# CHANGES IN PERFORANT PATH - DENTATE GYRUS EVOKED POTENTIALS DURING CLASSICAL FEAR CONDITIONING IN THE ANESTHETIZED RAT



PHILIP A. MURPHY

# Changes in Perforant Path – Dentate Gyrus Evoked Potentials during Classical Fear Conditioning in the Anesthetized Rat

by

© Philip A. Murphy

A thesis submitted to the

School of Graduate Studies

in partial fulfilment of the

requirements for the degree of

Master of Science

Department of Psychology, Faculty of Science

Memorial University of Newfoundland

2005

St. John's Newfoundland and Labrador

## ACKNOWLEDGEMENTS

I would like to thank members of my committee: Professor John Evans, whose dedication, guidance and encouragement was always there; Professor Carolyn Harley, whose commitment to my thesis proved invaluable; and Professor Charles Malsbury, whose feedback was well appreciated.

I would also like to acknowledge all my former lab mates especially Dr. Susan Walling and Dr. Robert Brown for their assistance.

Thanks are also extended to Mr. Steve Milway and Mr. Avery Earle, for their assistance in providing technical solutions.

Special thanks are given to Mrs. Bernice St. Croix, former Secretary for Professor John Evans, and Mrs. Brenda Noftle, Department Secretary, Psychology, whose support and moments of humour were greatly appreciated.

I would also like to thank my parents, Sandy and Phil, who were always proud of my accomplishments and never forced me to do something that I didn't want to pursue.

# ABSTRACT

**Changes in Perforant Path – Dentate Gyrus Evoked Potentials During Classical Fear Conditioning in the Anesthetized Rat**

Neural changes accompanying fear conditioning in the hippocampus, specifically the dentate gyrus, have been described in awake rats. Doyère et al. (1995) examined the time course of synaptic modifications in perforant path-dentate gyrus connections during learning. They found an increase in slope (mV/ms) of the field excitatatory synaptic potential (EPSP), a reflection of system drive, for the conditioned group while a decrease was noted for the pseudoconditioned group. As for the reactivity of the granule cells in the dentate gyrus, population spike decreases were found in both groups. The primary goal of this study was to apply the conditioning methods of Doyère et al. (1995) and measure the perforant path-dentate gyrus responses during an anesthetized state using urethane.

Following a 30 min initial baseline period, animals in the conditioned group received 32 tone-footshock pairings over a 90 min conditioning period, followed by a 60 min rest period, and then a 90 min extinction period during which 32 tones were presented. Mean EPSP slope and population spike responses were plotted over time and compared to the responses recorded in a pseudoconditioned group where animals received 32 deliberate CS-US unpairings during the 90 min conditioning period.

The EPSP slope responses in both groups did not vary significantly from baseline level over the 300 min period and were not affected by the manner in which tone and footshock presentations were received. As for the population spike response, no group differences were noted during the conditioning period but a significant group by block interaction was found for the 60 min post-conditioning period. In contrast to the slope responses, significant group effects were found in the extinction period and the final 30 min period of recording. Consistent results were noted following analysis of the calculated ratios of the population spike/slope responses. Correlation analyses of the

slope and population spike suggested that conditioning led to an increase in cell excitability such that a smaller slope was associated with a larger population spike. This effect occurred after conditioning and disappeared with extinction.

The overall pattern suggested that conditioning changes perforant path-dentate gyrus connections in the urethane anesthetized rat and that the changes are unlike those that occur during the awake state. While EPSP slope changes were non significant in both pseudoconditioned and conditioned rats, the difference in spike amplitude profile with conditioning relative to pseudoconditioning implies that pairing-related modifications can occur in the anesthetized state.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## DEDICATION

I would like to dedicate this thesis to my wife, Tina Giles Murphy, who is the main reason I have made it this far. Her love, support, and patience through this entire process will never be forgotten and for that I will always be grateful.

# INTRODUCTION

The process of acquiring knowledge or information refers to learning, while the retention or storage of that knowledge or information is memory. When an animal changes its behaviour learning can be inferred. Exposing animals to specific types of controlled sensory experiences has enabled researchers to classify learning into two different classes: nonassociative and associative. The former refers to what happens when an animal is exposed repeatedly to a single stimulus, while the latter happens when an animal experiences paired stimuli. Nonassociative learning is thought to reflect encoding of individual stimulus properties while associative learning reflects encoding of the relationship among stimuli or between stimuli and behavior.

Psychologists and neuroscientists have been studying the formation of associations for over a century. Behavioural and electrophysiological studies are two separate approaches taken by researchers to identify and understand underlying principles of associative learning. Within behavioural studies, one model that has been valuable is the exploration of the formation of associations in Pavlovian conditioning. As for electrophysiological work, long term potentiation, or LTP, has helped researchers understand the neurobiology of learning through a more direct exploration of the brain. Important contributions from both approaches are discussed below.

## Pavlovian Conditioning

Pavlovian conditioning, a type of associative learning, has been studied for well over a century with initial experiments by the Russian physiologist, Pavlov (1927).

Pavlovian conditioning is said to occur when a previously neutral stimulus, such as a tone or light, becomes associated with an already existing reflex to the extent that it will, by itself, evoke a response. This new reflex is said to be conditional, in that its ability to evoke a response depends upon the stimulus having been associated with a previously existing reflex.

Much of the progress in understanding emotion in associative learning, has come from studies of fear, especially fear conditioning. The fear-conditioning procedure, a subset of Pavlovian conditioning, involves the association of a neutral stimulus (e.g., a 10 sec presentation of tone) with an aversive unconditioned stimulus (US), (e.g., an electric footshock). After repeated pairings, the presentation of the tone alone predicts the occurrence of the shock and acts as a conditioned stimulus (CS), eliciting a state of fear. Other stimuli that have been used as CSs in fear-conditioning experiments include light, odours and tactile stimuli (e.g., air puff). These stimuli can range from a few seconds to a few minutes, and because of their brevity are discrete CSs. Subjects also become conditioned to the less temporally restricted features of their environment such as odour and colour. These stimuli are termed contextual stimuli. Fear in both discrete and contextual situations can be acquired very rapidly, even in a single trial (LeDoux, 1991, 1992, 1996).

Scientists have used fear-modulated behaviours as models to understand how emotions influence behaviour. Investigation into this field has assisted the development of strategies to treat and cure anxiety disorders (e.g., specific phobias, panic attacks, post-traumatic stress, and generalized anxiety). Also, since fearful experiences are rapidly

learned and long remembered, fear conditioning has become model of choice for unravelling the processes and mechanisms underlying learning and memory (Fendt and Fanselow, 1999).

The extensive research done in this area has resulted in numerous behavioural tests or models to study fear. These behaviours fall into two general classes: learned and unlearned. Tests of unlearned fear rely on stimuli that elicit fear even when the animal has had no prior experience with the stimulus. The most frequently used stimuli in these tasks are natural predators such as the cat for a rat (Adamec, 1991), or exposure to a novel place (e.g., one that is brightly lit or elevated (Graeff et al., 1993)). Approaches that use learned fear tests have utilized conditioned behaviours elicited by stimuli that have been associated with an aversive event, such as an electric footshock. These Pavlovian fear stimuli elicit many of the same behaviours that innate fear stimuli do. Some of these behavioural responses include freezing, startle, tachycardia, defensive burying, and ultrasonic vocalization (Davis, 1992).

Pavlovian conditioning has been a popular paradigm for the study of learning and has played an important role in the understanding of emotion. Many stimuli are able to arouse emotional responses, such as fear. Davis' extensive work on fear conditioning (Davis, 1989; 1990; 1992; Davis et al., 1993) has shown that rapidly acquired and long lasting conditioned emotional responses, such as freezing or startle, provide a valuable model for examining the neural basis of emotional learning and memory.

**Long Term Potentiation (LTP)**

One of the first notions of synaptic memory came from David Hartley (1751/1971) in which he suggested that mental associations or memories about the relation between stimuli are a result of vibrations between nerves. Other theories including those of James (1890), Cajal (1894), and Freud (1895) further clarified the synaptic theory of memory. However, it is the ideas and work of the Canadian, Donald Hebb, that is most cited as providing a framework for identifying the neurological basis of memory and learning in the brain. Hebb's (1949) original notion stated:

> "when an axon of a cell A is near enough to excite cell B or repeatedly or consistently takes part in firing it, some growth process or metabolic changes take place in one or both cells such that A's efficiency, as one of the cells firing B, is increased".

Applying this theory to memory formation, it is hypothesized that in order for two stimuli to be associated, the neurons must receive information about both stimuli. Hebb's theory provided an explanation of how changes happened between neurons and thus provided a mechanism for memory formation. Bliss and Lomo (1973) first observed the phenomenon of long term potentiation (LTP) by applying a train of high-frequency (100 Hz) stimulation to the perforant path-dentate gyrus synapse in rabbit hippocampi. They produced a long-lasting potentiation of both the EPSP slope and the population spike amplitude components of the perforant path evoked potential. In addition to supporting the Hebbian hypothesis, this research provided a potential mechanism for translating neural activity generated by environmental stimuli into changes in synaptic efficiency. Bliss and Lomo's results, discovered in the hippocampus, allowed subsequent researchers

to explore other neural regions such as auditory (Kudoh and Shibuki, 1994) and visual (Berry et al., 1989; Artola and Singer, 1987) cortices, and several other pathways; demonstrating the generalizability of the model. The notion that LTP involved an interaction at the cellular level between synaptic inputs provided a route for the formation of associations and, hence, offered a mechanism of learning.

**Rogan and LeDoux's Work**

Much of the research on LTP (e.g., Collingridge, 1983; Lynch, 1983; and Nicoll, 1988) primarily provides a possible basis for the way that synapses are changed when learning occurs. Using a different strategy, Rogan looked for plasticity in a circuit (the thalamo-amygdala pathway) undergoing learning rather than looking for 'model' plasticity and trying to relate it to learning (Rogan & LeDoux, 1995). Initially, considering LeDoux's earlier findings on fear conditioning, Rogan asked if LTP could be induced in this well-established learning pathway. After getting supportive results, he then wondered if LTP could change the processing of sounds. By testing LTP using a natural sound stimulus, instead of electrical stimulation of the nerve fibers, Rogan discovered that LTP enhanced the amygdala's response to a sound (Rogan, Staubli, & LeDoux; 1997a). This study was the first to show that the alteration of transmission in a potentiatied pathway changes the manner in which external stimuli are processed. Rogan then asked if LTP occurred in the brain during natural learning like fear conditioning (Rogan et al, 1997b). By substituting fear conditioning for LTP induction, he discovered similar changes in the amygdala's response to conditioned sound. So, in this preparation, both LTP and conditioning yielded similar changes in the amygdala. This supports the

view that LTP is a naturally occurring neural process. Hence LTP appears a valuable model for exploring the formation of associations and the physiological study of conditioning.

**The Role of the Hippocampal Formation**

Much of the work on fear conditioning has pinpointed the amygdala as an important component of the neural system involved in the acquisition, storage, and expression of fear memory (LeDoux, 2000). However, there is another major neural substrate that has been increasingly examined in connection with fear learning and memory processes in recent decades. It is now generally accepted that the hippocampal formation is an essential component of the brain systems underlying the explicit recollection of past events and the processing of relational information including that involved in fear learning (Phillips & LeDoux, 1992; 1994; and LeDoux, 2000).

The hippocampal formation is defined by a collection of neural substrates including the entorhinal cortex, subicular complex, dentate gyrus and three fields of Ammon's Horn (which includes areas CA1, CA2 and CA3, see Figure 1). The major input into the dentate gyrus arises from the axons of the medial and lateral entorhinal cortex via the perforant pathway (also known as the angular bundle). Cajal (1911) first described the perforant pathway as a collection of fibers, leaving the entorhinal cortex and perforating the underlying white matter and adjacent layers of the subiculum, on their way to the molecular layer of the subiculum. From there the fibers cross the hippocampal fissure into the molecular layer of the dentate gyrus. From the granule cells of the dentate gyrus, information is passed to area CA3 by way of the granule cell axons, the mossy

fibers. From here, CA3 pyramidal cell axons collateralize and either project within CA3, or they project to area CA1 through the Schaffer collaterals. The dentate gyrus-CA3-CA1 projections have typically been the most studied and are often referred to as the "tri-synaptic pathway". CA1 fibers output to entorhinal cortex through the subiculum and back to the deep layers of the entorhinal cortex. In addition, there are other direct connections from the entorhinal cortex to CA1 and CA3. A brief overview of the structures of the hippocampal formation will be discussed with particular emphasis on the dentate gyrus, which is the primary focus of this project.

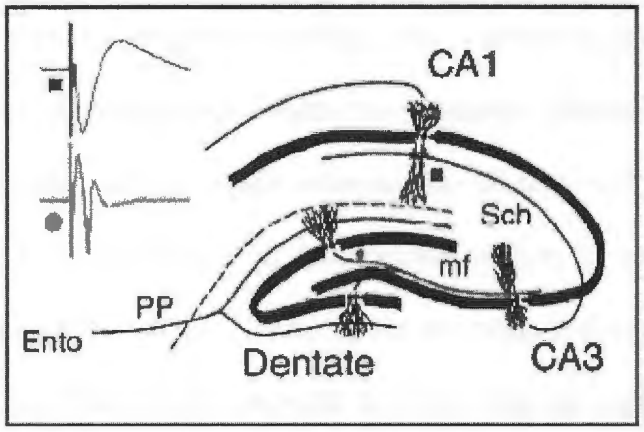


Figure 1. Schematic diagram of the tri-synaptic pathway in hippocampus.

Entorhinal Cortex

The entorhinal cortex is comprised of six cortical layers, which are highly laminated. Superficial layers project extrinsically to the hippocampus with Layer II being the primary output into the dentate gyrus via the perforant pathway, and Layer III projecting to area CA1 and the subiculum (Steward and Scoville, 1977; Witter and

Groenewegen, 1990; Desmond et al., 1994; Leung, 1995; Paré & Llinas, 1995; Yeckel & Berger, 1995; Canning & Leung, 1997; and Naber et al., 1999). These projections are glutamatergic (White et al., 1977), although GABA-ergic perforant path projections have also been observed (Germroth et al., 1989). The deep layers (V-VI) of the entorhinal cortex receive relatively little intrinsic innervation from the superficial layers of the entorhinal cortex; instead these layers primarily receive output from area CA1 and the subiculum (Kloosterman et al., 2003; Naber et al., 2001).

The entorhinal cortex is divided into lateral and medial areas which make different levels of contact on the granule cell dendritic tree. The lateral to medial bands in the entorhinal cortex relate to the septal (anterior) – to – temporal (posterior) portions along the hippocampal longitudinal axis which have separate functions. The laterally originating pathway provides sensory inputs, whereas, the medially originating pathway most likely, is involved in the transfer of motivational signals or reflections of the organism's intrinsic state (Witter et al., 2000a). It can be concluded from this matrix of connections that the entorhinal cortex network is more than an input/output station mediating corticohippocampal interplay. The lateral entorhinal cortex (LEC) and the medial entorhinal cortex (MEC) hippocampal loops mediate the processing of different sensory information (Burwell & Amaral, 1998, Witter et al., 2000a). The entorhinal cortex is uniquely positioned to monitor what hippocampal processing does to a particular input. Witter et al. (2000b) stated that the entorhinal cortex might detect differences between an incoming stimulus and the overall outcome of hippocampal processing of a closely related stimulus that entered earlier in time. The entorhinal cortex

may also provide short-term maintenance of information getting sent back to the hippocampus (Iijima et al, 1996). Buzsaki (1996) suggested adaptive behavioural responses might be generated by a hippocampal output signal to adjacent temporal association cortex. Witter (2000b) concluded that whatever the proposed entorhinal cortex function, it may depend on the activity of a specific set of cortical afferents. In summary, the entorhinal cortex can be divided into at least two longitudinal zones, which project to different parts along the hippocampal longitudinal axis on the basis of the perforant pathway afferents. The organization regarding projections from the perirhinal and postrhinal cortices is beyond the concerns of the present thesis. Additional information is found in Witter et al's (2000a; 2000b) discussions.

Dentate Gyrus and Hilus

The dentate gyrus, or fascia dentata, consists of three layers. The principal cell layer consists of densely packed granule cells; the molecular layer consists of a complex arborization of granule cell dendrites; and the polymorphic layer, commonly designated as hilus or the hilar region consists of a variety of interneurons and displaced pyramidal cells.

Granule cells are characterized by a spiny dendritic arborization that projects unidirectionally into the molecular layer. Information originating in the entorhinal cortex projects to the dentate gyrus where it synapses on the granule cell dendrites in a highly typified manner. Glutamatergic projections arising from the lateral entorhinal cortex synapse on the distal 1/3 of the molecular layer while afferents of the dentate gyrus originating from the medial entorhinal cortex synapse on the middle 1/3 of the granule

cell dendritic tree (Steward, 1977). The inner 1/3 of the molecular layer also receives intrinsic, presumably excitatory, input from both ipsilateral and contralateral hilar regions (Blackstad, 1956; Zimmer, 1971). Electrical stimulation of the medial and lateral entorhinal cortex produces two identifiably different EPSP profiles in the dentate gyrus, with lateral perforant path stimulation evoking an EPSP and population spike of longer latency than that seen with medial perforant path stimulation (Abraham and McNaughton, 1984;McNaughton and Barnes, 1977).

The hilar region possesses numerous cell types including "aspiny" interneurons and, the most prevalent cell in the hilar region, the "spiny" mossy cells. Dendrites of mossy cells most often extend only within the polymorphic region, but can penetrate the granule cell layer and terminate in regions as far as the outer molecular layer (Scharfman, 1991). Numerous types of inhibitory interneurons have been identified in the hilus. Many of these cells are immunoreactive for GABA, as well as parvalbumin, calbindin, somatostatin, and substance P (Boyett and Buckmaster, 2001;Sik et al., 1997;Sloviter et al., 2001). Though the role of dentate gyrus-CA3 connection has typically been classified as excitatory, inhibitory GABAergic cells in the hilus receive direct excitatory input from the granule cells, which may serve to suppress activity in area CA3 (Penttonen et al., 1998). The granule cells also receive a GABA-ergic projection from terminals of "basket cells" located under the granule cell layer (Kosaka et al., 1984). Other inhibitory influences on dentate granule cells arise from "chandelier cells" of the molecular layer and somatostatin-positive cells in the hilus (Morrison et al., 1982).

Although the dentate gyrus receives the largest projection from the entorhinal cortex, it also receives a number of projections from subcortical structures including: the septum (Amaral and Kurz, 1985), the supramammilary area of the hypothalamus, as well as brain stem monoaminergic projections. The only projection leaving the principal cells of the dentate gyrus is the mossy fiber projection originating from the unmyelinated axons of the granule cells synapsing with CA3. A single mossy fiber makes extensive contact with CA3 pyramidal cell dendrites in stratum lucidum and its mossy fiber projections extend throughout the entire CA3 field to the point where CA3 and CA2 converge.

Ammon's Horn

The principal cells of the hippocampus, or Ammon's Horn, are the pyramidal cells. These cells have two dendritic arborizations, the basal dendrites that extend into stratum oriens and the apical dendrites, which extend towards the hippocampal fissure. The principal cells of CA3 are typically larger than those found in region CA1. CA3 neurons collateralize within CA3 as well as terminating in CA2 and CA1. They also project to the same regions contralaterally and a small number project to the hilar region (Amaral and Witter, 1995). As an example of the largely unidirectional flow of the hippocampus, CA3 neurons do not appear to project to the entorhinal cortex though they receive direct projections from this area (Witter, 2000a). CA3 neurons project to CA1 through axons known as the Schaffer collaterals. These projections are topographically organized and vary according to the transverse location of origin of the projecting CA3 neuron.

The CA2 region of the hippocampus is unique in that it can only be delineated from CA1 and CA3 by using specific histological techniques. An interesting component of CA2 principal cells is that they appear to contain a large amount of calcium binding proteins, particularly parvalbumin (Leranth and Ribak, 1991). There are few studies investigating the functional significance of these cells. The appearance of CA2 neurons is similar to the pyramidal cells of CA3 though they receive no input from dentate gyrus mossy fibers. Behaviorally, there is little evidence to determine their role in behavior or memory systems (Corbett and Crooks, 1997).

As mentioned above, the primary inputs into region CA1 of the hippocampus arise from the Schaffer collateral pathway terminating in stratum oriens and stratum radiatum and from layer III of entorhinal cortex and terminating in stratum moleculare. Other minor projections exist including some from the amygdala (Finch, 1996). CA1 gives rise to two principal outputs, one to the subiculum, the second to the deep layers (V-VI) of entorhinal cortex (Calderazzo et al., 1996). Output from CA1 appears also to be topographically organized, these outputs include connections to the retrosplenial and perirhinal cortex, as well as to the anterior olfactory nucleus, the olfactory bulb, amygdala and hypothalamus (Amaral and Witter, 1995). Field CA3, on the other hand, projects bilaterally upon the lateral septum. The lateral septal nucleus in turn, projects partly upon the medial septal nucleus and nucleus of the diagonal band, and partly to the lateral hypothalamus and the mamillary complex. The medial septal-diagonal band complex projects back, through the fimbria and dorsal fornix, to fields CA3 and CA4 of the hippocampus, to the dentate gyrus, to the subicular complex, and to the entorhinal

area. The entorhinal cortex has long been regarded as a relay station that provides the major source of afferent input to the hippocampus. The perforant path input to the dentate gyrus from layer II has traditionally been regarded as the major pathway by which information is transferred. However, electrophysiological studies (Buzsaki and Eidelberg, 1982; Doller and Weight, 1982; Yeckel and Berger, 1990) indicate that other elements of the perforant path that project directly to the CA1 and CA3 are more important than previously thought, and that the properties of different neuronal elements in the entorhinal cortex may determine the way the information is passed on to, and processed by, the hippocampus (Jones, 1993).

**The Role of the Dentate Gyrus in Learning and Memory**

As discussed previously, Bliss and Lomo's work on LTP has identified the hippocampal formation and the tri-synaptic circuit as a useful model for exploring the neurobiology and neurophysiology of learning and the storage of memories supporting associative learning. LeDoux has demonstrated, through the fear-conditioning paradigm, that the hippocampal formation is an essential component of the brain system underlying the explicit recollection of past events and the processing of relational information (Phillips & LeDoux, 1992; Phillips & LeDoux, 1994; and LeDoux, 2000). LeDoux (2002) suggested that the consequences of activating these circuits are different because the amygdala has hard-wired responses and the hippocampus elicits a multitude of responses. It is clear that the hippocampus plays an important role in learning and memory. However, in terms of exploring the dentate gyrus's unique role in memory a

challenge remains. It has been difficult to separate the dentate gyrus from the rest of the tri-synaptic pathway in order to study, selectively, the behavioral role of the granule cells alone. The next section outlines work that further explored the specific role of the dentate gyrus in learning and memory by focusing on associative learning and examining the synaptic changes during fear conditioning. These results taken from Doyère et al. (1995) are the basis of this present study.

**Doyère's Work**

Doyère et al. (1995) examined the time course of synaptic modifications during learning. Changes in the perforant path-dentate gyrus evoked field potentials were measured in rats that were given a classical conditioning (paired tone and footshock) or pseudoconditioning (unpaired tone and footshock) task. Differential changes in the evoked response were observed during the 4 days of training. An increase in slope (mV/ms) of the EPSP was seen in the conditioned group, and began to appear after five tone-shock paired trials. This effect outlasted the 22 min training session by 20 minutes. In contrast, the EPSP slope decreased during training and the decrease lasted for over an hour for the pseudoconditioned group. A prolonged decrease in population spike (mV) was seen in both groups. The increase and duration of the EPSP change reduced and shortened over the course of training for the conditioned group, whereas the decrease in the EPSP for the pseudoconditioned group increased. To test if the rats had learned the tone-footshock association, an operant conditioning task was given. Lever pressing for food reward was suppressed during the presentation of the tone for the conditioned group. However, this difference in suppression was only seen in the first block of trials. No

difference was found between groups during the second block of trials, suggesting rapid extinction. Temperature, stress, arousal, and muscular effort were controlled as possible causes of the differential changes in the EPSP. It was concluded that synaptic changes, as indexed by the perforant path-dentate gyrus measures, vary in magnitude and time-course according to the temporal relationship between the conditioned stimulus (CS) and the unconditioned stimulus (US).

**Rationale and Objectives**

The primary goal of this study was to repeat the methodology of Doyère et al. (1995) in an acute preparation in order to examine the time course of synaptic modifications in perforant path-dentate gyrus connections during conditioning procedures and determine if Doyère et al. (1995) findings can be repeated in anesthetized rats. This would demonstrate that associative learning in the anesthetized state could be convincingly demonstrated, and that synaptic modifications in the perforant path-dentate gyrus take place in the anesthetized rat, which would facilitate the ability to dissect the origin of such changes. Doyère et al. (1995) trained their animals and recorded evoked potentials across 4 periods separated by 24 hrs. This present study investigated changes in perforant path-dentate gyrus in rats subjected to the same number of CS-US pairings, but occurring in one session. Rats in both of Doyère's groups showed a trend to increases in EPSP slope during shock presentation that reversed during tone alone presentation. A group similar to Doyère's pseudoconditioned group received 32 deliberately unpaired presentations of tone and footshock. If the group that receives co-terminating sound and shock pairings demonstrates greater changes in slope and or population spike than the

pseudoconditioned group, it would be reasonable to conclude, that it was the pairing of the stimuli that induced the changes in dentate gyrus. If Doyère et al's results can be replicated in animals receiving anesthesia it would suggest the hippocampus is modulated during the anesthetized state for learning as it is during LTP. A secondary goal of the experiment was to record perforant path-dentate gyrus measures when a period of extinction trials (32 tone alone presentations) is administered to further explore changes in the CS-US association during the anesthetized state.

There have only been a few studies that suggest that learning can take place under anesthetized conditions. Using urethane anesthesia, Pirch et al. (1985a and 1985b) observed conditioning of single units and slow potentials in rat frontal cortex following pairings to of 2-sec tone with medial fibre bundle stimulation. However, these responses could only be detected if they first had been initiated in awake animals as a result of hundreds of conditioning trials. Weinberger et al. (1984) found support for associative learning during the unconscious state when injections of epinephrine, in addition to barbiturate anesthesia were administered. Edeline and Neuenschwander-El Massioui (1988) demonstrated that Pavlovian conditioning can occur under ketamine, an anesthetic with dissociative effects on the nervous system, and be retained for at least a week. Finally, experiments using other anesthetics give supporting evidence that learning can take place in the anesthetized state. These include those using halothane in mice (Pang et al., 1996) and propofol in humans (Deeprose, Andrade, Varma, and Edwards, 2004).

# METHODS

## Animals

This experiment used naïve male (n=14; 250-325 g) Sprague-Dawley rats, purchased from the Memorial University of Newfoundland Vivarium Laboratory (St. John's, NF, Canada). The rats were housed individually in standard home cages (a Plexiglas box measuring 42 X 30 X 42 cm with wood chip bedding covering the floor) at the Biotechnology Animal Care Facility for a minimum of two days prior to any experimentation. They were given food and water *ad libitum* in a temperature-controlled room on a 12 hr light/dark cycle (lights on at 7 am). Animals were weighed daily immediately prior to urethane injection. All procedures carried out on the animals conformed to Canadian Council on Animal Care (CCAC) standards and were approved by the Institutional Committee on Animal Care.

## Group Selection

Two groups, each consisting of seven naïve animals were used to examine the changes in perforant path evoked response associated with Pavlovian conditioning in the anesthetized model. The experimental or conditioned group received 32 CS-US pairings of tone and footshock over a 90 min period followed by a 60 min no pairing period and then a 90 min extinction period where 32 tone presentations were given without footshock. The interval between each tone-footshock pairing and tone alone presentation was variable (2-4 min). The control or pseudoconditioned group received the identical presentation of stimuli except during the conditioning period, where they received 32 deliberately unpaired presentations of tone and footshock. The CS-US unpairings were

generated through a random list which included reverse order of CS and US stimuli. The interval between the presentation of tone and footshock pairs varied between 2-4 mins similar to the pairing configurations and intervals used by Doyère et al. (1995). The parameters for the tone and shock stimuli are described in the succeeding sections.

**Surgery**

Animals were initially anesthetized with urethane (1.5 mg/100 ml, intraperitoneal, 24 gauge syringe). The dorsal surface of the head was then shaved and given a local injection of marcaine (0.5 ml, subcutaneously, 24 gauge syringe). When the animal no longer responded to foot pinch it was placed on a heating pad in a stereotaxic instrument. A rectal probe was used to maintain body temperatures at 36-37°C. Hollow ear bars were used to allow acoustic delivery via ear bud headphones.

Before any incision or electrode placement, a test was done for hind limb reflexes. If a reflex was observed, animals were given a supplement (20% initial injection) of urethane. Additional supplements were given if needed. A midline incision was made, with the head in the skull flat position. Bregma and lamda reference points were identified and marked using the stereotaxic arm. Two small holes were then drilled into the skull for the dentate gyrus (3.5 mm posterior to bregma and 2.0 mm lateral) and perforant path (7.2 mm posterior to bregma and 4.1 mm lateral). A small stainless steel jeweller's screw was positioned in the skull as a ground reference.

**Electrophysiology**

A concentric bipolar stimulating electrode (Kopf Instruments; NE-100) was placed in the perforant path, while a glass recording saline micropipette (25-50 μm tip

diameter) was positioned in the dentate gyrus. The perforant path was stimulated (50 to 800 μA, 0.2 ms) every thirty seconds with an Isolated Current Source (Neuro Data Instruments Corp., 0-1 mA, Model S1490).

For electrocardiogram (EKG) measurements, hypodermic needles (10 gauge) were threaded under the skin in the upper dorsal right shoulder and ventral left abdomenal regions. Alligator clips were attached to the needles and connected to a Grass Hi Z Probe (Model PSII) that connected to the third amplifier input. A Grass RPS $107^{E}$ regulated power supply (±12V/0.7A) (Quincy, MASS), and three Grass PS Series A.C. Pre Amplifiers (Model PSIIK) were used to amplify the perforant path-dentate gyrus evoked response and the EKG signals were recorded (see Table 1 for respective settings).

**Table 1**

**Amplifier Settings for Perforant Path-Dentate Gyrus evoked potential and EKG**

| | *Calibrator* | *Low Filter* | *High Filter* | *Amplification* | *Filter* |
|---|---|---|---|---|---|
| ***Perforant Path - Dentate Gyrus*** | 50 mV | 1 | 3 | 50X | Out |
| ***EKG*** | 10 mV | 30 | 1 | 500X | Out |

Following perforant path-dentate gyrus placements two stereo wire leads, which originated from the Lafayette (Indiana) Master Shocker (Model A-615A), were connected to the ventral portion of both hindpaws with electrolyte gel and electric tape.

Input-output (I/O) curves for the evoked response were recorded by increasing the perforant path stimulating current in increments of 50 μA and observing the evoked

response changes. The stimulation current ranged from 100 - 500 μA to achieve 50% of the maximum population spike (PS) amplitude. Following completion of the I/O curves, baseline responses (50% of maximum PS) were taken. To rule out acute surgery effects, no perforant path-dentate gyrus stimulation or any experimental manipulation was done during the first 3 hr post injection (Gilbert and Mack, 1999). A 30 min baseline period prior to the first CS-US pairing was recorded before the start of the conditioning period. Once baseline recordings were established, a computer program was initiated to deliver 32 pairings of tone-shock over 90 min. The following is the sequence of events that occurred during each CS-US pairing:

1. 5 sec of EKG, then
2. 5 sec of CS tone (8 KHz, 70 dB) that co-terminated with a US footshock (0.5 sec, 0.5 mA), then
3. 10 sec of EKG, then
4. 0.2 ms perforant path stimulation every 30 sec for entire recording period as in Doyère et al. and
5. 5 sec of EKG.

Perforant path stimulations took place every 30 sec including before pairings and continued throughout the 32 CS-US or CS protocol sequence which was interpolated as outlined below. Animals received 32 pairings of tone-shock over approximately 90 min. To generate the 8 kHz tone, a Hewlett Packard Audio Oscillator (Model 200ABR) and a Hewlett Packard Function Generator (Model 3310B) were used. A Grason-Stadler (Model 455C) noise generator was used for the background noise which was on during

the entire experiment. A Sony Integrated Stereo Amplifier (Model TA-3650), with mode set at mono and the low and high filters off, was used to amplify the tone. The generators and amplifier were powered by an Anatek (Model 25-2S) regulated DC power supply. The inter-trial-interval (ITI) varied randomly between 2 and 4 min and no pairings coincided with PP stimulation. Thirty-two pairings were chosen to incorporate Doyère et al. (1995) 4-session conditioning procedure into a single session. Following the last pairing, 60 min of perforant path-dentate gyrus recording alone was taken, followed by 90 min of recording with 32 CS (tone alone) presentations, and finally 30 min of perforant path-dentate gyrus recording alone to end the recording session.

**Calibrations**

Calibration of the ear bud stereo headphones (Sony - Model MDRE819V) that were attached to the hollow ear bars was performed before the animal was prepared for the stereotaxic set-up. Decibel readings were measured with a 7-Range Analog Display Sound Level Meter (SLM) (Radio Shack – cat no. 33-4050) that was positioned in the centre of the hollow ear bars. With headphones attached, each ear bar was clamped in a vice and the centre of the SLM meter was positioned horizontally directly against the ear bar opening that made contact to the animal's skull.

**Histology**

After electrophysiological recordings were completed, the brains of the animals were removed for histological examination. A 0.5 mA current was delivered to the PP stimulation electrode to produce a lesion to verify placement (C.H. Stoelting Co., CAT NO. 58040). The animals were then given an overdose of urethane and decapitated. The

brains were removed and immersed in chilled methylbutane solution and placed in a freezer at a temperature of –77 °C. The brains were blocked and sliced on a cryostat at 40 μm and sections were mounted on glass slides. They were stained using a cresyl violet staining procedure and cover slipped. Sections were observed under microscope to verify placement of electrodes.

**Data Collection**

The digitized evoked response data and the EKG data were cut into ASCII format (DataWave Technologies) and later examined in Microsoft Excel. The two measures that were analyzed from the evoked responses were slope (mV/ms) or synaptic response to cortical input and population spike (mV) the cellular response to cortical input (Figures 2a and 2b). The slope was calculated by plotting the 10 points that make up the straightest line prior to the initial peak in the trace of the total 200 points recorded for each potential. The difference between the maximum point on the initial peak to the minimum point in the valley of the trace was used to calculate the population spike. Responses were normalized by taking the mean of the 30 min baseline period prior to the first CS-US pairing.

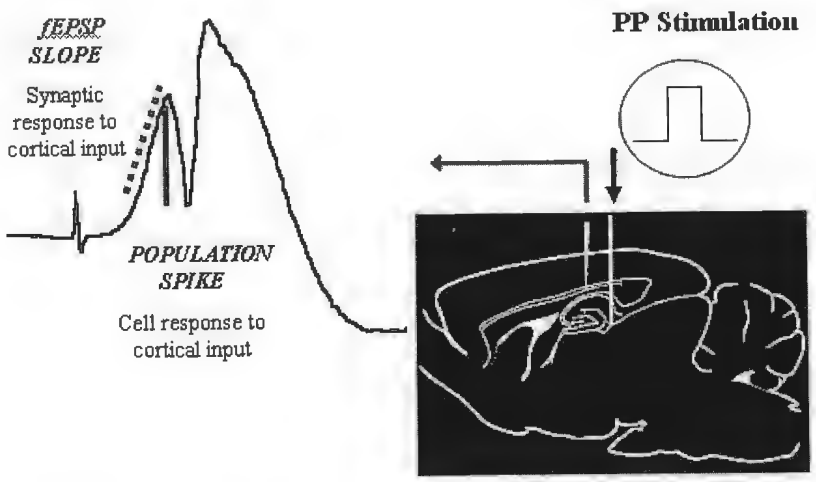


Figure 2.a. Perforant Path – Dentate Gyrus Evoked Potential

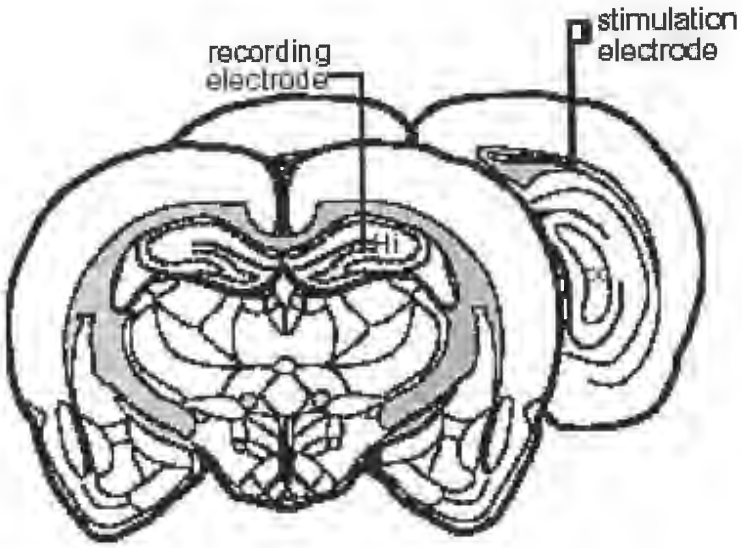


Figure 2.b. Schematic of stimulation (perforant path) and recording (dentate gyrus) electrodes in the Rat brain

**Data Analysis**

For both groups, repeated measures ANOVA across time was performed on the normalized responses; alpha = 0.05. Pearson correlation calculations were also done to measure the association between the normalized slope and population spike changes for each group over time; alpha = 0.05.

# RESULTS

## Histology

Marked placements of the dentate gyrus (Figure 3a) and perforant path (Figure 3b) were verified through brain slice examination.

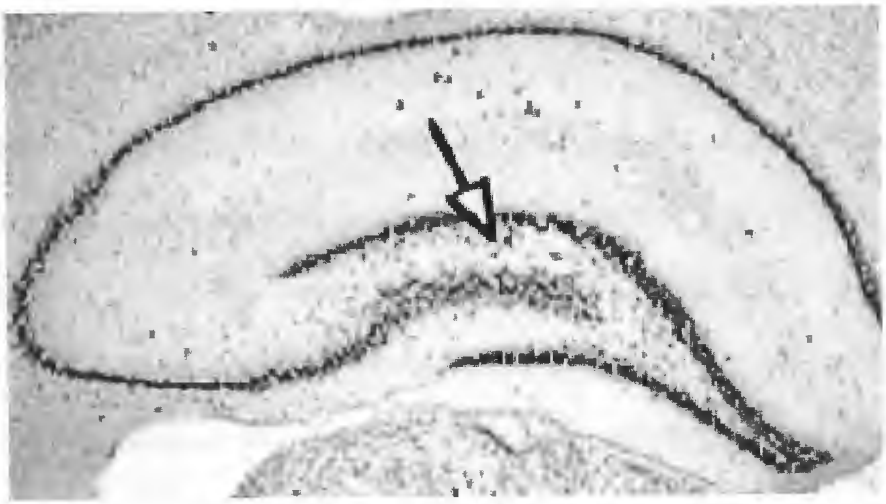

Figure 3a. An example of Dentate Gyrus Recording electrode placement

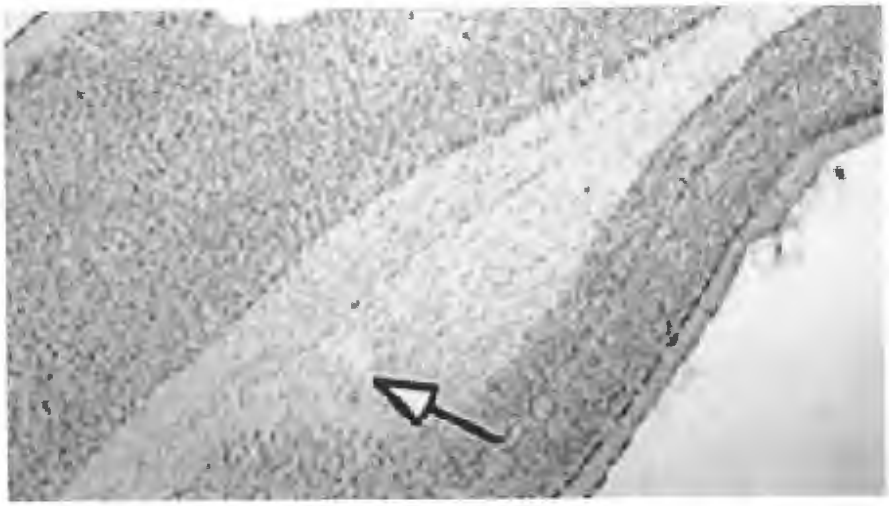

Figure 3b. An example of Perforant Path Stimulation placement

**Omission of Animals**

Examination of the normalized population spike responses showed that two subjects in the pseudoconditioned group were more than twice a standard deviation away from the group mean in the extinction period. These were treated as outliers and omitted from further analysis.

**Slope Response**

The raw data indicate that during the initial 30 min baseline period the mean size of the raw slope (mV/ms) for the conditioned and pseudoconditioned groups was 5.62 (± 0.96 S.D.) and 7.92 (± 2.12 S.D) respectively. These were not significantly different ($F_{1,10}$=6.82, $p>0.05$). Normalized data from the slope responses for both the conditioned group (N=7) and the pseudoconditioned group (N=5) are shown in Figure 4 where a similar pattern was noted throughout parts of the 300 min of recording. The conditioned group responses remained at baseline levels 60 min in the conditioning period, increased slightly above baseline, and then leveled off during the extinction period. The same result occurred for the pseudoconditioned group with the exception on the initial decline in slope responses at the start of the conditioning period which lasted only 30 min before reversing back towards baseline.

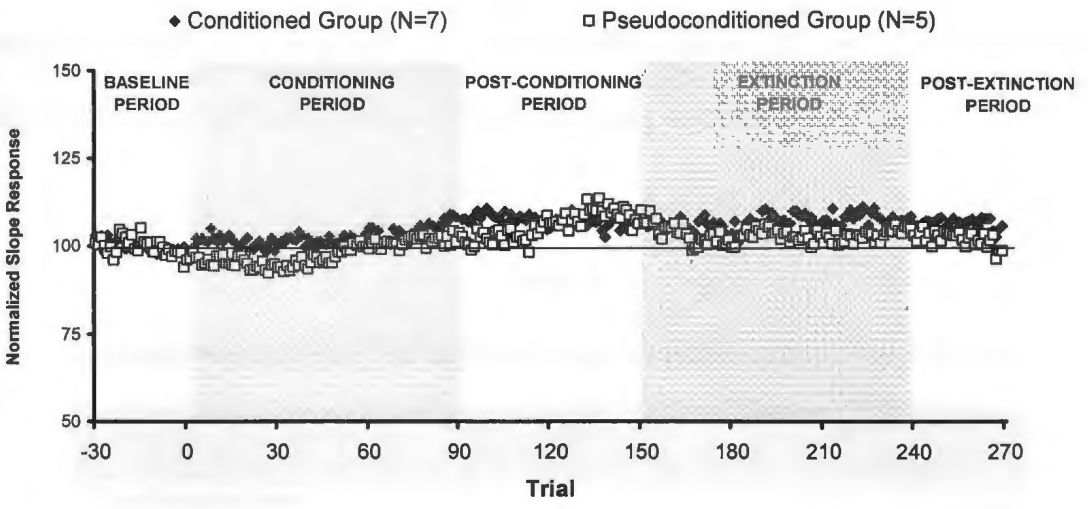


Figure 4 Averaged Normalized Slope Response across Time for Conditioned and Pseudoconditioned Groups.

For each group of animals, the slope responses were normalized and grouped into 30 record blocks, each consisting of 10 min periods (refer to Tables 2 and 3 for a descriptive summary and Figure 5). An Analysis of Variance found no differences between the two groups ($F_{1,10}$=0.10, $p>0.05$) over the entire recording period. Overall, the slope responses did not significantly vary from baseline level and were not affected by the manner in which tone and footshock presentations were received. A 2 group x 9 blocks x 30 trials ANOVA of the responses in the conditioning period showed no effect of group ($p=0.149$), block ($p=0.091$), trial ($p=0.435$) or interactions. The same result occurred for the 60 min period that followed. However a three-way interaction between group, block and trial was close to significant ($p=0.054$). The conditioned group showed greater slope responses in the earlier trials that decreased over the 60 min while the

opposite occurred for the pseudoconditioned group with an increase over trials and blocks. Separate analysis of the 90 min extinction period and the 30 min period that followed found no effects or interactions on the slope responses.

**Table 2**

**Characteristics of the Normalized Slope Sample – Conditioned Group**

| *Recording Block (10 min)* | *Mean* | *SD* | *Minimum* | *Maximum* |
|---|---|---|---|---|
| ***Conditioning Period*** | | | | |
| ***1st 10 min*** | 100.9 | 2.2 | 97.4 | 104.9 |
| ***2nd 10 min*** | 101.4 | 1.2 | 99.3 | 103.1 |
| ***3rd 10 min*** | 100.2 | 1.5 | 98.3 | 102.3 |
| ***4th 10 min*** | 101.8 | 1.2 | 99.8 | 104.0 |
| ***5th 10 min*** | 101.2 | 0.8 | 100.5 | 102.8 |
| ***6th 10 min*** | 101.5 | 1.4 | 99.7 | 103.5 |
| ***7th 10 min*** | 102.7 | 2.1 | 100.3 | 105.2 |
| ***8th 10 min*** | 103.7 | 1.6 | 100.6 | 105.8 |
| ***9th 10 min*** | 106.9 | 1.4 | 105.2 | 108.9 |
| ***Post-Conditioning Period*** | | | | |
| ***1st 10 min*** | 107.9 | 2.0 | 103.7 | 110.6 |
| ***2nd 10 min*** | 108.1 | 1.2 | 106.7 | 109.8 |
| ***3rd 10 min*** | 107.3 | 1.3 | 104.6 | 108.9 |
| ***4th 10 min*** | 107.1 | 1.5 | 105.0 | 110.2 |
| ***5th 10 min*** | 106.2 | 1.8 | 102.5 | 108.8 |
| ***6th 10 min*** | 106.5 | 1.4 | 104.3 | 108.5 |
| ***Extinction Period*** | | | | |
| ***1st 10 min*** | 106.3 | 0.8 | 104.9 | 107.3 |
| ***2nd 10 min*** | 105.5 | 1.8 | 102.3 | 108.3 |
| ***3rd 10 min*** | 105.8 | 2.0 | 102.0 | 108.9 |
| ***4th 10 min*** | 105.1 | 2.1 | 101.0 | 107.8 |
| ***5th 10 min*** | 107.5 | 2.0 | 103.8 | 110.5 |
| ***6th 10 min*** | 107.8 | 1.9 | 104.4 | 110.0 |
| ***7th 10 min*** | 107.5 | 1.8 | 105.3 | 110.5 |
| ***8th 10 min*** | 108.9 | 1.6 | 105.9 | 111.1 |
| ***9th 10 min*** | 107.3 | 0.7 | 106.5 | 108.3 |
| ***Post-Extinction Period*** | | | | |
| ***1st 10 min*** | 106.6 | 1.0 | 104.8 | 108.0 |
| ***2nd 10 min*** | 106.3 | 1.0 | 104.7 | 107.7 |
| ***3rd 10 min*** | 105.8 | 1.9 | 102.4 | 108.0 |

**Table 3**

**Characteristics of the Normalized Slope Sample – Pseudoconditioned Group**

| *Recording Block (10 min)* | *Mean* | *SD* | *Minimum* | *Maximum* |
|---|---|---|---|---|
| ***Conditioning Period*** | | | | |
| ***1st 10 min*** | 95.8 | 1.3 | 94.1 | 98.4 |
| ***2nd 10 min*** | 96.1 | 1.2 | 94.4 | 97.4 |
| ***3rd 10 min*** | 94.1 | 1.0 | 92.4 | 95.7 |
| ***4th 10 min*** | 94.4 | 1.2 | 92.7 | 96.9 |
| ***5th 10 min*** | 95.8 | 1.2 | 93.4 | 97.4 |
| ***6th 10 min*** | 99.6 | 1.1 | 98.2 | 101.5 |
| ***7th 10 min*** | 100.5 | 0.9 | 99.1 | 101.8 |
| ***8th 10 min*** | 101.6 | 1.6 | 98.7 | 103.1 |
| ***9th 10 min*** | 101.9 | 1.3 | 99.9 | 104.3 |
| ***Post-Conditioning Period*** | | | | |
| ***1st 10 min*** | 101.9 | 1.9 | 98.8 | 104.8 |
| ***2nd 10 min*** | 102.5 | 1.8 | 100.1 | 104.9 |
| ***3rd 10 min*** | 103.7 | 3.0 | 98.1 | 108.6 |
| ***4th 10 min*** | 107.0 | 1.5 | 104.2 | 109.3 |
| ***5th 10 min*** | 110.2 | 2.4 | 105.5 | 113.5 |
| ***6th 10 min*** | 108.5 | 2.1 | 104.4 | 111.0 |
| ***Extinction Period*** | | | | |
| ***1st 10 min*** | 106.4 | 2.6 | 102.0 | 110.2 |
| ***2nd 10 min*** | 103.0 | 2.8 | 98.8 | 106.5 |
| ***3rd 10 min*** | 101.9 | 1.3 | 100.3 | 104.1 |
| ***4th 10 min*** | 102.9 | 1.9 | 99.7 | 105.6 |
| ***5th 10 min*** | 103.8 | 1.5 | 101.2 | 106.2 |
| ***6th 10 min*** | 102.3 | 1.5 | 99.7 | 104.5 |
| ***7th 10 min*** | 102.0 | 1.5 | 100.2 | 104.4 |
| ***8th 10 min*** | 102.4 | 1.2 | 100.6 | 104.0 |
| ***9th 10 min*** | 104.5 | 0.9 | 103.0 | 106.0 |
| ***Post-Extinction Period*** | | | | |
| ***1st 10 min*** | 102.7 | 1.7 | 99.8 | 105.4 |
| ***2nd 10 min*** | 102.7 | 1.3 | 99.7 | 104.0 |
| ***3rd 10 min*** | 100.3 | 2.2 | 96.2 | 103.4 |

Figure 5 shows the slope responses for each group plotted across trial block. There were no differences between the conditioned and pseudoconditioned groups during the conditioning period or the extinction period. Interestingly, the variability in the responses increased following the conditioning period for both groups.

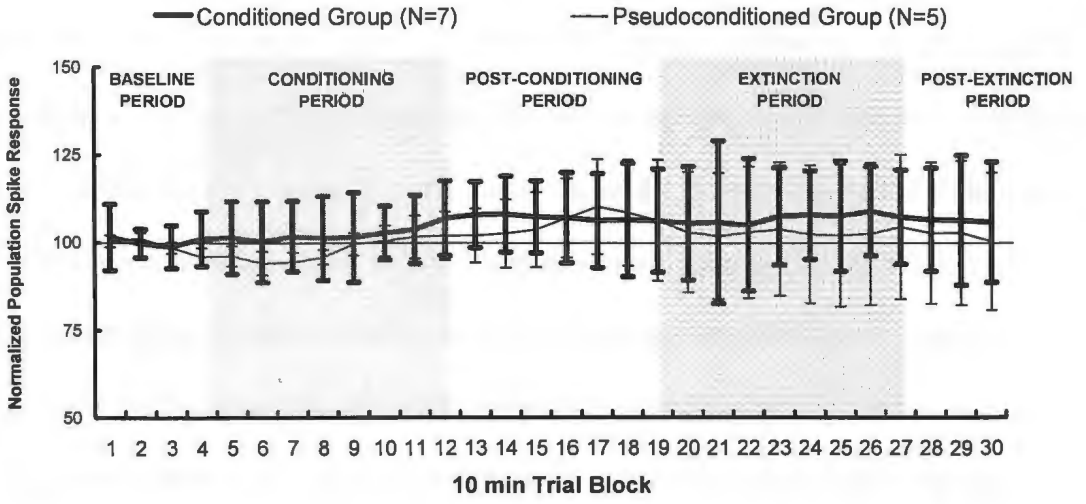


Figure 5. Averaged Normalized Slope Response across Trial Block for Conditioned and Pseudoconditioned Groups.

**Population Spike Response**

The population spike magnitude (mV) of the raw responses reported across the two groups were 5.14 (± 1.68 S.D.) and 5.23 (± 2.39 S.D) respectively. These mean responses were similar in size prior to start of the conditioning period ($F_{1,10}$=0.01, $p>0.05$).

Normalized data from the population spike responses for both the conditioned group (N=7) and the pseudoconditioned group (N=5) are shown in Figure 6. Throughout the 300 min recording session, the conditioned group showed a delayed increase in population spike amplitude whereas the pseudoconditioned group showed a decreasing response following conditioning that continued throughout the remainder of the recordings. For both groups this started after the conditioning and prior to extinction.

During the conditioning period the population spike responses remained at baseline levels for both groups. During the post-conditioning baseline period the population spike responses for the conditioned group increased over 40% over baseline levels, while the pseudoconditioned group showed a decreasing trend in responses that reached approximately 15% below baseline levels. During the extinction period, the population spike responses for the conditioned group remained above baseline while the responses for the pseudoconditioned group decreased from baseline at the start of the CS alone trials, reaching approximately 40% below baseline levels. Following the extinction period the pseudoconditioned group leveled off at about 30-35% below baseline while the responses in the conditioned group continued to increase to 50% above baseline levels.

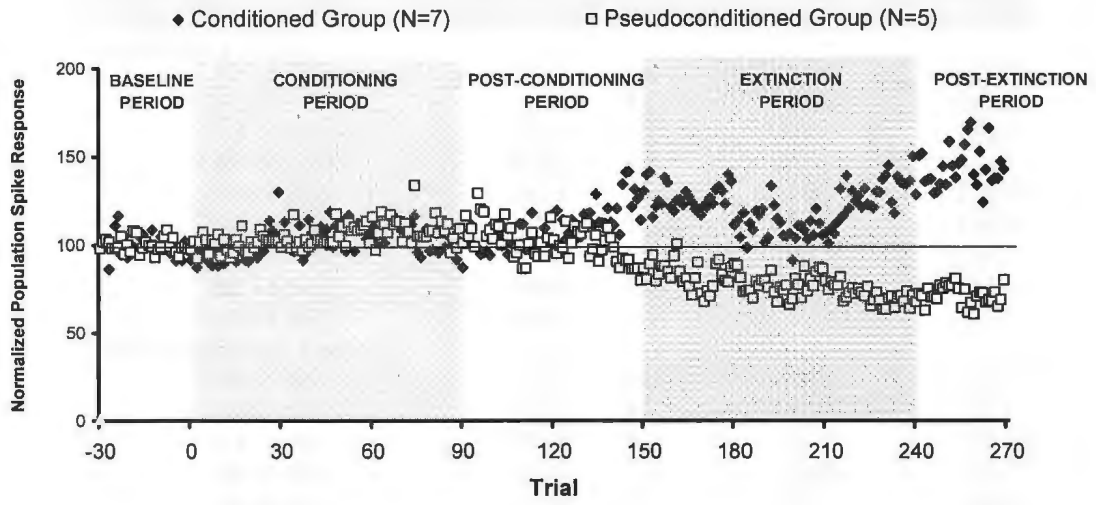


Figure 6. Averaged Normalized Population spike Response across Time for Conditioned and Pseudoconditioned Groups.

Similar to the normalized slope responses, the population spike responses were grouped into 30 blocks, each consisting of 10 min periods (refer to Tables 4 and 5 for a

descriptive summary). Figure 7 shows the population spike responses for each group plotted across trial block. A mixed model ANOVA showed no effect of group, however there was a significant group by block interaction ($F_{26,260}$=5.13, p<0.01).

The only significant difference between the population spike responses was a group by block interaction during the 60 min post-conditioning period ($F_{5,50}$=3.53, p<0.01) and group effects in both the extinction period ($F_{1,10}$=7.60, p<0.01) and the final 30 min period of recording ($F_{1,10}$=12.56, p<0.01).

**Table 4**

**Characteristics of the Normalized Population Spike Sample – Conditioned Group**

| *Recording Block (10 min)* | *Mean* | *SD* | *Minimum* | *Maximum* |
|---|---|---|---|---|
| ***Conditioning Period*** | | | | |
| ***1st 10 min*** | 93.6 | 4.5 | 87.4 | 100.6 |
| ***2nd 10 min*** | 94.4 | 3.6 | 89.4 | 99.2 |
| ***3rd 10 min*** | 103.6 | 11.7 | 92.7 | 130.2 |
| ***4th 10 min*** | 102.5 | 8.1 | 91.3 | 114.4 |
| ***5th 10 min*** | 107.5 | 5.8 | 98.8 | 118.8 |
| ***6th 10 min*** | 107.7 | 7.0 | 96.5 | 116.8 |
| ***7th 10 min*** | 108.0 | 4.8 | 100.5 | 114.9 |
| ***8th 10 min*** | 105.0 | 7.6 | 93.1 | 116.4 |
| ***9th 10 min*** | 101.3 | 6.1 | 91.7 | 111.3 |
| ***Post-Conditioning Period*** | | | | |
| ***1st 10 min*** | 98.4 | 6.6 | 87.2 | 111.2 |
| ***2nd 10 min*** | 102.8 | 6.4 | 93.3 | 111.9 |
| ***3rd 10 min*** | 105.0 | 8.4 | 94.5 | 118.2 |
| ***4th 10 min*** | 107.1 | 7.1 | 96.6 | 119.3 |
| ***5th 10 min*** | 116.4 | 5.4 | 111.3 | 128.8 |
| ***6th 10 min*** | 124.7 | 12.3 | 105.7 | 141.4 |
| ***Extinction Period*** | | | | |
| ***1st 10 min*** | 129.1 | 9.0 | 115.6 | 141.1 |
| ***2nd 10 min*** | 123.1 | 4.1 | 116.8 | 130.1 |
| ***3rd 10 min*** | 128.6 | 6.4 | 121.3 | 139.7 |
| ***4th 10 min*** | 112.2 | 7.0 | 98.3 | 120.0 |
| ***5th 10 min*** | 109.5 | 11.8 | 91.0 | 133.2 |
| ***6th 10 min*** | 109.3 | 5.3 | 103.0 | 120.5 |
| ***7th 10 min*** | 117.4 | 12.7 | 100.9 | 139.0 |
| ***8th 10 min*** | 126.7 | 5.3 | 121.1 | 137.0 |

| | | | | |
|---|---|---|---|---|
| *9th 10 min* | 135.4 | 9.2 | 117.8 | 149.9 |
| *Post-Extinction Period* | | | | |
| *1st 10 min* | 137.7 | 8.4 | 128.3 | 151.6 |
| *2nd 10 min* | 149.8 | 11.9 | 133.9 | 168.9 |
| *3rd 10 min* | 141.9 | 11.5 | 123.8 | 165.9 |

**Table 5**

**Characteristics of the Normalized Population spike Sample – Pseudoconditioned Group**

| *Recording Block (10 min)* | *Mean* | *SD* | *Minimum* | *Maximum* |
|---|---|---|---|---|
| *Conditioning Period* | | | | |
| *1st 10 min* | 100.7 | 4.5 | 92.4 | 107.3 |
| *2nd 10 min* | 98.9 | 5.5 | 92.9 | 111.0 |
| *3rd 10 min* | 103.0 | 2.6 | 98.5 | 108.7 |
| *4th 10 min* | 102.2 | 5.9 | 96.7 | 117.1 |
| *5th 10 min* | 104.3 | 5.2 | 100.3 | 117.4 |
| *6th 10 min* | 108.3 | 4.6 | 99.5 | 113.4 |
| *7th 10 min* | 111.2 | 6.3 | 97.1 | 118.7 |
| *8th 10 min* | 108.5 | 9.4 | 101.2 | 133.8 |
| *9th 10 min* | 107.3 | 6.8 | 95.6 | 118.2 |
| *Post-Conditioning Period* | | | | |
| *1st 10 min* | 111.2 | 9.6 | 98.9 | 129.2 |
| *2nd 10 min* | 104.6 | 7.6 | 93.1 | 117.3 |
| *3rd 10 min* | 98.4 | 9.1 | 86.7 | 115.6 |
| *4th 10 min* | 102.8 | 6.7 | 92.4 | 113.4 |
| *5th 10 min* | 102.3 | 7.4 | 90.7 | 112.3 |
| *6th 10 min* | 89.0 | 5.0 | 79.9 | 98.7 |
| *Extinction Period* | | | | |
| *1st 10 min* | 85.6 | 4.6 | 79.6 | 94.2 |
| *2nd 10 min* | 83.1 | 9.3 | 71.3 | 100.5 |
| *3rd 10 min* | 79.9 | 7.9 | 67.8 | 91.4 |
| *4th 10 min* | 78.6 | 6.4 | 69.6 | 89.2 |
| *5th 10 min* | 74.3 | 5.9 | 66.0 | 85.4 |
| *6th 10 min* | 80.4 | 6.1 | 69.7 | 87.7 |
| *7th 10 min* | 75.5 | 4.2 | 68.0 | 81.7 |
| *8th 10 min* | 70.5 | 4.2 | 63.2 | 76.1 |
| *9th 10 min* | 68.5 | 3.6 | 63.4 | 73.4 |
| *Post-Extinction Period* | | | | |
| *1st 10 min* | 71.1 | 4.2 | 62.7 | 76.8 |
| *2nd 10 min* | 71.5 | 7.4 | 60.6 | 80.8 |
| *3rd 10 min* | 70.7 | 4.2 | 65.0 | 80.0 |

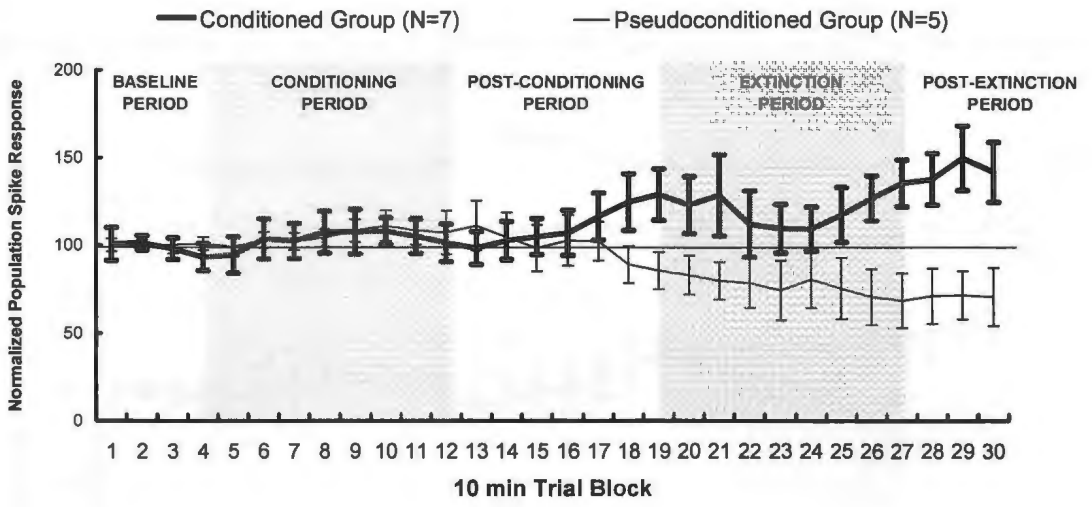


Figure 7. Averaged Normalized Population spike Response across Trial Block for Conditioned and Pseudoconditioned Groups.

**Population spike/ EPSP Slope Ratio Response**

Ratios between the population spike and slope responses were calculated to explore coupling changes (refer to Tables 6 and 7). Figure 8 shows the population spike/slope ratio responses for each group plotted across trial block (Margineanu et al., 1994). Over the entire recording period, division of the normalized population spike responses by the normalized slope responses found a significant group by block interaction ($F_{26,260}$=4.22, $p<0.001$). No effects or interactions were noted during the 90 min conditioning period. However, a group by block interaction was found ($F_{5,50}$=4.73, $p=0.001$) during the 60 min post-conditioning period that followed. Finally, no significant effects or interactions were found for the 90 min extinction period but a

significant group effect was noted for the 30 min post-extinction ($F_{1,10}$=8.55, $p<0.05$). This was consistent with the initial analysis of the normalized population spike responses.

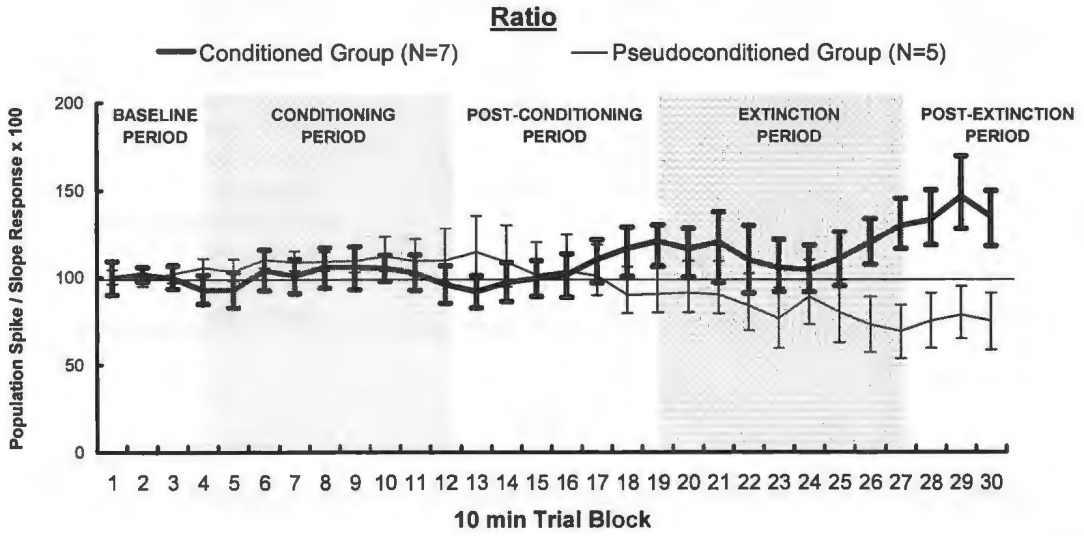


Figure 8. Population Spike / Slope Response Ratios across Trial Block for Conditioned and Pseudoconditioned Groups.

**Table 6**

**Characteristics of the Ratio Sample – Conditioned Group**

| Recording Block (10 min) | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| ***Conditioning Period*** | | | | |
| ***1st 10 min*** | 0.933 | 0.053 | 0.866 | 1.025 |
| ***2nd 10 min*** | 0.930 | 0.039 | 0.869 | 0.986 |
| ***3rd 10 min*** | 1.040 | 0.140 | 0.919 | 1.381 |
| ***4th 10 min*** | 1.009 | 0.086 | 0.882 | 1.147 |
| ***5th 10 min*** | 1.060 | 0.063 | 0.959 | 1.181 |
| ***6th 10 min*** | 1.058 | 0.072 | 0.937 | 1.140 |
| ***7th 10 min*** | 1.054 | 0.053 | 0.958 | 1.123 |
| ***8th 10 min*** | 1.024 | 0.089 | 0.887 | 1.163 |
| ***9th 10 min*** | 0.960 | 0.061 | 0.864 | 1.069 |
| ***Post-Conditioning Period*** | | | | |
| ***1st 10 min*** | 0.920 | 0.058 | 0.856 | 1.032 |
| ***2nd 10 min*** | 0.971 | 0.064 | 0.875 | 1.070 |
| ***3rd 10 min*** | 0.994 | 0.086 | 0.890 | 1.141 |
| ***4th 10 min*** | 1.015 | 0.073 | 0.937 | 1.169 |

| | | | | |
|---|---|---|---|---|
| *5th 10 min* | 1.103 | 0.065 | 1.034 | 1.238 |
| *6th 10 min* | 1.167 | 0.131 | 0.970 | 1.347 |
| *Extinction Period* | | | | |
| *1st 10 min* | 1.208 | 0.084 | 1.080 | 1.313 |
| *2nd 10 min* | 1.164 | 0.045 | 1.078 | 1.231 |
| *3rd 10 min* | 1.202 | 0.073 | 1.110 | 1.333 |
| *4th 10 min* | 1.099 | 0.091 | 0.952 | 1.223 |
| *5th 10 min* | 1.056 | 0.127 | 0.881 | 1.287 |
| *6th 10 min* | 1.042 | 0.059 | 0.969 | 1.165 |
| *7th 10 min* | 1.109 | 0.124 | 0.938 | 1.325 |
| *8th 10 min* | 1.201 | 0.058 | 1.137 | 1.341 |
| *9th 10 min* | 1.299 | 0.093 | 1.096 | 1.406 |
| *Post-Extinction Period* | | | | |
| *1st 10 min* | 1.332 | 0.085 | 1.245 | 1.490 |
| *2nd 10 min* | 1.467 | 0.149 | 1.306 | 1.725 |
| *3rd 10 min* | 1.351 | 0.096 | 1.170 | 1.510 |

**Table 7**

**Characteristics of the Ratio Sample – Pseudoconditioned Group**

| Recording Block (10 min) | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| *Conditioning Period* | | | | |
| *1st 10 min* | 1.056 | 0.052 | 0.941 | 1.129 |
| *2nd 10 min* | 1.034 | 0.055 | 0.970 | 1.144 |
| *3rd 10 min* | 1.100 | 0.027 | 1.063 | 1.157 |
| *4th 10 min* | 1.087 | 0.069 | 1.028 | 1.265 |
| *5th 10 min* | 1.090 | 0.058 | 1.036 | 1.236 |
| *6th 10 min* | 1.094 | 0.047 | 1.012 | 1.151 |
| *7th 10 min* | 1.122 | 0.073 | 0.966 | 1.212 |
| *8th 10 min* | 1.094 | 0.101 | 1.003 | 1.355 |
| *9th 10 min* | 1.097 | 0.087 | 0.950 | 1.221 |
| *Post-Conditioning Period* | | | | |
| *1st 10 min* | 1.145 | 0.108 | 1.014 | 1.367 |
| *2nd 10 min* | 1.089 | 0.093 | 0.929 | 1.236 |
| *3rd 10 min* | 1.013 | 0.102 | 0.881 | 1.177 |
| *4th 10 min* | 1.037 | 0.070 | 0.896 | 1.128 |
| *5th 10 min* | 1.010 | 0.098 | 0.885 | 1.141 |
| *6th 10 min* | 0.900 | 0.049 | 0.828 | 1.002 |
| *Extinction Period* | | | | |
| *1st 10 min* | 0.905 | 0.063 | 0.828 | 1.011 |
| *2nd 10 min* | 0.912 | 0.115 | 0.760 | 1.093 |
| *3rd 10 min* | 0.901 | 0.111 | 0.763 | 1.109 |
| *4th 10 min* | 0.839 | 0.137 | 0.720 | 1.125 |
| *5th 10 min* | 0.764 | 0.066 | 0.676 | 0.878 |
| *6th 10 min* | 0.890 | 0.116 | 0.711 | 1.067 |
| *7th 10 min* | 0.801 | 0.071 | 0.716 | 0.948 |
| *8th 10 min* | 0.729 | 0.051 | 0.639 | 0.808 |
| *9th 10 min* | 0.691 | 0.046 | 0.627 | 0.793 |

| | | | | |
|---|---|---|---|---|
| ***Post-Extinction Period*** | | | | |
| ***1st 10 min*** | 0.751 | 0.074 | 0.639 | 0.864 |
| ***2nd 10 min*** | 0.786 | 0.126 | 0.607 | 0.960 |
| ***3rd 10 min*** | 0.751 | 0.060 | 0.678 | 0.884 |

## Correlation Findings

Table 8 shows the results of the correlation analysis of the slope and population spike for each group. During the conditioning period, no association was found between the measures for the conditioned group ($r=0.463$, $p<0.05$) while a positive association was found for the pseudoconditioned group ($r=0.393$, $p=0.000$). Negative associations were found for each group during the 60 min period that followed the CS-US pairings ($r=-371$, $p=0.004$ for and $r=-0.302$, $p=0.019$), respectively. No significant correlations were noted for the 90 min extinction period and the 30 min period that followed.

**Table 8**

**Correlation Findings for Slope and Population Spike Responses**

| *Recording Period* | *r value* | *p value* | *Significant?* | *Direction* |
|---|---|---|---|---|
| ***Conditioning (90 min)*** | | | | |
| Conditioned Group | .463 | $p > .05$ | No | Positive |
| Pseudoconditioned Group | .393 | $p < .001$ | Yes | Positive |
| ***Post-Conditioning (60 min)*** | | | | |
| Conditioned Group | -.371 | $p < .05$ | Yes | Negative |
| Pseudoconditioned Group | -.302 | $p < .05$ | Yes | Negative |
| ***Extinction Period (90 min)*** | | | | |
| Conditioned Group | -.049 | $p > .05$ | No | Negative |
| Pseudoconditioned Group | .164 | $p > .05$ | No | Positive |
| ***Post-Extinction (30 min)*** | | | | |
| Conditioned Group | -.027 | $p > .05$ | No | Negative |
| Pseudoconditioned Group | .040 | $p > .05$ | No | Positive |

**Heart Rate**

Following the test of a number of samples of inter beat interval (IBI), of subjects from each group, the max variation in IBI was 1 millisecond. Given this limited variability no further analysis was performed.

# DISCUSSION

## Slope Response

The results show no significant normalized slope measure differences between the conditioned and pseudoconditioned groups during the 90 min conditioning period and the other recording periods. During the CS-US pairings, the responses for the conditioned rats remained at baseline levels where the maximal percent change in EPSP slope above baseline was 8.9%, while a 4.3% maximal increase was seen in the pseudoconditioned group. During the 60 min rest period that followed the training period, the slope responses for the conditioned group were above the responses recorded for the pseudoconditioned group. However, this was only short lasting (approximately 30 min). Responses in both groups returned to baseline levels during the 90 min extinction period and the 30 min rest period that completed the recording trials.

Generally the input drive on the granule cells did not change with the presentation of CS-US pairings and CS alone presentations. Due to the lack of slope differences found in the present experiment, it can be concluded that the synaptic drive in the dentate gyrus was not affected by the relationship of the tone and footshock stimuli received in the anesthetized rats.

## Population Spike Response

For both groups, the population spike response remained at baseline levels throughout the entire conditioning period and much of the post-conditioning period. Approximately 30 min into the CS alone presentations the population spike responses in the conditioned group increased significantly reaching approximately 50% above

baseline by the end of the extinction period (60 min later) and over 165% above baseline by the end of the recording period (another 30 min later). The population spike response of the pseudoconditioned group decreased away from baseline levels throughout the CS alone trials and the final 30 min of recording reaching approximately 35% below baseline.

Analysis indicated no significant group difference during the conditioning period, but did reveal a group by block interaction during the 60 min rest period that followed. Significant group differences were found during the extinction period and the final 30 min period of recording. This suggests that the difference in the pairing of tone and footshock did have an effect specific to the population spike measure, as reflected in the post-acquisition period.

**Population Spike / EPSP Slope Ratio Response**

Ratios between the population spike and slope responses were calculated to investigate the coupling changes throughout the different recording periods. The ratio results paralled the population spike response results, with no significant differences found between the two groups during the conditioning period and a group by block interaction in the 60 min period that followed training. In contrast to the population spike results, no significant main effects or interactions was found in the 90 min extinction period but in comparison, a significant difference between the conditioned and pseudoconditioned groups was noted for the final 30 min period of recording. The fact that smaller slopes are associated with larger population spikes, as found in the

conditioned group, but not the pseudoconditioned group, suggests increased excitability may be a signature of associative learning.

**Comparison to Doyère et al's Findings**

Doyère et al. (1995) reported an increase in slope for the conditioned group (maximal % change from 4.03 to 13.06%) and a decrease in slope for the pseudoconditioned group (between 3.34 and 12.61%), both of which developed rapidly (i.e., after five tone shock paired trials) and lasted 40 and 60 min respectively before returning to baseline measures. They concluded that the EPSP slope increase was an associative effect and not due to sensory stimulation, environmental novelty or sensitization. They also argued that the temporal relationship between environmental events determines the change in the synaptic efficacy during conditioning in the dentate gyrus.

Although the increase was slower in development, it is noteworthy to mention that the anesthetized conditioned group did show an increasing trend in EPSP slope during the conditioning period similar to that of Doyère et al. (1995) conditioned group, along with comparable magnitudes (8% vs. 9% respectively). The EPSP slope response in Doyère's experiments returned toward baseline, a pattern also seen here. In addition, the initial slope depression in the pseudoconditioned group during the conditioning period resembles Doyère et al.'s data. However these changes were not significant in the present study.

The type of anesthetic in the present experiment may explain why there was no difference found in the slope measure between the groups. Urethane has been known to depress hippocampal evoked responses (Riedel et al., 1994; Shirasaka and Wasterlain, 1995; Maggu and Meli, 1986). Instability of dentate gyrus field potentials, or field potential drift, as reported by Rick and Milgram (1999), may have prevented the conditioned group from responding differently than the pseudoconditioned group. They suggested that common drifting changes, towards the positive direction in the acute preparation, can be 4-6% per hour in individual subjects. The use of an anesthetized preparation versus the chronic preparation of Doyère et al. (1995) may account for the general differences between field potential responses. Kamondi et al. (1988) suggested the excitability of the hippocampus is different in awake animals compared to the anesthetized preparation. Therefore, it is not surprising that the present results, at least in part, were in contrast to what was demonstrated by Doyère et al. (1995). The post-conditioning, results, however, support the argument that the increase in population spike was not solely due to urethane or other factors (e.g., cell death, edema, spreading depression, blood loss, and physical stabilization of electrode) mainly because the responses differed across the two groups. However, the fact that there was no useful EKG data makes it unclear whether the rats were "experiencing" the stimuli presented to them although footshock does activate arousal systems in urethane anesthetized rats (Valentino, Foote, and Aston-Jones, 1983). The lack of EEG measures doesn't allow confirmation that the depth of the anesthesia was too deep to elicit pain responses from

the footshock. EEG measures would have been useful as an index of response to footshock.

As previously mentioned, there have only been a few studies that suggest that learning can take place under anesthetized conditions. Of the studies that used urethane anesthesia (Pirch et al., 1985a and 1985b) the potentials were recorded in the frontal cortex not the dentate gyrus, and could only be detected if they first had been initiated in awake animals as a result of hundreds of conditioning trials.

With respect to the population spike responses, no between group differences were reported in the experiment of Doyère et al. (1995). The same was found during conditioning for the present experiment, with the exception of the extinction period and the final 30 min. However in the awake rats, a decrease (i.e., between 10 and 15%) in the population spike of the dentate gyrus granule cells was noted during the conditioning period lasting throughout the remainder of the session trials. In the urethane anesthetized rats, the decreasing trend was delayed by about 1 hour post conditioning and only seen for the pseudoconditioned group, whereas a delayed increase was noted for the conditioned group. If the recording period had been stopped 60 min after conditioning, as was done in the awake study, the difference in reactivity in cells wouldn't have become known. It would be interesting to see an additional 2 hours of recording done in the awake model, as was done here.

Doyère et al. (1995) tested for learning the CS-US association by measuring the conditioned suppression of an appetitive response to food. Upon presentation of the CS stimulus, the conditioned group in contrast to the pseudoconditioned group, demonstrated

suppression in lever-pressing for food reward, thus was indicating that they had learned the tone-footshock association. The present experiment used urethane because it sustains anesthesia over a long period without the need for supplementary dosages. However, animals cannot be recovered from this form of anesthesia by CCAC regulations, thus preventing any testing for learning in the awake state. It would also be interesting if an extinction period was implemented in the awake rats 1 hour following the last CS-US paired presentation. This would allow for comparisons with the present extinction results.

Due to the fact that the only group differences seen coincided with the 90 min period of CS alone trials, there is no way to determine how much of the group difference in the population spike responses was due to the conditioning manipulation or the onset of the tone alone presentations. However, the results indicate that the pattern of population spike responses for the conditioned group reversed prior to the start of the extinction period, whereas the negative trend for the pseudoconditioned rats was unaffected by the change in stimuli presentation. Therefore, it may be plausible to argue that a change in population spike responding following the start of extinction is indicative of the granule cells processing the association of the CS and US stimuli presented to the conditioned group. That is, although there was no obvious disparity of population spike responses during the conditioning period, the group difference at the end of the experimental recording reflects the possibility that the conditioned group did process the CS-US association differently than the pseudoconditioned group, rather than a simple effect of tone alone information. Hence the evidence suggests conditioned rats did "learn" the association of the CS-US pairings previously presented to them. Powell,

Maxwell and Penney (1996) observed another form of plasticity under anesthesia. They found decreases in tone-evoked neuronal activity during extinction trials compared with the previous CS/US paired trials while assessing Pavlovian eyeblink conditioning in the medial prefrontal cortex.

It was already explained that urethane may have accounted for the non significant changes in the slope responses. The urethane preparation may also account for the delayed differential reactivity to the CS-US pairings in the present experiment.

**Future Work**

Other Anesthetics - Future work in this area should attempt to replicate the results with an anesthetic that would allow for safe recovery and testing of the learned association between the CS and the US. This experimenter had difficulty with maintaining a consistent level of unconsciousness to allow for stable perforant path-dentate gyrus recordings in preliminary studies when using ketamine. Ketamine might not be the best choice for learning experiments because it blocks the NMDA receptors, but Edeline and Neuenschwander-El Massioui (1988) demonstrated, as previously mentioned, that a Pavlovian conditioning demonstrated in hippocampal recordings can occur under ketamine which lasted a week. However, this method was only employed on a few animals. Learning of an CS-US association under anesthesia is possible as indicated previously. Therefore additional work using inhaled anesthetics (e.g., halothane) may allow for a briefer training period (i.e., fewer than 4 days as employed by Doyère et al., 1995) prior to testing in the awake state.

Post-Conditioning Period – A post-conditioning period of 60 min following the conditioning period was designed to determine if the slope and population spike responses would change following the CS-US pairings and allow some time for consolidation of the learned association to occur. However, if the 32 pairings does enable a strong learning of the CS-US association in the anesthetized rat, perhaps the test of learning through extinction could be administered earlier. The apparent changes in the present experiment appeared at the end of the recordings; therefore this effect should have been followed. If the slope and population spike reacted differently with a shorter baseline, then it might be possible to have an increased control of the potential effects of baseline drifting as caused by the anesthetic and add additional support for a mechanism of associative learning in the dentate gyrus. This experimenter is aware that drifting could occur even with manipulations; therefore an additional control group with no manipulations may separate out the variance due to drifting versus manipulation effects. Additional recording between (and following) the conditioning and extinction periods may clarify the optimal procedure for establishing the best time to administer extinction trials and for demonstrating their impact on DG responses returning (or not returning) to baseline levels. Doyère et al. (1995) reported changes that didn't last as long compared to the present experiment, but they were still able to provide evidence for long-term memory in their suppression testing.

Mechanism Involved – This experiment suggests increased excitability is involved in the acquisition and/or extinction of paired conditioning. Exploring the effects of

pharmaceutical agents that facilitate or block the action of receptors or transmitters known to be involved in associative learning such as N-methyl-D-aspartate (NMDA) receptors (Kim et al., 1991; Miserendino et al., 1990; Baker and Azorlosa, 1996; Falls et al., 1992; Johnson et al., 2000) and/or norepinephrine receptors (Stein, Belluzzi, and Wise, 1975; Davis, 1980; Neuman and Harley, 1983; Lacaille and Harley, 1985; Stanton and Sarvey, 1985; Harley and Milway, 1986; Babstock and Harley, 1992; Lee et al., 1993; Wilson, Pham and Sullivan, 1994; Harley and Evans, 1998; Jeltsch et al., 2001; Southwick et al., 2002) on dentate gyrus measures would now be useful to isolate possible substrates of the pairing effect observed here. NE, for example produces long-term increases in cell excitability, although it has not previously been shown that they depend on pairing.

Other Neural Substrates - Considerable evidence has implicated the amygdala in Pavlovian fear conditioning. A widely held view is that the hippocampus is required for the formation and retrieval of context-fear associations, whereas the amygdala is required for conditioning and recall of associations to contextual and discrete cues (Maren and Fanselow, 1996; Rogan and LeDoux, 1996). Specifically, the basolateral amygdala (BLA) has been argued to be the central locus of all fear conditioning (Fanselow and Ledoux, 1999). However certain forms of fear conditioning persist despite lesions to the BLA (Selden et al., 1991; Killcross et al., 1997; Cahill et al., 1999; Maren, 1999), reflecting either the involvement of other amygdala nuclei in fear memory recall (Killcross et al., 1997) or a more limited involvement of the amygdala in the acquisition,

but not the storage, of fear memories (McGaugh et al., 1996; Cahill and McGaugh, 1998).

Vazdarjanova and McGaugh (1999) suggested that the strength of Pavlovian contextual fear conditioning could be modulated by post-training infusion of muscimol (a GABA-A agonist that functionally inactivates the amygdala). It would be interesting to see the impact on dentate gyrus measures of amygdala inactivation. The impact of another substrate may also be worthwhile exploring. Welsh and Harvey (1998) anaesthetized the inferior olive with lidocaine while rabbits simultaneously: (i) performed conditioned nictitating membrane responses to a flashing light to which they had already been trained; and (ii) underwent their first experience with classical conditioning of the same response to a tone.. They demonstrated that an acute disruption in olivary function can block associative learning and suggested that the inferior olive may have a general role in regulating temporal processing.

On a final note, it would be worthwhile to examine the impact of dentate gyrus changes during anesthetic following a contextual conditioning paradigm. However this can only be accomplished with contextual training in the awake animal and then later testing for perforant path-dentate gyrus changes while under urethane. In exploring this potential paradigm, the present experimenter found inconsistent results in a few animals; hence the data are not presented.

**Conclusion**

Doyère et al. (1995) argued that synaptic potentiation demonstrated by their conditioned group as increased slope responses represented the processing of aspects of

the memory trace, and hence a possible mechanism for acquisition. The present experiment provides evidence that the dentate gyrus reacts differently to paired and unpaired presentations of tone and footshock during the anesthetized state, as indexed specifically by the population spike amplitude measure. However, unlike what was found in the awake group study, the differences between the groups of the present experiment are only seen post acquisition as opposed to during the conditioning period. The results lend support for a more active role of the responsiveness of the granule cells versus the overall synaptic drive of the dentate gyrus in processing information about the association of a CS and US during the anesthetized state. Further exploration and consideration of other connected substrates such as the amygdala and other parts of the tri-synaptic circuit, would shed more light on the molecular properties and mechanisms involved in one form of associative learning in the acute preparation. Associative learning, and the extent to which it is independent of consciousness, continues to be debated.

While Doyère and her colleagues showed transient increases in synaptic strength that appeared selective to conditioning, an equally impressive result of this study was an increase in cell excitability among pseudoconditioned rats that was not seen among conditioned rats. Decreased cell excitability with pseudoconditioning was seen in the present study. This opposite pattern of results suggests pairing selectively modulates and attenuates or facilitates generalized cell excitability depending on the preparation. This could restrict such changes to the paired tone stimulus itself (something which was not evaluated in either study). Recording during the extinction period suggests a diminution in excitability, but whether this would occur with time independent of tone presentations

as in Doyère et al.'s (1995) experiment is not clear. It would be of interest to monitor averaged evoked responses to the tone stimulus itself in this paradigm.

# REFERENCES


1. Abraham, W.C., Mason, S.E. (1988). Effects of the NMDA receptor/channel antagonists CPP and MK801 on hippocampal field potentials and long-term potentiation in anesthetized rats. Brain Research, 462:40-46.

2. Abraham, W.C. and McNaughton, N. (1984). Differences in synaptic transmission between medial and lateral components of the perforant path. Brain Research, 303, 251-260.

3. Adamec, R.E. (1991). Individual differences in temporal lobe sensory processing of threatening stimuli in the cat. Physiology and Behavior, 49: 455-64.

4. Amaral, D.G. and Kurz, J. (1985). An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat. The Journal of Comparative Neurology, 240: 37-59.

5. Amaral, D.G. and Witter, M.P. (1995). The hippocampal formation In: The Rat Brain Paxinos G (Ed), Academic Press 2nd edition, 443-493.

6. Artola, A. and Singer, W. (1987). Long-term potentiation and NMDA receptors in rat visual cortex. Nature, 330: 649-652.

7. Babstock, D.M. & Harley, C.W. (1992). Paragigantocellularis stimulation induces beta-adrenergic hippocampal potentiation. Brain Research Bulletin, 28 (5):709-14.

8. Baker, J.D. & Azorlosa, J.L. (1996). The NMDA antagonist MK-801 blocks the extinction of Pavlovian fear conditioning. Behavioral Neuroscience, 110 (3):618-20.

9. Berry, R.L., Teyler, T.J., and Han, T.Z. (1989). Induction of LTP in rat primary visual cortex: tetanus parameters. Brain Research. 481, 221-227.

10. Blackstad, T.W. (1956). Commisural connections of the hippocampal region in the rat, with special reference to their mode of termination. The Journal of Comparative Neurology, 105, 417-537.

11. Bliss, T.V., Goddard, G.V., Riives, M. (1983). Reduction of long-term potentiation in the dentate gyrus of the rat following selective depletion of monoamines. Journal of Physiology, 334:475-91.

12. Bliss, T.V. & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. Journal of Physiology, 232 (2): 331-56.

13. Boyett, J.M. and Buckmaster, P.S. (2001). Somatostatin-immunoreactive interneurons contribute to lateral inhibitory circuits in the dentate gyrus of control and epileptic rats. Hippocampus, 11, 418-422.

14. Burwell, R.D. & Amaral, D.G. (1998). Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. The Journal of Comparative Neurology, 391 (3): 293-321.

15. Buzsaki, G. & Eidelberg, E. (1982). Direct afferent excitation and long-term potentiation of hippocampal interneurons. Journal of Neurophysiology, 48 (3): 597-607.

16. Buzsaki, G. (1996). The hippocampo-neocortical dialogue. Cerebral Cortex, 6 (2): 81-92.

17. Cahill, L., McGaugh, J.L. (1998). Mechanisms of emotional arousal and lasting declarative memory. Trends in Neuroscience, 21 (7):294-9.

18. Cahill, L., Weinberger, N.M., Roozendaal, B., McGaugh, J.L. (1999). Is the amygdala a locus of "conditioned fear"? Some questions and caveats. Neuron, 23 (2):227-8.

19. Calderazzo,L., Cavalheiro,E.A., Macchi,G., Molinari,M., and Bentivoglio,M. (1996). Branched connections to the septum and to the entorhinal cortex from the hippocampus, amygdala, and diencephalon in the rat. Brain Research Bulletin, 40, 245-251.

20. Canning, K.J. & Leung, L.S. (1997). Lateral entorhinal, perirhinal, and amygdala-entorhinal transition projections to hippocampal CA1 and dentate gyrus in the rat: a current source density study. Hippocampus, 7 (6): 643-55.

21. Collingridge, G.L., Kehl, S.J., & McLennan, H. (1983). The antagonism of amino acid-induced excitations of rat hippocampal CA1 neurones in vitro. Journal of Physiology, 334: 19-31.

22. Corbett, D. and Crooks, P. (1997). Ischemic preconditioning: a long term survival study using behavioural and histological endpoints. Brain Research, 760, 129-136.

23. Davis, M. (1980). Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. Neuroscience Biobehavioral Reviews, 4 (2):41-63.

24. Davis, M. (1989). Sensitization of the acoustic startle reflex by footshock. Behavioral Neuroscience, 103 (3): 495-503.

25. Davis, M. (1990). Animal models of anxiety based on classical conditioning: the conditioned emotional response (CER) and the fear-potentiated startle effect. Pharmacology Theory, 47 (2): 147-65.

26. Davis, M. (1992). The role of the amygdala in fear and anxiety. Annual Review of Neuroscience, 15: 353-75.

27. Davis, M., Falls, W.A., Campeau, S. & Kim, M. (1993). Fear-potentiated startle: a neural and pharmacological analysis. Behavioral Brain Research, 58 (1-2): 175-98.

28. Deeprose, C., Andrade, J., Varma, S. & Edwards, N. (2004). Unconscious learning during surgery with propofol anaesthesia. British Journal of Anaesthesia, 92 (2):171-7.

29. Desmond, N.L., Scott, C.A., Jane, J.A. & Levy, W.B. (1994). Ultrastructural identification of entorhinal cortical synapses in CA1 stratum lacunosum-moleculare of the rat. Hippocampus, 4 (5): 594-600.

30. Doller, H.J. & Weight, F.F. (1982). Perforant pathway activation of hippocampal CA1 stratum pyramidale neurons: electrophysiological evidence for a direct pathway. Brain Research, 237 (1): 1-13.

31. Doyère, V., Redini-Del Negro, C., Dutrieux, G., Le Floch, G., Davis, S. & Laroche, S. (1995). Potentiation or depression of synaptic efficacy in the dentate gyrus is determined by the relationship between the conditioned and unconditioned stimulus in a classical conditioning paradigm in rats. Behavioral Brain Research, 70 (1): 15-29.

32. Edeline, J.M. & Neuenschwander-el Massioui, N. (1988). Retention of CS-US association learned under ketamine anesthesia. Brain Research, 457 (2): 274-80.

33. Falls, W.A., Miserendino, M.J. & Davis, M. (1992). Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. Journal of Neuroscience, 12 (3):854-63.

34. Fanselow, M.S. & LeDoux, J.E. (1999). Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron, 23 (2):229-32.

35. Fendt, M. & Fanselow, M.S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. Neuroscience and Biobehavioral Review, 23 (5): 743-60.

36. Finch, D.M. (1996). Neurophysiology of converging synaptic inputs from the rat prefrontal cortex, amygdala, midline thalamus, and hippocampal formation onto

single neurons of the caudate/putamen and nucleus accumbens. Hippocampus, 6 (5):495-512.

37. Freud, S. (1895). Project for a scientific psychology. First published in Strachey, J. (ed.). The standard edition of the complete psychological works of Sigmund Freud. London: The Hogarth Press and the Institute of Psycho-Analysis.

38. Germroth, P., Schwerdtfeger, W.K., and Buhl, E.H. (1989). GABAergic neurons in the entorhinal cortex project to the hippocampus. Brain Research, 494, 187-192.

39. Gilbert, M.E., Mack, C.M. (1999). Field potential recordings in dentate gyrus of anesthetized rats: stability of baseline. Hippocampus, 9 (3):277-87.

40. Graeff, F.G., Viana, M.B. & Tomaz, C. (1993). The elevated T maze, a new experimental model of anxiety and memory: effect of diazepam. Brazil Journal of Medical Biology Research, 26 (1): 67-70.

41. Harley, C.W. and Evans, S. (1988). Locus coeruleus-induced enhancement of the perforant path-evoked potential: Effects of intradentate beta blockers. In Cellular Mechanisms of Conditioning and Behavioral Plasticity, C. D. Woody, D. L. Alkon, and J. L. McGaugh, eds. (New York: Plenum Publishing), pp. 415-423.

42. Harley, C.W. & Milway, J.S. (1986). Glutamate ejection in the locus coeruleus enhances the perforant path-evoked population spike in the dentate gyrus. Experimental Brain Research, 63 (1):143-50.

43. Hartley, David. (1749/1971). Observations on Man, His Frame, His Duty, and His Expectations. London, reprinted New York: Garland.

44. Hebb, D. O. (1949). The organization of behavior: A neuropsychological approach. New York: John Wiley & Sons.

45. Iijima, T., Witter, M.P., Ichikawa, M., Tominaga, T., Kajiwara, R. & Matsumoto, G. (1996). Entorhinal-hippocampal interactions revealed by real-time imaging. Science, 272 (5265): 1176-9.

46. James, W. (1890). Principles of psychology. New York, NY: Holt.

47. Jeltsch, H., Bertrand, F., Lazarus, C., and Cassel, J.C. (2001). Cognitive performances and locomotor activity following dentate granule cell damage in rats: role of lesion extent and type of memory tested. Neurobiology of Learning & Memory, 76 (1):81-105.

48. Johnson, D.M., Baker, J.D. & Azorlosa, J.L. (2000). Acquisition, extinction, and reinstatement of Pavlovian fear conditioning: the roles of the NMDA receptor and nitric oxide. Brain Research, 857 (1-2):66-70.

49. Jones, R.S. (1993). Entorhinal-hippocampal connections: a speculative view of their function. Trends in Neuroscience, 16 (2): 58-64.

50. Kamondi A, Horvath Z, Bors L, Buzsaki G. (1988). Perforant path activation of the hippocampus: spatial distribution, effects of urethane and atropine. Acta Physiolica Hungarica, 71 (1):19-29.

51. Killcross, S., Robbins, T.W. & Everitt, B.J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. Nature, 388 (6640):377-80.

52. Kim, J.J., DeCola, J.P., Landeira-Fernandez, J. & Fanselow, M.S. (1991). N-methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning. Behavioral Neuroscience, 105 (1):126-33.

53. Kloosterman, F., Witter, M.P., and Van Haeften, T. (2003). Topographical and laminar organization of subicular projections to the parahippocampal region of the rat. The Journal of Comparative Neurology, 455, 156-171.

54. Kosaka,T., Hama,K., and Wu,J.Y. (1984). GABAergic synaptic boutons in the granule cell layer of rat dentate gyrus. Brain Research. 293, 353-359.

55. Kudoh, M. & Shibuki, K. (1994). Long-term potentiation in the auditory cortex of adult rats. Neuroscience Letters, 171(1-2): 21-3.

56. Lacaille, J.C., Harley, C.W. (1985). The action of norepinephrine in the dentate gyrus: beta-mediated facilitation of evoked potentials in vitro. Brain Research, 358 (1-2):210-20.

57. LeDoux, J.E. (1991). Emotion and the limbic system concept. Concepts in Neuroscience, 2: 169-99.

58. LeDoux, J.E. (1992). Emotion and the amygdala, in The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction, Aggleton J.P. (ed), New York, Wiler-Liss, pp 339-352.

59. LeDoux, J.E. (1996). The Emotional Brain. New York: Simon & Schuster.

60. LeDoux, J.E. (2000). Emotion circuits in the brain. Annual Review of Neuroscience, 23:155-84.

61. LeDoux, J.E. (2002). Synaptic Self: How Our Brains Become Who We Are. Viking. New York.

62. Lee, E.H., Lee, C.P., Wang, H.I., and Lin, W.R. (1993). Hippocampal CRF, NE, and NMDA system interactions in memory processing in the rat. Synapse, 14 (2):144-53.

63. Leranth, C. and Ribak, C.E. (1991). Calcium-binding proteins are concentrated in the CA2 field of the monkey hippocampus: a possible key to this region's resistance to epileptic damage. Experimental Brain Research, 85, 129-136.

64. Leung, L.W. (1995). Simulation of perforant path evoked field and intracellular potentials in hippocampal CA1 area. Hippocampus, 5 (2): 129-36.

65. Lynch, G., Larson, J., Kelso, S., Barrionuevo, G., & Schottler, F. (1983). Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature, 305 (5936): 719-21.

66. Maggi, C.A., and Meli, A. (1986). Suitability of urethane anesthesia for physiopharmacological investigations. Part 1: general considerations. Experientia, 42 (5):109-113.

67. Maren, S. & Fanselow, M.S. (1996). The amygdala and fear conditioning: has the nut been cracked? Neuron, 16 (2):237-40.

68. Maren, S. (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. Journal of Neuroscience, 19 (19):8696-703.

69. Margineanu, D.G., Gower, A.J., Gobert, J., and Wulfert, E. (1994). Long-term adrenalectomy reduces hippocampal granule cell excitability in vivo. Brain Research Bulletin, 33 (1):93-8.

70. McGaugh JL, Cahill L, Roozendaal B. (1996). Involvement of the amygdala in memory storage: interaction with other brain systems. Proceedings of the National Academy of Sciences, 93 (24):13508-14.

71. McNaughton, B.L. & Barnes, C.A. (1977). Physiological identification and analysis of dentate granule cell responses to stimulation of the medial and lateral perforant pathways in the rat. Journal of Comparative Neurology, 175 (4): 439-54.

72. Miserendino, M.J., Sananes, C.B., Melia, K.R. & Davis, M. (1990). Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. Nature, 345 (6277):716-8.

73. Morrison,J.H., Benoit,R., Magistretti,P.J., Ling,N., and Bloom,F.E. (1982). Immunohistochemical distribution of pro-somatostatin-related peptides in hippocampus. Neuroscience.Letters, 34, 137-142.

74. Naber, P.A., Witter, M.P. & Lopez da Silva, F.H. (1999). Perirhinal cortex input to the hippocampus in the rat: evidence for parallel pathways, both direct and indirect. A combined physiological and anatomical study. European Journal of Neuroscience, 11 (11): 4119-33.

75. Naber, P.A., Lopes da Silva, F.H., and Witter, M.P. (2001). Reciprocal connections between the entorhinal cortex and hippocampal fields CA1 and the subiculum are in register with the projections from CA1 to the subiculum. Hippocampus, 11, 99-104.

76. Neuman, R.S. & Harley, C.W. (1983). Long-lasting potentiation of the dentate gyrus population spike by norepinephrine. Brain Research, 273 (1):162-5.

77. Nicoll, R.A., Kauer, J.A., & Malenka. R.C. (1988). The current excitement in long-term potentiation. Neuron, 1(2): 97-103.

78. Pang, R., Turndorf, H. & Quartermain, D. (1996). Pavlovian fear conditioning in mice anesthetized with halothane. Physiology and Behavior, 59 (4-5): 873-5.

79. Pare, D. and Llinas, R. (1995). Intracellular study of direct entorhinal inputs to field CA1 in the isolated guinea pig brain in vitro. Hippocampus, 5 (2): 115-9.

80. Pavlov, Ivan P. (1927). Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex (G. V. Anrep, Trans.).

81. Penttonen, M., Kamondi, A., Acsady, L., and Buzsaki, G. (1998). Gamma frequency oscillation in the hippocampus of the rat: intracellular analysis in vivo. European Journal of Neuroscience, 10, 718-728.

82. Phillips, R.G. & LeDoux, J.E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behavioral Neuroscience, 106 (2): 274-85.

83. Phillips, R.G. & LeDoux, J.E. (1994). Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. Learning & Memory, 1 (1): 34-44.

84. Pirch, J.H., Corbus, M.J. & Ebenezer, I. (1985b). Conditioned cortical slow potential responses in urethane anesthetized rats. International Journal of Neuroscience, 25 (3-4): 207-18.

85. Pirch, J.H., Corbus, M.J. & Rigdon, G.C. (1985a). Conditioning-related single unit activity in the frontal cortex of urethane anesthetized rats. International Journal of Neuroscience, 25 (3-4): 263-71.

86. Powell DA, Maxwell B, Penney J. (1996). Neuronal activity in the medial prefrontal cortex during Pavlovian eyeblink and nictitating membrane conditioning. Journal of Neuroscience, 16 (19):6296-306.

87. Ramón y Cajal, S. (1894) La fine structure des centres nerveux: the Croonian Lecture, Proc. Roy. Soc. Lond., 55: 443-468.

88. Ramón y Cajal, S. 1911. Histologie du Système Nerveux de l'Homme et des Vertebrés, Malonie, Paris.

89. Rick JT, Milgram NW. (1999). Instability of dentate gyrus field potentials in awake and anesthetized rats. Hippocampus, 9 (3):333-9.

90. Riedel, G., Seidenbecher, T., Reymann, K.G. (1994). LTP in hippocampal CA1 of urethane-narcotized rats requires stronger tetanization parameters. Physiology and Behavior, 55 (6):1141-6.

91. Rogan, M.T. & LeDoux, J.E. (1995). LTP is accompanied by commensurate enhancement of auditory-evoked responses in a fear conditioning circuit. Neuron, 15 (1): 127-36.

92. Rogan, M.T. & LeDoux, J.E. (1996). Emotion: systems, cells, synaptic plasticity. Cell, 85 (4):469-75.

93. Rogan, M.T., Staubli, U.V. & LeDoux, J.E. (1997). AMPA receptor facilitation accelerates fear learning without altering the level of conditioned fear acquired. Journal of Neuroscience, 17 (15): 5928-35.

94. Rogan, M.T., Staubli, U.V. & LeDoux, J.E. (1997). Fear conditioning induces associative long-term potentiation in the amygdala. Nature, 390 (6660): 604-7.

95. Scharfman,H.E. (1991). Dentate hilar cells with dendrites in the molecular layer have lower thresholds for synaptic activation by perforant path than granule cells. Journal of Neuroscience, 11, 1660-1673.

96. Selden, N.R., Everitt, B.J., Jarrard, L.E. & Robbins, T.W. (1991). Complementary roles for the amygdala and hippocampus in aversive conditioning to explicit and contextual cues. Neuroscience, 42 (2):335-50.

97. Shirasaka, Y., & Wasterlain, C.G. (1995). The effect of urethane anesthesia on evoked potentials in dentate gyrus. European Journal of Pharmacology, 282 (1-3):11-7.

98. Sik, A., Penttonen, M., and Buzsaki, G. (1997). Interneurons in the hippocampal dentate gyrus: an in vivo intracellular study. European.Journal of Neuroscience, 9, 573-588.

99. Sloviter,R.S., Ali-Akbarian,L., Horvath,K.D., and Menkens,K.A. (2001). Substance P receptor expression by inhibitory interneurons of the rat hippocampus: enhanced detection using improved immunocytochemical methods for the preservation and colocalization of GABA and other neuronal markers. Journal of Comparative Neurology, 283-305.

100. Southwick, S.M., Davis, M., Horner, B., Cahill, L., Morgan, C.A. 3rd, Gold, P.E., Bremner, J.D. & Charney, D.C. (2002). Relationship of enhanced norepinephrine activity during memory consolidation to enhanced long-term memory in humans. American Journal of Psychiatry, 159 (8):1420-2.

101. Stanton, P.K. & Sarvey, J.M. (1985). Blockade of norepinephrine-induced long-lasting potentiation in the hippocampal dentate gyrus by an inhibitor of protein synthesis. Brain Research, 361 (1-2):276-83.

102. Stein, L., Belluzzi, J.D. & Wise, C.D. (1975). Memory enhancement by central administration of norepinephrine. Brain Research, 84 (2):329-35.

103. Steward, O. (1977). Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. Journal of Comparative Neurology, 167, 285-314.

104. Steward, O. and Scoville, S.A. (1977). Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. Journal of Comparative Neurology, 169, 347-370.

105. Valentino, R. J., Foote, S. L. and Aston-Jones, G. (1983). Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. Brain Research, 270: 363-367.

106. Vazdarjanova, A. & McGaugh, J.L. (1998). Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. Proceedings of the National Academy of Sciences, 95 (25):15003-7.

107. Weinberger, N.M., Gold, P.E. & Sternberg, D.B. (1984). Epinephrine enables Pavlovian fear conditioning under anesthesia. Science, 223 (4636): 605-7.

108. Welsh, J.P. & Harvey, J.A. (1998). Acute inactivation of the inferior olive blocks associative learning. European Journal of Neuroscience, 10 (11):3321-32.

109. White, W.F., Nadler, J.V., Hamberger, A., Cotman, C.W. and Cummins J.T. (1977). Glutamate as transmitter of hippocampal perforant path. Nature, 270, 356-357.

110. Wilson, D.A., Pham, T.C., and Sullivan, R.M. (1994). Norepinephrine and posttraining memory consolidation in neonatal rats. Behavioral Neuroscience, 108(6):1053-8.

111. Witter, M.P., Naber, P.A., Van Haeften, T., Machielsen, W.C., Rombouts, S.A., Barkhof, F., Scheltens, P. & Lopes da Silva, F.H. (2000b). Cortico-hippocampal communication by way of parallel parahippocampal-subicular pathways. Hippocampus, 10 (4): 398-410.

112. Witter, M.P., Wouterlood, F.G., Naber, P.A. & Van Haeften, T. (2000a). Anatomical organization of the parahippocampal-hippocampal network. Annals of New York Academy Sciences, 911: 1-24.

113. Witter, M.P. and Groenewegen, H.J. (1990). The subiculum: cytoarchitectonically a simple structure, but hodologically complex. Progress in Brain Research, 83, 47-58.

# APPENDIX A:

## Summary of Statistical Testing Results

## 1. NORMALIZED SLOPE

### 1.A. ALL TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 1002123.18 | 10 | 100212.32 | | |
| group | 9792.87 | 1 | 9792.87 | .10 | .761 |
| WITHIN CELLS | 455518.62 | 260 | 1751.99 | | |
| BLOCK | 28765.31 | 26 | 1106.36 | .63 | .919 |
| group BY BLOCK | 5413.23 | 26 | 208.20 | .12 | 1.000 |
| WITHIN CELLS | 1850.45 | 90 | 20.56 | | |
| TRIAL | 110.11 | 9 | 12.23 | .60 | .798 |
| group BY TRIAL | 134.47 | 9 | 14.94 | .73 | .683 |
| BLOCK BY TRIAL | 3794.65 | 234 | 16.22 | .91 | .831 |
| group BY BLOCK BY TRIAL | 3703.29 | 234 | 15.83 | .89 | .885 |

### 1.B. CONDITIONING TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 21874.96 | 10 | 2187.50 | | |
| group | 5333.23 | 1 | 5333.23 | 2.44 | .149 |
| WITHIN CELLS | 30753.98 | 80 | 384.42 | | |
| BLOCK | 5501.98 | 8 | 687.75 | 1.79 | .091 |
| group BY BLOCK | 889.16 | 8 | 111.14 | .29 | .968 |
| WITHIN CELLS | 1097.43 | 90 | 12.19 | | |
| TRIAL | 111.22 | 9 | 12.36 | 1.01 | .435 |
| group BY TRIAL | 79.84 | 9 | 8.87 | .73 | .683 |
| BLOCK BY TRIAL | 640.43 | 72 | 8.89 | .67 | .982 |
| group BY BLOCK BY TRIAL | 1012.94 | 72 | 14.07 | 1.06 | .343 |

## 1.C. POST-CONDITIONING TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 241849.96 | 10 | 24185.00 | | |
| group | 412.92 | 1 | 412.92 | .02 | .899 |
| WITHIN CELLS | 11585.99 | 50 | 231.72 | | |
| BLOCK | 1047.18 | 5 | 209.44 | .90 | .486 |
| group BY BLOCK | 2493.68 | 5 | 498.74 | 2.15 | .074 |
| WITHIN CELLS | 1850.78 | 90 | 20.56 | | |
| TRIAL | 71.36 | 9 | 7.93 | .39 | .939 |
| group BY TRIAL | 104.21 | 9 | 11.58 | .56 | .824 |
| WITHIN CELLS | 7888.99 | 450 | 17.53 | | |
| BLOCK BY TRIAL | 954.40 | 45 | 21.21 | 1.21 | .173 |
| group BY BLOCK BY TRIAL | 1094.30 | 45 | 24.32 | 1.39 | .054 |

## 1.D. EXTINCTION TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 826917.93 | 10 | 82691.79 | | |
| group | 3413.79 | 1 | 3413.79 | .04 | .843 |
| WITHIN CELLS | 12043.72 | 80 | 150.55 | | |
| BLOCK | 751.50 | 8 | 93.94 | .62 | .755 |
| group BY BLOCK | 965.85 | 8 | 120.73 | .80 | .603 |
| WITHIN CELLS | 2115.89 | 90 | 23.51 | | |
| TRIAL | 318.99 | 9 | 35.44 | 1.51 | .157 |
| group BY TRIAL | 253.88 | 9 | 28.21 | 1.20 | .305 |
| WITHIN CELLS | 15248.62 | 720 | 21.18 | | |
| BLOCK BY TRIAL | 1331.04 | 72 | 18.49 | .87 | .763 |
| group BY BLOCK BY TRIAL | 989.70 | 72 | 13.75 | .65 | .989 |

## 1.E. POST-EXTINCTION TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 312068.24 | 10 | 31206.82 | | |
| group | 1636.19 | 1 | 1636.19 | .05 | .824 |
| WITHIN CELLS | 547.03 | 20 | 27.35 | | |
| BLOCK | 173.22 | 2 | 86.61 | 3.17 | .064 |
| group BY BLOCK | 61.27 | 2 | 30.64 | 1.12 | .346 |
| WITHIN CELLS | 2014.74 | 90 | 22.39 | | |
| TRIAL | 204.11 | 9 | 22.68 | 1.01 | .436 |
| group BY TRIAL | 80.94 | 9 | 8.99 | .40 | .931 |
| WITHIN CELLS | 3914.24 | 180 | 21.75 | | |
| BLOCK BY TRIAL | 273.20 | 18 | 15.18 | .70 | .810 |
| group BY BLOCK BY TRIAL | 221.95 | 18 | 12.33 | .57 | .919 |

## 2. NORMALIZED POPULATION SPIKE

### 2.A. ALL TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 1018961.43 | 10 | 101896.14 | | |
| group | 436701.51 | 1 | 436701.51 | 4.29 | .065 |
| WITHIN CELLS | 1192217.00 | 260 | 4585.45 | | |
| BLOCK | 74133.11 | 26 | 2851.27 | .62 | .926 |
| group BY BLOCK | 612077.04 | 26 | 23541.42 | 5.13 | .000 |
| WITHIN CELLS | 24593.90 | 90 | 273.27 | | |
| TRIAL | 2243.62 | 9 | 249.29 | .91 | .518 |
| group BY TRIAL | 1810.94 | 9 | 201.22 | .74 | .675 |
| WITHIN CELLS | 865221.86 | 2340 | 369.75 | | |
| BLOCK BY TRIAL | 83384.38 | 234 | 356.34 | .96 | .638 |
| group BY BLOCK BY TRIAL | 64137.78 | 234 | 274.09 | .74 | .998 |

### 2.B. CONDITIONING TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 151744.46 | 10 | 15174.45 | | |
| group | 1476.49 | 1 | 1476.49 | .10 | .761 |
| WITHIN CELLS | 170119.05 | 80 | 2126.49 | | |
| BLOCK | 19112.77 | 8 | 2389.10 | 1.12 | .357 |
| group BY BLOCK | 2847.80 | 8 | 355.98 | .17 | .995 |
| WITHIN CELLS | 19540.82 | 90 | 217.12 | | |
| TRIAL | 948.43 | 9 | 105.38 | .49 | .881 |
| group BY TRIAL | 2402.39 | 9 | 266.93 | 1.23 | .287 |
| WITHIN CELLS | 204489.64 | 720 | 284.01 | | |
| BLOCK BY TRIAL | 23562.82 | 72 | 327.26 | 1.15 | .191 |
| group BY BLOCK BY TRIAL | 12499.84 | 72 | 173.61 | .61 | .995 |

## 2.C. POST-CONDITIONING TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 320103.43 | 10 | 32010.34 | | |
| group | 10290.38 | 1 | 10290.38 | .32 | .583 |
| WITHIN CELLS | 111269.82 | 50 | 2225.40 | | |
| BLOCK | 4010.73 | 5 | 802.15 | .36 | .873 |
| group BY BLOCK | 39286.52 | 5 | 7857.30 | 3.53 | .008 |
| WITHIN CELLS | 18963.62 | 90 | 210.71 | | |
| TRIAL | 2017.04 | 9 | 224.12 | 1.06 | .397 |
| group BY TRIAL | 1106.41 | 9 | 122.93 | .58 | .807 |
| WITHIN CELLS | 172953.81 | 450 | 384.34 | | |
| BLOCK BY TRIAL | 20864.64 | 45 | 463.66 | 1.21 | .176 |
| group BY BLOCK BY TRIAL | 15057.82 | 45 | 334.62 | .87 | .710 |

## 2.D. EXTINCTION TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 665801.55 | 10 | 66580.15 | | |
| group | 505745.50 | 1 | 505745.50 | 7.60 | .020 |
| WITHIN CELLS | 398311.41 | 80 | 4978.89 | | |
| BLOCK | 24260.66 | 8 | 3032.58 | .61 | .768 |
| group BY BLOCK | 33075.82 | 8 | 4134.48 | .83 | .578 |
| WITHIN CELLS | 27993.54 | 90 | 311.04 | | |
| TRIAL | 2523.51 | 9 | 280.39 | .90 | .528 |
| group BY TRIAL | 2130.34 | 9 | 236.70 | .76 | .652 |
| WITHIN CELLS | 312070.10 | 720 | 433.43 | | |
| BLOCK BY TRIAL | 25791.67 | 72 | 358.22 | .83 | .845 |
| group BY BLOCK BY TRIAL | 19878.12 | 72 | 276.09 | .64 | .991 |

## 2.E. POST-EXTINCTION TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 361431.06 | 10 | 36143.11 | | |
| group | 454053.11 | 1 | 454053.11 | 12.56 | .005 |
| WITHIN CELLS | 32397.65 | 20 | 1619.88 | | |
| BLOCK | 2434.70 | 2 | 1217.35 | .75 | .485 |
| group BY BLOCK | 2002.93 | 2 | 1001.46 | .62 | .549 |
| WITHIN CELLS | 49320.48 | 90 | 548.01 | | |
| TRIAL | 4286.97 | 9 | 476.33 | .87 | .556 |
| group BY TRIAL | 3411.87 | 9 | 379.10 | .69 | .715 |
| WITHIN CELLS | 84483.76 | 180 | 469.35 | | |
| BLOCK BY TRIAL | 5632.92 | 18 | 312.94 | .67 | .841 |
| group BY BLOCK BY TRIAL | 9461.91 | 18 | 525.66 | 1.12 | .336 |

## 3. SPIKE/SLOPE RATIO

### 3.A. ALL TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 118.38 | 10 | 11.84 | | |
| group | 17.93 | 1 | 17.93 | 1.51 | .247 |
| WITHIN CELLS | 129.02 | 260 | .50 | | |
| BLOCK | 6.97 | 26 | .27 | .54 | .969 |
| group BY BLOCK | 54.45 | 26 | 2.09 | 4.22 | .000 |
| WITHIN CELLS | 3.87 | 90 | .04 | | |
| TRIAL | .25 | 9 | .03 | .64 | .761 |
| group BY TRIAL | .26 | 9 | .03 | .67 | .738 |
| WITHIN CELLS | 112.75 | 2340 | .05 | | |
| BLOCK BY TRIAL | 11.22 | 234 | .05 | 1.00 | .509 |
| group BY BLOCK BY TRIAL | 8.94 | 234 | .04 | .79 | .989 |

### 3.B. CONDITIONING TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 11.58 | 10 | 1.16 | | |
| group | 1.64 | 1 | 1.64 | 1.41 | .262 |
| WITHIN CELLS | 24.50 | 80 | .31 | | |
| BLOCK | 1.36 | 8 | .17 | .55 | .813 |
| group BY BLOCK | .33 | 8 | .04 | .13 | .998 |
| WITHIN CELLS | 2.49 | 90 | .03 | | |
| TRIAL | .14 | 9 | .02 | .55 | .833 |
| group BY TRIAL | .29 | 9 | .03 | 1.18 | .320 |
| WITHIN CELLS | 25.97 | 720 | .04 | | |
| BLOCK BY TRIAL | 2.88 | 72 | .04 | 1.11 | .258 |
| group BY BLOCK BY TRIAL | 1.69 | 72 | .02 | .65 | .988 |

## 3.C. POST-CONDITIONING TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 49.82 | 10 | 4.98 | | |
| group | .00 | 1 | .00 | .00 | .981 |
| WITHIN CELLS | 8.93 | 50 | .18 | | |
| BLOCK | .16 | 5 | .03 | .18 | .968 |
| group BY BLOCK | 4.23 | 5 | .85 | 4.73 | .001 |
| WITHIN CELLS | 2.25 | 90 | .02 | | |
| TRIAL | .26 | 9 | .03 | 1.15 | .338 |
| group BY TRIAL | .07 | 9 | .01 | .32 | .967 |
| WITHIN CELLS | 19.98 | 450 | .04 | | |
| BLOCK BY TRIAL | 2.54 | 45 | .06 | 1.27 | .121 |
| group BY BLOCK BY TRIAL | 1.84 | 45 | .04 | .92 | .619 |

## 3.D. EXTINCTION TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 73.91 | 10 | 7.39 | | |
| group | 28.19 | 1 | 28.19 | 3.81 | .079 |
| WITHIN CELLS | 34.76 | 80 | .43 | | |
| BLOCK | 2.31 | 8 | .29 | .67 | .720 |
| group BY BLOCK | 4.20 | 8 | .52 | 1.21 | .306 |
| WITHIN CELLS | 3.65 | 90 | .04 | | |
| TRIAL | .41 | 9 | .05 | 1.13 | .349 |
| group BY TRIAL | .44 | 9 | .05 | 1.20 | .302 |
| WITHIN CELLS | 42.28 | 720 | .06 | | |
| BLOCK BY TRIAL | 3.76 | 72 | .05 | .89 | .730 |
| group BY BLOCK BY TRIAL | 3.01 | 72 | .04 | .71 | .964 |

## 3.E. POST-EXTINCTION TRIALS

| Source of Variation | SS | DF | MS | F | Sig of F |
|---|---|---|---|---|---|
| WITHIN CELLS | 39.37 | 10 | 3.94 | | |
| group | 33.64 | 1 | 33.64 | 8.55 | .015 |
| WITHIN CELLS | 4.54 | 20 | .23 | | |
| BLOCK | .51 | 2 | .25 | 1.11 | .348 |
| group BY BLOCK | .16 | 2 | .08 | .36 | .703 |
| WITHIN CELLS | 8.03 | 90 | .09 | | |
| TRIAL | .67 | 9 | .07 | .83 | .588 |
| group BY TRIAL | .39 | 9 | .04 | .48 | .884 |
| WITHIN CELLS | 11.97 | 180 | .07 | | |
| BLOCK BY TRIAL | .82 | 18 | .05 | .68 | .825 |
| group BY BLOCK BY TRIAL | 1.46 | 18 | .08 | 1.22 | .250 |