden Platform Morris Water Maze Performance in Inbred Mouse Strains (Ctd.)

Inbred Strain	*MWM Learning*	*MWM Probe Trial (Memory)*	*Notes*	*Reference(s)*
CBA/J	Poor	Poor	Poor visible platform.	(Nguyen et al., 2000a)
DBA/2	Intermediate	Poor	Good visible platform (Nguyen et al., 2000a; Owen et al., 1997). Good performance on probe trial in selected studies (Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Owen et al., 1997).	(Francis et al., 1995; Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Logue et al., 1997; Nguyen et al., 2000a; Owen et al., 1997; Upchurch and Wehner, 1988; Upchurch and Wehner, 1989)
DBA/2J-I	Intermediate	Intermediate	Good visible platform.	(Owen et al., 1997)
FVB/N	Poor	Poor	Poor visible platform.	(Fox et al., 1999; Owen et al., 1997; Royle et al., 1999; Voikar et al., 2001)
LP	Intermediate	Intermediate	Good visible platform.	(Leil et al., 2002; Owen et al., 1997)
SJL	Poor	Poor	Poor visible platform.	(Owen et al., 1997)

Table A3: Hippocampal Synaptic Transmission in Inbred Mouse Strains

Inbred Strain	*Intact*	*Impaired*	*Notes*	*Reference(s)*
129S6/SvEv		4 trains HFS (20sec ITI), PPF.	Hippocampal slices, area CA1.	(Gerlai, 2002)
129T2/SvEmsJ	4 trains HFS (3sec ITI), FSK+IBMX , LTD, I/O, PPF, general biophysical and electrophysiological attributes.	1 train HFS, TBS, 4 trains HFS (20sec ITI and 5 min ITI).	Hippocampal slices, area CA1.	(Nguyen et al., 2000a; Nguyen et al., 2000b)
129 Ola	HFS-induced LTP followed for one hour. PPF.		In vivo, medial perforant pathway.	(Bampton et al., 1999)
C3H Albino	HFS-induced LTP, PPF.		In vivo, medial perforant pathway.	(Bampton et al., 1999)
C57 Albino	HFS-induced LTP, PPF.		In vivo, medial perforant pathway.	(Bampton et al., 1999)
C57BL/6	LTP induced by 1 train or 3 trains HFS.		In vivo perforant path.	(Matsuyama et al., 1997)
	1 train HFS, 3 trains HFS, TBS LTP.		In vivo, dentate gyrus.	(Jones et al., 2001)
	4 trains HFS (20sec ITI), PPF.		Hippocampal slices, area CA1.	(Gerlai, 2002)
	1 train HFS, TBS, 4 trains HFS (3 sec, 20sec, and 5min ITI). FSK+IBMX, LTD, PPF, I/O. General biophysical and electrophysiological attributes.		Hippocampal slices, area CA1.	(Nguyen et al., 2000a; Nguyen et al., 2000b)
CBA/J	1 train HFS, LTD, PPF, I/O, general biophysical and electrophysiological attributes.	TBS, 4 trains HFS (3sec, 20sec, and 5min ITI), FSK+IBMX.	Hippocampal slices, area CA1.	(Nguyen et al., 2000a; Nguyen et al., 2000b)

Table A3: Hippocampal Synaptic Transmission in Inbred Mouse Strains (Ctd.)

Inbred Strain	*Intact*	*Impaired*	*Notes*	*Reference(s)*
DBA/2	TBS LTP.	1 train HFS, 3 trains HFS LTP.	In vivo, dentate gyrus.	(Jones et al., 2001)
	LTP induced by 3 trains HFS.	LTP induced by 1 train HFS.	In vivo perforant path.	(Matsuyama et al., 1997)
	PPF.	HFS-induced LTP.	In vivo, medial perforant pathway. Good induction, impaired maintenance.	(Bampton et al., 1999)
	PPF.	4 trains HFS (20sec ITI).	Hippocampal slices, area CA1. Good induction, impaired maintenance.	(Gerlai, 2002)
	1 train HFS, 4 trains HFS (5min ITI), LTD, I/O, general biophysical and electrophysiological attributes.	TBS, 4 trains HFS (3sec and 20sec ITI), FSK+IBMX, PPF.	Hippocampal slices, area CA1.	(Nguyen et al., 2000a; Nguyen et al., 2000b)
FVB/N	HFS-induced LTP, PPF.		In vivo, medial perforant pathway.	(Bampton et al., 1999)

Abbreviations: HFS, high frequency stimulation; ITI, inter-train interval; FSK+IBMX, application of a forskolin + 3-isobutyl-1-methylxanthine cocktail; I/O, input-output relation; PPF, paired-pulse facilitation; TBS, theta-burst stimulation.

Table A4: ↑ indicates good memory performance or good LTP; ↓ indicates poor memory performance or poor LTP. In vivo LTP was induced by high frequency stimulation to the medial perforant pathway (MPP). Area CA1 hippocampal slice LTP was induced by 4 trains of 100-Hz stimulation applied at 20sec intervals. CFC, contextual fear conditioning; MWM, Morris water maze.

Table A4: Memory Performance Correlates with Hippocampal LTP in Some Inbred Strains

Inbred Strain	*CFC*	*MWM Probe Trial*	*Hippo. LTP*	*Type of LTP*	*References*
129S6/SvEv	↑	↑	↓	Area CA1, hippo. slices	(Balogh and Wehner, 2003; Cook et al., 2002; Gerlai, 2002; Holmes et al., 2002; Owen et al., 1997)
129 Ola		↑	↑	MPP, in vivo	(Bampton et al., 1999; Royle et al., 1999)
129T2/SvEms	↑	↑	↓	Area CA1, hippo. slices	(Cook et al., 2002; Nguyen et al., 2000a,b; Wolff et al., 2002)
C57BL/6	↑	↑	↑	Area CA1, hippo. slices	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Chaudhury and Colwell, 2002; Chen et al., 1996; Contet et al., 2001; Cook et al., 2002; Fitch et al., 2002; Francis et al., 1995; Francis et al., 2003; Fox et al., 1999; Gerlai, 1998; Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Leitinger et al.., 1994; Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a,b; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Radulovic et al., 1998; Royle et al., 1999; Stiedl et al., 1999; Upchurch and Wehner, 1988; Upchurch and Wehner, 1989; Valentinuzzi et al., 1998; Voikar et al., 2001; Wolff et al., 2002; Yoshida et al., 2001; Young et al., 2000)
CBA	↓	↓	↓	Area CA1, hippo. slices	(Balogh and Wehner, 2003; Bolivar et al., 2001; Nguyen et al., 2000a,b; Nie and Abel., 2001)
DBA/2	↓	↓	↓	Area CA1, hippo. slices	(Ammassari-Teule et al., 2001; Balogh et al., 2002; Balogh and Wehner, 2003; Bolivar et al., 2001; Fitch et al., 2002; Francis et al., 1995; Francis et al., 2003; Gerlai, 1998; Gerlai, 2002; Gutekunst et al., 1993; Holmes et al., 2002; Logue et al., 1997; Lu and Wehner, 1997; Nguyen et al., 2000a,b; Nie and Abel, 2001; Owen et al., 1997; Paylor et al., 1994; Stiedl et al., 1999; Upchurch and Wehner, 1988; Upchurch and Wehner, 1989; Valentinuzzi et al., 1998; Young et al., 2000)
FVB/N	↓	↓	↑	MPP, in vivo	(Bampton et al., 1999; Bolivar et al., 2001; Fox et al., 1999; Owen et al., 1997; Royle et al., 1999; Voikar et al., 2001)

Table A5: PKC Isozymes

	C57BL/6	*DBA/2*
α	132 ± 10	136 ± 10
β_I	156 ± 5	172 ± 8
β_{II}	235 ± 29	246 ± 6
γ	295 ± 32	200 ± 20*
δ	131 ± 15	118 ± 7
ε	174 ± 19	147 ± 15

Adapted from Bowers et al. (1995). Membrane-bound PKC isozymes were compared between C57BL/6 and DBA/2 hippocampi and are expressed as percentage of whole brain (means ± SEM of five to 10 mice). * $p<0.05$.

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