AN EXAMINATION OF PLACE CELLS IN THE HIPPOCAMPUS IN THE DELAY BOX AND THE GOAL BOX DURING PERFORMANCE OF A BLACK/WHITE ALLEY DISCRIMINATION TASK ACQUIRED WITH A DELAY OF REINFORCEMENT

CENTRE FOR NEWFOUNDLAND STUDIES

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0-612-66745-6

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An examination of place cells in the hippocampus in the delay box and the goal box during performance of a black/white alley discrimination task acquired with a delay of reinforcement

by

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9466137

A thesis submitted to the

school of graduate studies

in partial fulfillment of the

requirements for the degree of

Master of Science

Biopsychology Program

Memorial Univeristy of Newfoundland

May /01

St. John's **Newfoundland**

Abstract

The hippocampus is important in spatial navigation in rodents. Less clear is the relationship between the cognitive map of physical space, and task requirements that take place within that space. This study addresses the issue by recording pyramidal cells of the hippocampal CA1 region as animals perform the Lawrence and Homel (1969) discrimination task. Proceeding from a start box, animals made a choice to run down either a black or white alley, which led to a grey delay box. Following a brief delay, animals entered the goal box to receive a reward for a correct alley choice. Although the goal box always occupied the same physical space, the colour varied with reward contingency in the experimental group. I hypothesized that animals would have two representations of the delay box, one based on anticipatory reward, and the other not. Results indicated that the animals had differential representations of the goal boxes, and that they viewed the delay box as a constant space.

Acknowledgments

This research stems from my interest in electrophysiology and its application in psychology and the neurosciences.

The idea for the present study originated through my association with Dr. Gerard Martin and Dr. Carolyn Harley. For their patience, understanding and insight I am greatly indebted to them.

Financial support for the project was provided by the Natural Sciences and Engineering Research Council of Canada in the form of grants issued to Dr. Harley and Dr. Martin. Financial support in the form of graduate fellowships was provided by the School of Graduate studies.

I would like to thank John Huxter, Bob Brown and Susan Walling for their endless assistance and advice regarding recording techniques and chronic surgery. My gratitude also goes out to Darlene Skinner and John Evans who provided a great deal of insight regarding behavioral training procedures, apparatus construction and statistical analysis. I would also like to thank the many MUCEP students who lent their ideas and skills to the many tasks involved in this project: David Laidley, Elysia Maret-Eckart, Frank Butler, Anna Wyse and David Jones.

The completion of this thesis would not have been possible without the generosity and compassion of Ray Hefford, Joan Reddigan and especially my parents James and Rosemarie Barry.

Finally, I would like to dedicate this thesis to John Edwards and Peter Henke, who first sparked my interest in research and encouraged me to follow my curiosity and carry on my studies

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Memory collects the countless phenomena of our existence into a single whole...our consciousness would be broken up into as many fragments as we had lived seconds but for the binding and unifying force of memory.

-Edwald Hering

In 1957, Scoville and Milner described a profound and selective impairment in human memory after the bilateral surgical removal of the medial temporal lobe including the hippocampal formation, the amygdaloid complex and adjacent cortical areas (entorhinal cortex, perirhinal cortex, and parahippocampal cortex). Neuropsychological evaluation of a severe epileptic patient in this series, referred to as H.M., showed that he suffered from profound anterograde amnesia after surgery. Though H.M. had a normal short-term or working memory, an intact procedural memory, and better implicit than explicit memory, he suffered from severe anterograde amnesia (memory of events after brain damage). These test results established the fundamental principle that the ability to acquire new memories is a distinct cerebral function, distinct from other perceptual and cognitive abilities (Milner, 1959; Cohen, Eichenbaum, Deacodo, and Corkin, 1985; Scoville and Milner, 1959). Yet, until the 1980's amnesia was incompletely understood. Better comprehension of the phenomenon was realized with the appreciation of memory not as a single faculty but composed instead of multiple separate systems, only one of which is impaired in amnesia. As seen in the case of H.M., damage to the medial temporal lobe impairs the ability to acquire information about facts and events (declarative memory) but spares the capacity for skill learning, certain kinds of conditioning and habit learning, as well as the phenomenon of priming. Declarative memory, such as knowledge of facts (semantic memory) and events (episodic memory), is accessible to conscious recollection and available to multiple response systems. Nondeclarative memory includes several kinds of abilities, such as remembering how to ride a bike, which are non-conscious and expressed through performance. Nondeclarative memories tend to be inflexible, bound to the learning situation, and not readily accessed

by response systems that did not participate in the original learning situation (Eichenbaum, Mathews. and Cohen, 1989; Saunders and Weiskrantz, 1989; Squire and Knowlton, 1995).

Due to the large areas that are typically removed during surgery of the temporal lobe, as in the case of H.M., it has been difficult to determine from human cases precisely which structures and connections within the medial temporal lobe are important for memory, but a favored candidate has been the hippocampal formation. The hippocampal formation comprises six cytoarchitechtonically distinct regions, including the dentate gyrus; hippocampus (or hippocampus proper), which is subdivided into three fields (CA3, CA2, and CA1); subiculum; presubiculum; parasubiculum; and entorhinal cortex, which contains two or more subdivisions. The subiculum, presubiculum and parasubiculum are sometimes grouped together as the subicular complex. The main justification for including these regions under the banner of the hippocampal formation is that they are linked, one to the next, by unique and largely unidirectional projections (Amaral and Witter, 1995).

The putative link between the hippocampus and memory led to the widespread study of this structure in lesion studies and with electrophysiological techniques. On considering the possible value of the different theories that had been advanced in a vast literature, particularly in the early days of experimentation, James Olds offered the opinion that "theories of hippocampal function were nearly as numerous as the paradigms that had been used to elaborate its function" (cited in Best and White, 1999, pp 348).One of the most important findings in this early research was made by O'Keefe and Dostrovsky in 1971 when the authors reported that the firing rates of a number of

hippocampal neurons appeared to be closely linked to an animal's location in the environment. In the nearly 30 years since that discovery, hundreds of studies have been published and thousands of words have been written, yet full understanding of the function of hippocampal cells and the role they play in learning remains somewhat elusive.

The principle debate with regard to hippocampal function lies mainly in whether or not the hippocampus functions solely in relation to spatial processing, as a cognitive map (O'Keefe and Nadel, 1978; Lenck-Santini, Save, Poucet, 2001), or if it also has a broader role in processing spatial and non-spatial information such as the "temporal order, similarity, or spatial arrangement as well as relations of cues to their significance and responses made to them, in other words, virtually any relationship worth remembering" (Nadel and Eichenbaum, 1999, p. 343). Though there is a large body of evidence for both cases, "the devil lies in the details". The present paper aims to examine both spatial and non-spatial views of hippocampal function and to explore a putative role for hippocampal place cells in the bridging of a temporal gap. We will also examine the role of the hippocampus in preventing interference in the acquisition of a black and white discrimination with delayed reinforcement.

Theories of Learning (Place Vs. Response)

In the early part of the century, psychology and animal learning was dominated by Watsonian Behaviorism, in which it was argued that all behavior is essentially reflexive. That is, all behavior could be understood as behavioral reactions (responses) to events in the environment (stimuli). This was later referred to as S-R psychology. Watson believed that understanding behavior did not require reference to the "mind" or to any

unobservable events occurring within the individual. It was enough to simply describe lawful environment-behavior relationships. According to Watson, the explanation of these relationships was obscured by any reference to the "mind" as no direct way existed to observe the animal mind (Watson, 1924). It would be decades before this type of strict behaviorism would be challenged.

The notion of cognitive mapping first began with an influential experiment conducted in 1946 by Tolman, Ritchie and Kalish. The authors used a "sun burst" maze with 18 arms with an extended goal arm inserted between arms 9 and 10. Rats were reinforced for running from the start location to the goal box. As the route to the goal involved several right and left turns, Hullian theorists of the day hypothesized that the animals were merely learning a rigid set of response or motor movements. In contrast to Hullian theory, Tolman hypothesized that the rats were learning to go to a particular place as specified by extra maze cues such as a desk lamp located near the goal area. According to Tolman this place was contained in the "cognitive map" of the rat. In order to test his theory Tolman blocked the arm to the goal area. If Tolman's theory was correct the rats would try an arm that led to the goal location. If Hullian theorists were correct, the rats would try to get in the arm that was blocked or choose an arm close to it. As Tolman had predicted rats tended to choose the arm that led to a similar location as the goal at a significantly greater percentage (36%) than any other of the 11 arms. This result and those of other experiments led Tolman to further elaborate on this theory in 'Cognitive Maps in Rats and Men' (1948) which outlined his theory that rats use cognitive 'field maps' of their environment in getting from one place to another. Tolman often used terms such as "purpose" and "cognition", arguing that the goal of behavior

was to reach some final goal (reaching that goal demonstrated the subjects' purpose). The main point of Tolman's theory was that animals derive knowledge of their environment and are able to form expectancies about the consequences of their behavior in that environment. This theory was in direct contrast to Watson's mechanistic S-R theories of behaviorism that predominated at the time (Watson, 1924). Tolman's approach to animal learning fostered a new brand of behaviorism often referred to as S-O-R behaviorism. The "O" in this formulation stands for hypothetical processes occurring inside the organism, which mediate the relationship between stimuli and response. As with most new theories, this more "cognitive" approach to learning and the notion of place learning was not received without a certain amount of controversy (e.g., Guthrie, 1935).

Perhaps acting as a spokesman for S-R theorists at the time, Guthrie (1935) referred to Tolman's rat as so immersed in thought at the choice point of a discrimination problem that it is quite unable to move a muscle in order to make a response. Guthrie seemed unable to understand how a hypothetical construct such as expectancy, an unobservable event, could generate a behavioral response such as an alley choice. According to Guthrie, these processes would leave the rat buried in associations as it has no mechanism by which to translate associations into choices and ultimately a behavioral response. Guthrie believed that animals were incapable of such cognitive abilities and were only capable of making appropriate responses when specific stimuli were present.

In the early days of the debate between response and mapping theorists, which took place on the miniature battlefield of the T-maze, Munn (1950) summarized most of the work to that time as generally confirming Tolman's hypothesis. He concluded that in a environment rich with cues (a "heterogeneous" environment) rats will learn to run to

one place from different directions more readily than they will learn to make the same turn to different places. Thus, in a rich environment, rats that learn to go to a place will reach criterion faster than rats that learn rigid motor responses. However, if rats were trained in a plain environment such as that used by Blodgett, McCutchan and Mathews (1949), the reverse would be found. These authors found that in learning a simple T-maze rats acquire a direction disposition and of lesser strength a response disposition. There was no evidence that the rats acquired a place disposition. The lack of place learning is possibly related to the fact that the only object in the room, besides the maze and some food boxes, was a chair. Further emphasizing this point, Restle (1957) concluded that:

> There is nothing in the nature of the rat which makes it a 'place' learner or a 'response' learner. A rat in a maze will use all relevant cues, and the importance of any class of cues depends on the amount of relevant stimulation provided as well as the sensory capacities of the animal. In place-response experiments, the importance of place cues depends on the amount of differential extra maze stimulation (p. 226).

In other words, Restle believed that place learning was no different than response learning; it merely utilized a different class of cues. However, this did not resolve the issue of why place learning seemed to have different properties than response learning, most noticeably the flexibility found in place learning. An animal could be started from a number of different points in a maze, such as a water-maze, and still be able to find a submerged platform. Thus place learning is flexible as the 'cognitive map' could be 'called up' from a variety of start points (Morris, 1981).

O'Keefe and Nadel (1978) further defined cognitive mapping with the distinction between routes and maps. Route learning, with its landmarks and the specific responses guided by these, is defined in contrast to map learning which implies the availability of

interrelated information with no necessary specification of guides or landmarks. In other words, route learning is a 'mindless' form of learning where an animal merely learns a series of stimuli and responses, whereas map learning implies a great deal of cognition on the part of the organism. According to O'Keefe and Nadel (1978) distal cues are important in specifying directions, since they do not change relative positions as the organism moves in its local environment; on the other hand, this very property means that distal cues, by themselves, distinguish amongst places in that environment. Places, would seem to be defined by extra-maze cues which are close enough to the animal for its movement to change the angles between them.

Though O'Keefe and Nadel revolutionized the way we look at navigation and cognitive mapping, there are still more questions than answers. There are still many contentious issues when it comes to teasing apart the aspects of an organism's environment that are most important in the formation of cognitive maps, as well as the processes with which the perception of spatial relations are transformed into neural reality. These processes are vital to every mobile animal and some of them are reviewed in the next section. It is important to note that the spatial memory processes discussed here are not meant to be synonymous with memory processes per se, but with what is most often remembered in spatial problem solving or memory content.

Processes In Navigation

Guided Navigation vs. Maps:

Since O'Keefe and Nadel's hypotheses about the usage of cues in spatial learning, several other experiments have since reiterated the role of cues in the animal's learning of 'place' as opposed to using cues as signals in a response chain. Suzuki, Augerinos, and

Black (1980) found that in order to form a cognitive map of their environment, animals did not treat available spatial information segmentally. Animals seem to establish a spatial map that locates stimuli in space relative to each other and the total environment. In other words, animals do not home in on one stimulus, but use a configuration of stimuli to fix their position. Likewise, Morris (1981) has found that entering a milk-maze from a number of different points did not affect the rat's ability to find a submerged platform even though they had learned to locate the platform initially from a single point of entry. This study demonstrates that animals did not learn a stimulus-response association (by swimming off in the same direction as they usually had) or a stimulus-stimulus association, (by following one particular cue). As in the previous study, the animals used a remembered relationship or configurations among all of the available cues in the environment to find the location of the submerged platform.

O'Keefe and Conway (1980) found that rats took significantly fewer trials in learning to find a goal arm in a plus maze if a number of salient cues were distributed around the maze than if the cues were clustered behind one arm; even if that arm happened to be the goal arm. It could be that the animals in the clustered-cue condition were learning to approach one or more of the cues, thus using them as guides. O'Keefe and Nadel (1978) defined guides as localized and stationary cues which direct attention to a particular landmark or object, and require that the animal approach them or maintain a certain angular relationship to them. O'Keefe and Conway (1980) suggested that the learning changes in guidance systems are incremental and subject to interference. Therefore, animals using a guidance strategy should not be able to remember the location of the cue longer than a few seconds. This would account for the fact that the rats in the

clustered condition of the experiment took significantly more trials than the distributed group to reach criterion. The rats in the distributed-cue condition, in contrast, could learn to find the goal arm by its relation to the controlled cues. In other words, by encoding the spatial relations among the distributed stimuli the animal could form a 'map' of the maze and would have far less trouble finding the correct goal arm. The formation of maps, in contrast to guided navigation, thus allows the animal to select any arbitrary direction and distance of locomotion relative to particular 'constellations' of landmarks.

Path Integration/ Dead Reckoning:

Path integration, in turn, also differs from mapping by the absence of 'place' recognition, except for the one or several points to which path integration, or an animal's internal sense of motion, is anchored (Alyan & Jander, 1994). When homing by two-dimensional path integration, a departing animal continuously monitors its displacements and uses this information to calculate the locomotion vector that would take it back to an anchoring point, where the process of path integration originally started (Etienne et al, 1991, cited in Alyan & Jander, 1994). The animal knows its starting location and orientation, but thereafter estimates its current location and direction by integration of internally generated information, such as vestibular or proprioceptive cues (also referred to as idiothetic cues). The process of path integration requires the animal to maintain an 'internal map' of its current location in relation to its movements through the environment. Continual monitoring of these internally generated cues is necessary for the system to maintain accuracy; otherwise errors would accumulate over time. The cognitive map is normally capable of integrating signals from both internally generated and landmark systems. However, when external landmark cues are unfamiliar or are not

available, such as when an animal enters a new environment, the animal must rely on path integration hypotheses to maintain its directional heading. Once an animal has become familiar with the new surroundings, it can then use landmark features unique to that environment in order to calculate its spatial orientation during subsequent exposures to the same surroundings (Gallistel, 1990; McNaughton et al, 1995; Taube & Burton, 1995).

An example of this phenomenon can be found in a study by Watson and Lashley (1915, cited in O'Keefe & Nadel, 1978) in which migratory birds were captured and displaced in a direction perpendicular to that in which they were originally flying. Naïve birds on their maiden voyage continued to fly in the same direction in which they had been headed and thus missed the goal by the amount to which they were transported. Experienced birds on the other hand, which had made the trip before, corrected for the distance they had been displaced and eventually found their way back to their nest. The naïve birds are relying solely on path integration, while the more experienced birds are able to adjust their position by using familiar landmarks. This is similar to McNaughton's theory in which path integration plays a primary role in spatial mapping and hippocampal function, while familiar visual cues are used to correct for drift in the path integration system. Yet, the addition of landmark cues to the cognitive map only occurs after extensive exposure to the environment (McNaughton et al, 1996). Without stable 'allothetic' cues path integration is prone to cumulative errors, because without a stable reference path integration must be based on each prior judgment of position (Knierim, Kudrimoti, and McNaughton, 1995).

Similar studies have been carried out with various insects such as ants and bees. When an ant sets out from its nest to find food, it follows a tortuous or convoluted path. The desert ant may end up 100 meters from its nest after a journey many times that length. When the foraging ant does find food, it turns and heads directly for home. The ant will move over the featureless desert ground until it is within a few meters of its nest, at which point it shows signs of looking for familiar landmarks. The ant does not retrace its convoluted path, but instead returns home by the 'beeline'. Therefore, the ant is not following a chemical trace that it laid down on the way out of the nest, and neither is it following a beacon as there are no stimuli or landmarks in the area of the nest entrance for the ant to home in on. The most likely explanation of how the animal finds its way back to its nest is through the use of path integration. The desert ant has been found to use its computed displacement to set its course for home, to hold that course by maintaining its orientation to the sun, and to measure how far it has gone along its route home. This enables the animal to know when to switch to using guides to narrow down its search to the 1 square centimeter of desert ground that will contain the opening to its nest. As in the previous study, foraging ants were trapped as they emerged from the nest and released at randomly chosen locations 2-5 meters away. Though they were displaced only a few meters the ants showed signs of being lost and not knowing which way to head. The ants searched for the nest in all directions. The behavior of the ants is thus true to the definition of path integration, in that the ants do not know where they are unless they themselves travel there. In other words, the motion of the ants allows them to use self-generated cues to calculate their position and distance from the nest, where the process of path integration first began (Wehner, 1981, cited in Gallistel, 1990).

Studies with rodents have also found that animals tend to rely more heavily on self-generated cues calculated through path integration processes, than cues external to the rats' maze environment. When the two sources of information are placed in conflict to what the rat had previously learned, the rat will tend to rely on self-generated cues (Alyan & Jander, 1994; Etienne, Teroni & Portenier, 1990).

Geometry - The Study of Metric and Sense Relations:

Working in concert with the aforementioned path integration processes, geometrical properties are believed to be the most important aspects in an organism's formation of a cognitive map (Gallistel, 1990; McNaughton et al, 1995). It may seem fairly surprising for an animal to show a preference for geometrical properties, as opposed to reliable visual cues, however, the preference for reliable visual cues is probably a human bias. In an evolutionary context it is more advantageous for an animal to remember an environment's geometrical properties, as landmarks in nature are extremely variable and subject to change. This is most easily illustrated in birds such as the nuthatch that cache food in the spring and then retrieve the cache in the winter when food supplies are short. Most of the landmarks surrounding the cache would probably be covered in a blanket of snow, thus making them unreliable as cues. Remembering the overall geometry of the cache location may thus be a more dependable strategy for the nuthatch (Gallistel, 1990; O'Keefe & Nadel, 1978).

Hermer and Spelke (1996) wanted to test the human bias toward the use of environmental cues in spatial navigation and found some very surprising results. Adults did not have too much trouble finding an object hidden in a particular corner of a rectangular room, though they would sometimes confuse the geometrically equal angles

of the room. The addition of a blue wall to the room was found to reliably prevent the confusion between the two equal angles of the room, thus allowing the adults to consistently find the hidden object in the correct corner. However, when the experiment was repeated with children the results were quite different. Children were found to show a significant increase in the number of errors related to confusing geometrically equal corners of the room, even with the introduction of the blue wall. This surprising result demonstrates that children prefer to use geometrical properties in contrast to other reliable cues such as wall color in solving a spatial problem.

In accordance with the above findings, Gallistel found that rats also depend more on geometric configurations than salient visual stimuli in a rectangular maze environment. Gallistel placed one of each of four salient visual stimuli above each corner of the rectangular maze. Each corner of the maze contained differing bait sizes (18, 6, 0, and 1 pellets) that could be reliably predicted by one of the four stimuli. In the original condition of the experiment the animals were required to learn to go to each corner of the maze in order of the specific bait sizes. In the test conditions of the experiment Gallistel rotated the cues 180 degrees and altered the configuration of the cues by conducting both affine and sense transformations. The affine transformations altered the metric relations of the maze (by changing the lengths of the walls and the angular distance of the corners thus changing the shape of the maze) while the sense transformations created a mirror image of the original rectangular maze (thus altering the sense of left and right in the maze as in a reflection). While the rotation of the maze did not interfere with the rats' performance of the task, as the stimuli were kept in the same configuration, the rotation and sense transformations were found to reduce the performance of the task to chance

levels. Gallistel concluded that the rats relied on uniquely metric relationships and the sense relations of the maze in order to code the position of the bait sizes in each arm and ignored the salient visual cues.

These findings reinforce the notion that cognitive spatial maps encode space in terms of the geometric relationships between objects in the environment, creating a Euclidean style map upon which the position of the animal and relevant locations can be found (Tolman et al, 1946, O'Keefe & Nadel, 1978).

However, in instances where the sense relations of an environment are altered, the distances and angles of the sides of the enclosure are unaltered but what was once left has become right. From the Gallistel study we can see that the animal treated this as a different environment. This is evident by the rats' random selection of goal arms in the rectangular maze after the sense transformations had taken place. Yet, can we say that the rats would form a different cognitive map for the same environment based on this transformation of sense information? It would be interesting to determine whether or not rats would treat the environment as a new place based solely on this distinction between left and right.

The Hippocampus As Cognitive Map

Behavioral and Lesion Studies (Influence On Spatial Ability):

As previously mentioned, the discovery of impaired memory in patient H.M. and other similar patients after bilateral removal of the hippocampus sparked a flurry of experiments aimed at discovering the role of the hippocampus in memory. Many of these early experiments were carried out with rats. As suggested by the case of H.M., hippocampal damage seemed to have the greatest effect on declarative (explicit) memory.

This led researchers to develop tasks for rats that were somewhat analogous to declarative memory in humans and would require the rats to remember particular events such as where they had been. Two of the most commonly used tasks are the radial maze and the Morris water maze.

Radial Maze: The radial maze was first introduced by Olton and Samuelson (1976), and usually consists of a central platform with several thin arms (ranging from 5 to sometimes 18) radiating out from the center. A 4 arm radial maze is often called a plus maze. The radial maze was first used as a test of working memory in animals (a modern term which is synonymous with 'short-term memory'). Working memory was thought to describe events that were specific to single trials in the radial maze such as which arms had already been visited by the animal. In contrast, reference memory was thought to represent events that were constant across trials such as which arms contained food. Returning to an arm that had already been visited or had bait taken from it was considered to be a working memory error while choosing an arm that had never been baited would be considered a reference memory error (Jarrard, 1983).

In order to compare O'Keefe and Nadel's cognitive mapping theory (1978) with Olton, Becker and Handelmann's working memory theory (1979), Jarrard (1993) devised a rather ingenious testing procedure using a radial maze task that that allows studying the acquisition of two kinds of information (spatial versus intramaze cues), and two different kinds of memory (working versus reference). In the spatial version of the task the eight arms of the radial maze differed only in their spatial location in the room. There were obvious extramaze cues that remained constant across trials including the door, shelves, and arrangement of cages. For any one animal the same four out of eight

arms were consistently baited over trials. In the intramaze cue task, different textured floor inserts (screen, wire, cloth, sandpaper etc.) were randomly moved among the eight arms and the rat was rewarded for choosing the same four cues independent of spatial location. So, correct performance in the cue task required the use of the intramaze cues while correct performance of the place task required the use of the distal cues. Jarrard stresses the point that that place and cue tasks appear to be of equal difficulty for normal rats since the tasks are learned at the same rate. However, the results of the experiment suggest that rats with ibotenic hippocampal lesions show both reference and working memory errors on the place task but not on the cue task. These results are more compatible with the idea that the hippocampus plays an especially important role in processing and remembering spatial information.

Morris water maze: The water maze was introduced by Morris (1981) and consists of a large pool of water mixed with milk, chalk, or paint in order to make the water opaque. Placed at a particular location in the pool there is typically a platform that the rat can stand on so it can get out of the water. The advantage of this task is that it is not an appetitive task and the rat will never become satiated. In other words, the rat will always be motivated to try and find the platform. Sometimes the platform is submerged just below the surface, in which case the task is referred to as the hidden platform water maze. Sometimes the platform is left above the water so it remains visible to the rat, in which case the task is referred to as the visible water maze. Similarly to the visible water maze task, the platform can be submerged but indicated by a salient cue in the vicinity of the platform, such as a lamp. This is referred to as the cued water maze task (Redish, 1999).

Morris (1981) found that rats placed in the pool quickly learn to swim to a platform in a consistent location, even if it is submerged and can not be seen. If the platform is removed, as it often is in 'probe trials' in this task, the animal will spend most of its time swimming in the location where the platform used to be. This shows that the rats know the 'location' of the platform and have an expectation of its location.

The results of several experiments have suggested that hippocampal or fimbria-fornix lesions impair navigation to hidden but not visible or cued platforms (Morris, Garraud, Rawlins, and O'Keefe, 1982; Eichenbaum, Stewart and Morris, 1990; Morris, Davis , and Butcher, 1990). This suggests that finding, or learning to approach the cued platform is a hippocampal independent process and does not involve the cognitive map. This is consistent with the observation that rats with hippocampal lesions are not impaired in a non-spatial simple discrimination task in which the rat has to learn to approach a white card for reward as opposed to a black card (Issacson, 1972).

However, the lesion technique, particularly in its early development, was often criticized as the extent and nature of the brain damage had varied considerably. In most of the lesion experiments damage was not limited to the hippocampus but also included varying amounts of damage to extrahippocampal structures and/or their projections (Jarrard, 1993). In addition, in cortically lesioned control groups, which are normally included in lesion experiments, it is hard to know what kind of conclusions to reach when the performance of these controls falls between the intact normals and the hippocampally lesioned group, as is often the case (see O'Keefe and Nadel, 1978 for a review of the early lesion literature).

Hippocampal Volume and Spatial Demands:

Birds: Comparative studies provide a unique source of evidence for the role of the hippocampus in spatial learning and memory. Within birds, the hippocampal volume of scatter hoarding species that cache food in many different locations is enlarged relative to the remainder of the telencephalon, when compared with that of species which cache food in a confined area, or do not cache at all. It has been suggested that selection pressures that give rise to food caching also lead to reallocation of nervous tissue in favour of the hippocampus (Clayton, 1998; Krebs, 1990). This can be seen in the larger relative hippocampal size of classes of birds such as Paridae, Sittidae, and Corvidae as opposed to classes of birds such as Passeridae and Cardinalinae (Krebs, 1990).

Some species of scatter-hoarding species have a more accurate and long lasting spatial memory , and a heavier reliance upon spatial cues than that of closely related species that do not store as much food, or any at all. An example of this can be see with Clark's Nutcracker a member of the Jay family, which lives at high altitudes and relies quite heavily on food stored during the summer (typically burying tens of thousands of seeds in thousands of locations) to survive the winter. Pinyon Jays, which live at a slightly lower elevation, bury less food and are less dependent on it during the winter. Scrub Jays and Mexican Jays, living at much lower altitudes, depend even less on cached food to survive the winter. Researchers have found that of these four species of Jay, Clarks' Nutcracker has the largest hippocampal volume and performs the best on radial maze tests of spatial memory. Pinyon Jays are second best in both respects while both the Scrub Jay and Mexican Jays performed the worst on tests of spatial memory. However, on non-spatial tasks such as tests of color memory, performance does not correlate with the size of hippocampus (Basil, Kamil, Balda, and Fite, 1996; Olson, Kamil, Balda, and

Nims, 1995). It has also been suggested that food-storing birds such as food-storing parids and corvids are also more resistant to memory interference and that they may remember additional information about the content and status of cache sites as well as its spatial location (Clayton, 1998).

Hippocampal volume may also be influenced by seasonal and environmental demands. Smulders, Shiflett, Sperling and DeVoogd, (2000) found that the volume of the hippocampal formation (as determined by the total number of large cells in the hippocampal formation) in black-capped chickadees varies across the seasons, in conjunction with the seasonal cycle in food hoarding. The seasonal variation in volume is due to an increase in the small and large cells (principally neurons) in the fall as opposed to April, June, or December. It is suggested that the increase in neuron number provides a 'larger neural network with which to process information about a large number of cache locations' (pp. 420) in order to more efficiently distribute food items for the upcoming winter months. The increase in the number of neurons in the hippocampal formation may be driven by shorter photoperiods that signal an approaching winter, but the maintenance of the cells is believed to be due to the experience of hoarding. This is why volume may be larger in the fall as opposed to the winter. Hoarding in the fall may maintain volume more than actually looking for the caches in the winter. Similarly, Abbot et al (1999) found that Leach's storm petrels that were taken from nesting burrows in wooded habitat had a larger relative hippocampal volume than those taken from burrows in an open meadow. This larger relative hippocampal volume may be associated with the increased spatial demands of returning to their nests at night in the darker, more navigationally complex woods. The light levels in the wooded environment and its 3-dimensional

structure and high burrow density makes returning to the burrow much more difficult than the open meadow.

As suggested by Smulders et al. (2000) increased hippocampal volume may be related to the 'experience' of hoarding. This was first suggested by Clayton and Krebs (1994) when they found that the volume of the hippocampus relative to the rest of the telencephalon in the food-storing marsh tit depends on retrieving stored food. One group of birds was allowed to search for, and then cache sunflower seeds. The other group could not cache as they were fed powdered sunflower seeds that could be eaten but not cached. (the powdered seeds would crumble when the birds tried to place them in their beaks).The authors found that the act of retrieving seeds that had previously been cached stimulated growth in the hippocampal volume while being denied the opportunity to cache and retrieve induces loss in hippocampal volume. It was further suggested that the act of storing and retrieving maintained hippocampal volume and prevents apoptosis.

Rodents: Gaulin and FitzGerald (1986) proposed that sex differences in spatial ability would evolve through sexual selection if the amount of spatial information to be processed was greater for one sex than the other. This hypothesis was tested by examining the different uses of territorial space in two species of vole, one of which was monogamous while the other was polygynous. The polygynous male vole is known to range widely during the breeding season in order to gain access to as many mates as possible, while females hold small constant territories. The monogamous pine vole, on the other hand, forms pair bonds during the breeding season and cohabitates in a single territory. Gaulin and Fitzgerald (1986) found that males of the polygynous species had larger home ranges than females while there were no sex differences in home-range size

of the monogamous pine vole. They hypothesized that sexually dimporphic spatial learning may have evolved in the polygynous species in response to the spatial demands upon the males that roam across several territories in order to find mates. Similarly, Galea, Kavaliers, and Osenkopp (1996) found that, in adult deer mice, male spatial performance (as judged by latency in finding a submerged platform in a water maze) increased in the breeding season as opposed to the non-breeding season. The opposite pattern was found for the female performance. The authors concluded that the expression of sexually dimorphic spatial ability in rodents appears to depend on the reproductive status of the animal and the associated levels of sex hormones, such as estradiol in females. Increased use of space, as measured by home range in the wild, appeared to correspond with better spatial learning in the Morris water maze in the lab (Galea et al., 1996). In line with expectations, the hippocampus of polygynous male species, such as the meadow vole, have been found to be significantly larger than the females', while no such sexual dimorphism is apparent in the monogamous pine vole species. Perhaps more spectacularly, the polygynous meadow voles' larger hippocampal size disappears outside the breeding season, when the male's home range is of comparable size to the female's home range (Jacobs, Gaulin, Sherry, and Hoffman, 1990; Jacobs, 1995).

Environmental complexity has also been suggested to influence the infrapyramidal hippocampal mossy-fiber connection (IIP-MF). This connection has been shown to be associated with spatial learning abilities in tasks such as the radial maze in mice (Prior, Schwegler, and Ducker, 1997). In order to clarify the role of this mossy fiber connection in a natural environment, Pleskacheva et al. (2000) studied the bank vole which is known to be adapted to a wide range of different habitats, and the root vole

which lives in homogenous grassland habitats with small home ranges. The authors found that the entire mossy fiber projection was 42% larger in the bank vole than the root vole. The IIP-MF projection in the bank vole was also found to be 230% larger than that of the root vole. When tested in a laboratory water maze task, path length used to find the submerged platform was significantly reduced in the bank vole as opposed to the root vole. The bank vole also scored much higher on scores that examined spatial search patterns. On the other hand, the root vole showed signs of strong thigmotaxis and circular swimming. This species also scored at chance level in the probe trial when the submerged platform was moved to the opposite quadrant in the water maze.

Humans: Maguire et al (2000) analysed structural MRI examinations on the brains of humans with extensive navigation experience, London taxi drivers, and compared them with those of control subjects who did not drive taxis. The posterior hippocampi of the taxi drivers were significantly larger relative to those of non-taxi driver controls. However, a more anterior hippocampal region was found to be larger in controls than taxi drivers. These results indicate that professional dependence on navigational skills in London taxi drivers is associated with a relative redistribution of gray matter in the hippocampus. The authors wondered if this difference was somehow reflective of a physiological predisposition toward a navigationally complex field of employment. In order to tease apart potential influences of predispositions from the influence of experience, the authors examined the correlation of hippocampal volume and the amount of time spent as a taxi driver. They found that the volume of gray matter in the right hippocampus was found to correlate significantly with the amount of time spent learning to be, and practicing as, a licensed taxi driver, positively in the right posterior

hippocampus and negatively in the right anterior hippocampus. This led the authors to conclude that the differences seen in hippocampal volume in taxi drivers and controls were due to experience and were acquired. These results further suggest a functional differentiation within the hippocampus. The posterior hippocampus seems to be preferentially involved when previously learned spatial information is used, whereas the anterior hippocampal region may be more involved (in combination with the posterior hippocampus) with the encoding of new environmental layouts. Thus, "the 'mental map' of the city is stored in the posterior hippocampus and is accommodated by an increase in tissue volume" (pp. 4402). Undoubtedly, in humans the functions of the hippocampus have adapted to also accommodate other types of memory, such as episodic memory (Tulving and Markovitsch, 1998), but the hippocampus retains an ability to store large-scale spatial information (O'Keefe and Nadel, 1978).

An Explanation of the Electrophysiological Approach and Hippocampal Place Cells:

In the earlier review of place versus response learning, it was noted how both types of learning are quite different and that place learning is related to location as judged, typically, by a constellation of cues. Yet, how is a place defined, learned about and internally represented? How does it mold ongoing behavior in adaptive ways? How do places influence learning about events that occur within their confines? An extremely important technique in answering these questions was developed by O'Keefe and Dostrovsky (1971). The authors reported that the firing rates of a number of hippocampal neurons appeared to be closely linked to an animal's location in the environment. Given the link between the location of the animal and the activity of the cell, the authors

referred to such neurons as 'place cells'. Without this remarkable discovery, it is unlikely that the cognitive map theory of hippocampal function, advanced by O'Keefe and Nadel in 1978, would have been developed. During the initial study of hippocampal cells, a 'neuroethology' approach was often taken. In this approach the experimenter would often observe the activity of a single cell in a number of different circumstances and during different behaviors emitted by the subject. The important advantage of this technique was that it made no assumptions about what had the greatest influence over the activity of the cell (O'Keefe and Nadel, 1978). This technique revealed two types of unit activity that are typically found in the hippocampus of the awake, freely moving rat: complex spike cells which fire in bursts in which several spikes occur with a short interspike interval (1.5-6msec) and successive spikes have differing (often decreasing) amplitudes; and theta cells that have high firing frequencies and action potentials of short duration. The complex spike cells were found to be pyramidal cells (the primary output cells of the hippocampus), while theta cells (or displace cells) were interneurons (Fox and Ranck, 1981). The primary correlate of the theta cell is some aspect of the animal's movements while that of the complex-spike cell is the animal's position in the environment (Hill, 1978, O'Keefe & Speakman, 1987). Complex-spike cells have thus come to be known as 'place' cells as they seem to fire preferentially in certain positions in an animal's environment. These locations of preferential firing have likewise become known as 'place fields'. O'Keefe (1979) offered a definition of a place cell based on the results of an earlier experiment:

> Hippocampal cells were recorded while the animals performed a place learning task in an environment where the cues for location were identified and controlled. Probe trials in which two of the usual place cues were available revealed that some of the place cells continued to fire in the place field when any

> two of the four cues were available. This suggests that the place cell is not "responding" to a particular sensory input but that it receives several redundant cues to the animal's position in an environment....a place cell is a cell which constructs the notion of a place in an environment by connecting together several multisensory inputs each of which can be perceived when the animal is in a particular part of an environment (pp. 425).

O'Keefe (1979) also points out that place units do not appear to fire in a certain part of the environment primarily because of something the animal does there, or because of its motivation for going to a certain part of the environment (i.e., hunger or thirst). Place cells appear to be primarily cognitive, signaling the animals' position in an environment irrespective of behavior, motivational state or the reward properties of a particular place. What does seem to be important in influencing the firing rate pattern of place cells are the presence or memory of cues or landmarks that define a particular place.

In order to further examine the role of extra-maze cues on the firing activity of hippocampal place cells (from CA1 and CA3), O'Keefe and Speakman (1987) recorded single unit activity from complex spikes from rats that were performing a spatial task in a four arm radial maze. The correct goal arm was varied but defined by 6 controlled spatial cues distributed around the enclosure (curtain which surrounded the maze). The authors found that 60% of the recorded place units had fields with significant relations to the controlled cues when they were rotated, while 29% had significant relations to the static background cues. In addition, 90% of the 30 units with place fields related to the controlled cues maintained their firing fields during a retention phase in which the cues were removed while the rat was in the start arm. This evidence for place field memory was in relation to the results from control trials in which the controlled cues were

removed before the rat was placed on the start arm. During these trials the units also maintained place fields, but the orientation of the fields was not aligned with the experimenter determined goal. However, the animal's choice, although incorrect in terms of the experimenter determined goal, could be predicted from its place field orientation. In other words, the place field indicated where the rat 'thought' the goal arm was.

The influence of landmarks or distal cues is also visible in the results of Cressant , Muller and Poucet (1997). These authors showed that place fields rotate with landmarks inside a small circular arena as long as the landmarks are pushed to the edge of the wall, that is if they are made to be orienting to distal stimuli. If the three cylindrical landmarks were clumped together in the interior of the arena, the place fields did not correlate with, or follow, their location.

Further exhibiting the control of cues over place fields, Bostock, Muller and Kubie (1991) substituted a new stimulus for a familiar stimulus in a familiar environment. The researchers recorded hippocampal place cells while rats searched for food pellets scattered on the floor of cylindrical apparatus with a white cue-card attached to the wall. Once a place cell had been recorded in the presence of a white card, the white card was replaced by a black card which was the same size and shape. Initially, both the black and white cue cards were found to have control over the firing fields. That is to say, that whenever the cue cards rotated, so did the location of the firing fields. Yet, during subsequent presentations of the black card the spatial firing patterns became distinct from the firing pattern found when using the white cue card. Once the differentiation of firing patterns had occurred in a given rat, all place cells subsequently recorded from the rat had different firing patterns in the presence of the white and black cards. This suggests that

there is an experience-dependent modification of place cell firing patterns. The alteration of the cue cards, in this case, caused the rats to eventually treat the same cylinder as two different places based on the color of the cue card.

Overall, changing environments cause place cells to "remap" the environment. In other words, a new, apparently random subset of cells becomes active. When a rat enters a novel or sufficiently changed environment, the hippocampus will remap almost immediately (Kubie and Muller, 1987; O'Keefe and Nadel, 1978; Wilson and McNaughton, 1993). A cell that had a place field in the first environment may become either silent or change the shape and location of its field; a previously silent cell may either remain silent or discharge in a new firing field. A logical extrapolation from such findings is that the emergence or disappearance of fields in a familiar environment that are qualitatively different from previous recordings in the same environment, may serve as an indication that the animal is treating the environment as novel. In other words, the rat may consider it a different place. Thus, the identity of an environment is encoded by a unique selection of pyramidal cells that will be used as place cells and furthermore, by a unique arrangement of the firing fields of the selected place cells. (Muller, Poucet, Fenton, and Cressant, 1999). This is the principle idea underlying the electrophysiological approach that will be taken with the recording of the hippocampal cells in the present study.

Hippocampal Involvement in Non-Spatial Tasks

Are Hippocampal Place Cells Influenced by Non-Spatial Cues?:

As stated in the previous section, hippocampal place cells are viewed not as a collection of separate place parameters, but as an index of a cohesive cognitive map in

the hippocampus. The primary significance of place cells, then, is that they act as elements of a Cartesian representation of the environment (O'Keefe, and Nadel, 1978). In short, the main idea contained in this notion is that the hippocampus contains an environmental framework in which the activation of each place cell (as indicated by the place field) represents the animal's presence at a particular set of co-ordinates within in the spatial reference frame. However, in the last twenty years of research, a large body of experiments have reported a role for the hippocampus in non-spatial tasks, as well as spatial tasks (Amsel, 1993; Eichenbaum, Dudchenko, Wood, Shapiro and Tanila, 1999; Hampson, Heyser and Deadwyler, 1993; Hampson and Deadwyler, 1996; Otto and Eichenbaum, 1992; Rawlins, 1985; Shapiro and Eichenbaum, 1999; Wallenstein, Eichenbaum and Hasselmo, 1998; Wood, Dudchenko and Eichenbaum, 1999). The typical stance taken by supporters of nonspatial correlates (e.g., Eichenbaum and colleagues) is that hippocampal firing fields are not best described as simply encoding spatial relations in a map. Eichenbaum states that hippocampal cells encode an ongoing record of the regularities, consistencies, and novelties that comprise the unfolding structure of episodes (Shapiro and Eichenbaum, 1999).

Directionality of place cells: McNaughton, Barnes, and O'Keefe (1983) suggested that an animal may call up different cognitive maps for the same environment based on the changing requirements of the task, or for the different paths the animal takes through a particular area. It has been found that in tasks involving repeated trajectories between fixed locations, hippocampal place fields are highly directionally sensitive, typically having non-significant firing when the rat faces in the direction opposite to the preferred

direction in the place field (Gothard, Skaggs, Moore & McNaughton, 1996; Markus, Qin, Leonard, Skaggs, McNaughton & Barnes, 1995, McNaughton et al, 1996). Gothard et al (1996a) showed that place cells can be bound to different behaviorally relevant landmarks, irrespective of spatial location. Does this mean that the hippocampus was functioning in a non-spatial manner? The authors interpreted this as evidence that subpopulations of hippocampal place cells encode location within multiple reference frames which focus on specific landmarks such as start and goal boxes. In this study 'reference frame' was intended to be synonymous with a map-like representation encoded in a specific distribution of place fields. Therefore, switching reference frames would be equivalent to switching maps. In a follow-up study (Gothard et al, 1996b), rats shuttled on a linear track between a fixed reward site at one end and a movable reward site at the other end. The authors found that cells that were bound to the start-box would fire only on an outward journey, while cells at the stable reference point fired only on the return journey to the start box. In other words, place cells have fields when the animal moves in one direction, but either become silent or encode a different place when the animal moves in another direction. Direction of travel thus appears to determine which map the animal uses. The most parsimonious explanation of these results is that the two places become associated with two separate reference frames, which switch as the rat reaches each food source. The rat thus seems to treat the outward and the inward path as two separate routes rather than as one path through the same environment. In contrast to the findings of Gothard et al (1996a), the results of Gothard et al (1996b) led the authors to conclude that landmark bound firing of place cells is caused by 'updated corrections' within a single map rather than to a switching of maps. Under these conditions, a journey

between two points seemed to be encoded on a single map rather than on a mosaic of different maps.

O'Keefe (1999) has suggested that if a sequence of behavioral tasks is repetitive and stereotyped, one would expect a good correlation between each behavior (such as ingoing and outgoing paths) and the firing of specific cells, since each behavior will tend to occur in the same location on each trial. In an ingenious experiment, Czurko, Hirase, Csicsvarki, and Buzaki, (1999) found that place cells could be both spatial and directional. They recorded from place cells with fields in a running wheel, a technique they referred to as "space clamping", and showed that the firing rates increased with the speed of running in the wheel. The spatial nature of these place cells was confirmed by manipulations that showed that moving the running wheel to a different location in the environment, or rotating it relative to the environment, caused the cell to stop firing altogether. It therefore seems that an animal determines its location in this situation based on the cues in the environment and that the place fields are influenced by direction. In conclusion, the directional nature of place cells in some cases does not necessarily mean that place cell activity can exist outside the parameters of a cohesive allocentric cognitive map that is determined in part by distal cues in the environment.

Place cells and 'partial remapping': In the spatial theory of hippocampal function, the remapping of an environment is believed to be 'all or none'. In the spatial theory, then, a complete remapping means that a certain environment is taken to be sufficiently different from other environments. It appears that even a small change in the environment, such as Bostock and Muller's changing the color of a cue (1991), can trigger a complete

remapping and suggests that a new map forms as a whole and is not formed piece by piece (Muller at al, 1999). However according to the theory advanced by Eichenbaum and colleagues a complete remapping implies that the constellation of stimuli have been altered to such an extent that the original combinations of stimulus features required to activate each place cell no longer exist, thus causing cells to change their firing rate, shift fields to a different place, change field shapes, or stop firing altogether. Therefore, only a fraction of cells will be altered by small changes in the environment and the extent to which components of a map change will vary with the extent to which the environment is changed. A small change in the environment would then cause, at most, a 'partial remapping' and major changes in the environment should be necessary before complete remapping would occur (Muller, Poucet, Fenton, and Cressant, 1999; Eichenbaum, Dudchenko, Wood, Shapiro, and Tanila, 1999). This section shall provide examples in which 'partial remapping', was found to occur, in contrast to the expectations of the spatial theory of hippocampal function.

Shapiro, Tanila and Eichenbaum (1997) presented data for the argument that place cells do not signal an animal's position as much as they are triggered by certain combinations of stimuli selected from among the available stimuli. According to the authors, location-specific firing is really more like 'stimulus-specific firing' that occurs in a specific place because the stimuli that activate a certain place cell are only available in that place and nowhere else. Their version of place cell activity, in contrast to O'Keefe's (1979), is that place cells are not bound together in a way that preserves the relative locations of their firing fields. Instead, the integrity of the place cell representation depends on the constancy of the environment. The Shapiro, Tanila and Eichenbaum

experiment demonstrated this argument by recording hippocampal place fields as rats explored a four-arm radial maze surrounded by curtains holding distal cues and distinct local olfactory, visual, and tactile cues in each of the four arms. Hippocampal cells were recorded as rats explored each of the arms in the maze while the cues were in a standard configuration. Probe tests were then carried out in order to see how changing the configuration of the various distal and local cues affected the firing of the place fields in the maze. This procedure is quite similar to the one mentioned earlier in the experiment by Suzuki et al (1980). Note that in this earlier experiment that altering the configuration of available cues disrupted the behavioral task. Shapiro et al (1997) included a 'double rotation trial' in which the constellation of local and distal cues were rotated 90 degrees in opposite directions. This probe helped to tease apart the influence of local, distal and other non-controlled cues on the firing of place cells. The activity of many cells seemed to track the distal or local cues while other cells seemed to track certain combinations of cues. One such place field rotated with the distal cue in the double rotation trial, but a second subfield also appeared in the middle of the same arm. In the following two trials, in which the configurations of both the local and distal cues were scrambled respectively, this cell appeared to encode two stimulus relationships. The small subfield seemed to track a particular distal stimulus (striped card) while the larger subfield seemed to track the local cues in a particular arm (rubber mat and mint scent). In other words, the place cell activity appeared to encode two subsets of environmental stimuli, a local and a distal cue. The authors argue that the cell activity did not therefore provide a good predictor of the animal's location in a map, but did provide a signal about the relative distance between the rat and two of the stimuli in the environment.

Skaggs and McNaughton (1998) performed an experiment in which pyramidal cells were recorded from rats foraging for food reward in an environment consisting of two nearly identical boxes, a North box and a South box, connected by a corridor. For each rat a 'higher than chance' fraction of cells had similarly shaped spatial firing fields in both boxes, but other cells had completely different fields in both boxes. The important finding in this paper is that when the rats were started in the South box during a probe trial, after having been started in the North box for every previous session, 50% of the subjects had place fields in the South box that had normally been associated with the North box. However, this only occurred the first time the rat was started in the South box, and thereafter the field reverted to the previous cell activity associated with the South box. It is likely that the differences found between the North and South maps resulted from a combination of the rats' expectations and a mechanism for remembering the rats' movements through the corridor joining both boxes. According to the authors, expectations would refer to the learned association that had developed in the part of the apparatus in which the rat was initially placed on every trial, except the last probe trial. The data from the experiment also provide compelling evidence that two distinct hippocampal maps can overlap without being identical, as the spatial representations activated in the two boxes for the rats in the study were neither identical nor completely distinct. The authors contend that the observation of partially overlapping maps is a challenge to the theory that hippocampal maps are preconfigured in relation to the path integrator mechanism and bound to exteroceptive cues as a product of learning (McNaughton et al., 1996). Skaggs and McNaughton (1998)conclude that situations that

are virtually identical in sensory and behavioral respects can have differing place cell activity, and that this activity can be determined in part by expectations.

In order to observe the response of place cells to both subtle and significant changes in an environment, Muller and Kubie (1987) observed place fields from complex spike cells in rats trained to search for food pellets tossed randomly onto the floor of a walled cylinder. They found that rotation of the cue card produced equal rotations of the firing fields of single cells. However, changing the width of the card did not affect the size, shape or position of the firing fields. Yet, the fields did sometimes rotate to a modest extent. Removing the cue altogether had very little effect on the size, shape, or radial position of the place fields but often caused the fields to rotate to unpredictable angles. In another set of manipulations, Muller and Kubie performed a particularly interesting manipulation of cues by scaling up the environment by two times its original size. They found that 36% of the cells that fired in both cylinders (the original and the new larger cylinder) also scaled up in size, in the sense that the field stayed at the same angular position and at the same relative radial position but was larger. However, 52% of the cells showed very different firing patterns from one cylinder to the other. In a similar manipulation, the experimenters found the same pattern of results when they scaled up a rectangular shaped environment. However, when the authors altered the geometry of an environment by changing a regular sized cylindrical environment to a regular sized rectangular environment, the firing pattern of cells in both environments were drastically different.

Place cells are Influenced by task: Markus et al. (1995) found that place fields were more directional when the rat searched for food at fixed locations in a particular environment, than when the rat foraged for food that was scattered in a random fashion. It thus seems that place fields are more directionally dependent when the animal is planning or following a route between fixed points of behavioral significance (much the same as in Gothard et al, 1996b). While moving between established reward sites in a directed search task, the reward sites themselves become points of reference upon which the rats focus their attention. However, it is a fairly reasonable assumption that the rats' attention was not focused on any particular landmark while performing the random foraging task. Apart from the increased directionality of place fields, changing the behavioral task was also accompanied by a change in firing location of about 33% of the recorded hippocampal cells. This suggests that some place cells encode certain behavioral aspects of the task required of the animal, or that place fields represent landmarks or points of reference within the environment as it navigates through different behavioral tasks. It can be inferred, then, that hippocampal neuronal activity encodes a complex interaction between locations, their significance and the behavior the rat is called upon to execute.

Place cells are influenced by tasks involving temporal and/or spatial discontiguity: Both amnesia patients and animals with hippocampal system damage exhibit what can be considered 'time dependent impairments' in behavioral tasks generally described as associative or relational in nature (Rawlins, 1985; Cohen and Eichenbaum, 1993). As mentioned previously, patient H.M. (after medial temporal lobe damage) exhibited a functioning working memory but was unable to recall events that were encountered only a brief period (about a minute or longer) before testing. Rawlins (1985) has suggested

that many of the experiments that are sensitive to hippocampal dysfunction have an inherent temporal discontiguity in their design. This temporal discontiguity, is thought to be represented by events that must be associated together as they do not overlap. This notion has most recently led Wallenstein, Eichenbaum and Hasselmo (1998) to advance a theory that, from a computational point of view, the hippocampus is involved most critically in learning and memory tasks in which discontiguous items must be associated, in terms of their temporal or spatial positioning or both.

One of the first experiments to note a connection between discontiguous events and the activity of hippocampal cells was an experiment by Foster et al (1987) which showed that hippocampal neurons fire differentially and in a time locked manner to the presentation of positive versus negative auditory stimuli in instrumental discrimination tasks. In particular, their activity was sensitive to the temporal sequence of the stimuli. This finding suggested that hippocampal cells may be activated by particular sensory stimuli but, unlike sensory neurons, are activated more in relation to the learned significance of the stimulus and temporal experiences with the stimulus than to its physical qualities. Similarly, Otto and Eichenbaum (1992) examined neuronal activity in the CA1 cell layer of rats in an odor-guided continuous delayed non-matching to sample task, focusing on the cells that selectively fired during the period of odor cue sampling and during the generation of a 'go' or 'no go' water port response. During half of the trials in the experiment, the odors represented were different from the original odor presentation (S+) and the correct response was to go to the water port for reward (R+). During the other half of the trials, the odor presented was the same as that presented on the previous trial (S-) and the correct response was to withhold ('no go') the water report

response (R-). The authors found that the firing patterns of many of the hippocampal cells reflected the outcome of the match or non—match and not the particular perceptual qualities upon which the comparison was based. Furthermore, the match/non-match cells were most pronounced when performance was accurate, suggesting that the hippocampus may be important in the behavioral decisions involved in the task. According to the authors, the activity of hippocampal cells in this task is best described as 'reflecting the active processing of cues resulting in a representation of the outcome of the match and non-match comparison' (p.331).

Another experiment which had a similar set of findings, but in a different task, was carried out by Hampson, Heyser, and Deadwyler (1993). They recorded CA1 and CA3 neurons from rats while they performed a delayed matching to sample task. Much the same as Otto and Eichenbaum (1992) they found that hippocampal cells fired differentially at different points of the task, specifically during the sample and match responses and during delivery of a water reward. Marked increases in firing rate were also found during the delay period, but such 'delay-specific firing' was not predictive of cell activity in other phases of the task. The authors concluded that sample-match comparisons are ultimately encoded and retrieved by hippocampal cells.

Perhaps the most convincing argument for the existence of a more 'relational' function of the hippocampus comes from a recent experiment by Wood, Dudchenko and Eichenbaum (1999). They recorded from hippocampal cells during a continuous non-matching to sample task (also referred to as successive olfactory discrimination) in which they tried to dissociate location, odor, and the match/mismatch aspects of the task. The rats were trained on an open platform to approach a small cup which contained sand

scented with one of nine different spices with distinct odors (e.g., thyme or paprika). In order to disentangle the influence of place, or perhaps in response to O'Keefe's criticisms, the cup was placed in each of nine locations in each trial. If a cup had a different odor from the cup on the previous trial (non-match), the rat could dig in the sand and find a cereal reward. On the other hand, if the cup had the same odor as the cup on the previous trial (match), the cup would not contain cereal and the rat would typically turn away from the food cup. An analysis of the cell activity showed that 7.8% of the cells responded to odor in the absence of any other correlate, 11% responded solely to location, and 13% responded solely to the match/mismatch aspect of the task. The key finding here is not only that the cells responded to the non-spatial stimuli, but that they were more likely to have non-spatial than spatial determinants, even though the authors used the same kind of rich stimulus environment typically used to study the formation of place fields in spatial tasks. Parenthetical

In a recent review, Eichenbaum et al (1999) lists some of the major properties of hippocampal neural activity based on the results of Wood et al. (1999) and other experiments in the last 15 years:

> Hippocampal spatial firing patterns do not consistently represent the animal's position among cues that compose an environment. Instead, the hippocampus creates distinct spatial representations, even for identical spatial cues under a variety of conditions where the animal might consider itself undergoing different experiences in the same environment....Hippocampal neuronal activity reflects a broad spectrum of specificities. Some cells encode unique events, characterized by particular conjunctions of stimuli, behaviors, and the locations where these occur. Other cells represent sequences of events within behavioral episodes or specific features of events that are common across different behavioral episodes (pp. 216).

Eichenbaum et al (1999) conclude that activity of hippocampal cells reflect sequences of events that serve to link large sets of successive events into representations of episodes that are unique in behavioral significance. In this light, place cells are thought to be 'codings' that might link one's current location to memories for previous episodes at those locations.

Behavioral Studies and Temporal Discontiguity:

In learning tasks where events are temporally contiguous, animals have to associate a significant event such as the delivery of an electric footshock or the delivery of food, with a stimulus that immediately precedes it, such as a lever press. In a temporally discontiguous task, the delay between the preceding stimulus and the significant event is increased . Obviously, the length of the delay can be varied in order to make a specific task more or less temporally discontiguous. In order for an animal to link the relevance of a specific stimulus to a significant event (such as reward or shock) in a temporally discontiguous task the animal must make a learned association between the initial stimulus and the significant event. The presence of a stimulus (in Pavlovian conditioning) or the acting out of a response (instrumental conditioning) is thought to create a 'neural trace' that fades over time (Rescorla and Holland, 1982). The trace can be thought of as being similar to the aftereffect of seeing the flash from a camera. At first the image is vivid, but gradually fades over time. In order for learning to take place, the remnants of this fading neural trace must overlap with a UCS, as in classical conditioning, or a consequence, as in operant conditioning. Rawlins (1985) points out the need for a 'temporary memory storage system' that is necessary in learning the appropriate rule in a temporally discontiguous task. This is most apparent when there is a

temporal gap between receiving information about how to respond, and being permitted to make the response. Although animals can learn tasks that require them to bridge temporal gaps, temporal discontiguity generally produces a clear retardation of learning in normal animals in both instrumental conditioning and classical conditioning (Rawlins 1985).

The Role of Interference: Early studies investigating the associations between dicontiguous events were able to find that associations could readily occur between events separated by an hour or more (Revusky and Garcia, 1970). Revusky and Garcia found that bridging the temporal gap in conditioned taste aversion occured more readily than more traditional manipulations involving delay gradients. Revusky (1971), based on these earlier findings, hypothesized that the associative processes underlying long-delay learning may be the same as those underlying the more traditional short delay paradigms. Revusky states that the usual degradation gradient in memory over delays is attributed 'not to a decay in the memory process itself but to associative interference' (pp. 159). The events of the reference situation, or the association the experimenter wants the animal to learn, often become associated with extraneous events occurring during a delay. Revusky hypothesizes that it is these extraneous events that interfere with the learning of an association over a delay. Thus, increasing the time interval between event A and event B will increase the number of intervening events that can occur during the delay. This increase in the intervening events will also increase the probability that events A and B will become associated with the extraneous events. Therefore, a delay of reward reduces the likelihood of an association between a response and reward by increasing the number of, and hence the possibility, that the intervening events will become associated with

either the response or the reward (Lett, 1973). Revusky (1971) proposed that if there was a delay between two elements of a learning paradigm, interference would be reduced if the animal was removed from the experimental apparatus and placed in a discriminably different chamber during the delay. It was believed that this relocation would prevent interference from developing inside the apparatus, as events that occurred outside the apparatus would not become associated with the events that occurred inside the apparatus. In an examination of this theory, Lett (1973) trained rats to select a specific side of a T-maze with a delay of reward ranging from 0.5 to 8 minutes. During the delay the rats were removed from the apparatus and returned to their home cages, regardless of whether they had made a correct choice or not. If the rats made a correct choice they would be returned to the apparatus for food reward. Remarkably, all of the subjects in the experiment were able to learn the position habit despite the lengthy delays between response and reward. These results can not be explained in terms of secondary reinforcement. Yet, the results can be viewed as 'reactivating' a memory of the previous response, making it available at the moment of reinforcement and thus strengthening the link between the previous response and reward. According to Rescorla and Holland (1982),' the response is viewed as leaving a memory that can either persist in time or be reactivated at the time of reinforcer delivery' (p.281). However, Rawlins (1985) explains the results of the experiment by noting that delay learning can be facilitated by the simple presentation of a salient event in close temporal proximity to the choice. A salient event may cause the animal to pay attention to its recent behavior, such as a recent choice between two differently colored alleys, and help bridge the temporal gap between choice point, a long delay, and reinforcement. However, Rawlins (1985) does not attempt to

explain these results in terms of his theory of the hippocampus as a temporary memory store. Might the hippocampus be involved in bridging large temporal gaps?

SecondaryReinforcers in Discontiguous learning: Historically, the necessity of response-reinforcer contiguity for learning was firmly established. Such classic experiments as that of Grice (1948) provided strong evidence that learning with apparent lack of contiguity was practically impossible unless the temporal gap was mediated by a secondary reinforcer. Grice found that if rats were kept in a delay area for 10 seconds before reward they would not learn to correctly choose a white alley which would lead to food reinforcement (obtained in a gray goal box) and to avoid choosing the black alley (which would lead to non-reinforcement in a similar gray goal box). It is as if the trace memory of which alley the rats had previously chosen deteriorated beyond the ten second delay, leaving the rats unable to associate the white alley with reinforcement and the black alley with non-reinforcement. Several rats were able to learn to discriminate between both alleys if the delay period was shortened to 5 seconds, although this took an average of 580 trials. However, if the rats were given distinct and differential endpoints, a black goal box after choosing the black alley, and a white goal box after choosing the white alley, the subjects were able to reach criterion of 18/20 correct choices in an average of 162 trials, even with a 5 second delay. Grice also found that the rats could learn this problem with only the gray goal box, in a five second delay, if different motor responses were required to travel through each choice alley (i.e., blocks in one alley and an incline in the other). Though these animals took many more trials to accurately learn the task (mean of 310) they were much better than the animals that were in the same condition without the addition of different motor responses in each of the choice alleys. Grice accounts for the

results with different colored goal boxes by explaining that the effect of differential goal boxes in bridging the temporal gap is due to secondary reinforcement effects bestowed upon the choice alleys. The stimulus present at the time of reward (white color of the goal box) was thought to acquire secondary reinforcing properties. Thus, upon orienting toward and entering the white alley, the subject received immediate secondary reinforcement that helped to guide its choice of alley.

Using the same paradigm Lieberman, McIntosh and Thomas (1979) carried out a study in which one group of rats was given either a light or a tone while they were in the delay area, regardless of which alley they chose, while another group of rats was not given any additional stimuli while in the delay area. The group of rats given additional stimuli, were found to learn to discriminate between the white and black alleys much better than the group which was given no added stimuli, even after a delay period of two minutes. This suggests that the added stimuli allowed the animal 's memory traces of the previously chosen arm to remain intact, and thus "bridge the gap" of the delay experienced in the delay area. With the added stimuli the animals were able to learn to choose the correct arm 90% of the time while the group without the tone or light during the delay remained at chance levels. The intermediate cues helped to 'mark' the choice response in memory. This would lead the subjects to more effectively 'rehearse' their previous choice. When the reward was given after the two minute delay, the memory of their response was stronger leading to a better association between a correct response and reward. However, this study may also be explained in terms of secondary reinforcement if the stimuli that occurred during the delay took on properties of the reward. This could, perhaps, be a more parsimonious explanation of Leiberman and Macintosh's results.

In an attempt to circumvent the influences of secondary reinforcement that were believed to allow animals to succeed in the Grice (1948) experiment, Lawrence and Homel (1961) conducted an experiment in which they also found that they could overcome a delay of reinforcement (a little over ten seconds) by giving rats different colored goal boxes that signaled, but did not duplicate, the color of the alley it had previously chosen. They did this by alternating the color of the choice alley and the color of the differential goal box. One group (B/W) would get a black goal box with food if they correctly chose the white alley, while they would get a white goal box with no food if they incorrectly chose the black alley. Another group of rats always got a gray goal box no matter which alley they chose, but would be reinforced for choosing the white alley. The B/W group was found to learn in approximately 120 trials while the gray goal box group only learned after approximately 300 trials or not at all. The secondary reinforcement effects of color could not account for these results. The B/W goal box group learned in significantly fewer trials, according to Lawrence and Homel, because the rats thought of the B/W goal boxes as different places. This differentiation aided the rats in discriminating between the correct white alley and the incorrect black alley. The differential endpoints based on which alley the rats had previously chosen, which led either to a black goal box which always contained food, or a white goal box which never contained food, was believed make the problem for the rats one of remembering how to get back to the correct goal and avoid the incorrect goal. This is in accordance with an earlier notion provided by Tolman and Gleitman (1949) that animals can encode stimuli that are intimately associated with instrumental rewards. The gray goal box group, on the other hand, did not have this advantage. For the gray goal box rats there is only one goal

box, regardless of which alley the rats had previously chosen. As this goal box contains food on some of the trials, it may become a positively valenced place to reach. However, as the alternative route (black alley) also leads to the same goal box, the problem for the rats in this group is why the gray goal box contains food on some trials and not on others. This situation was believed to inhibit the solution of the discrimination problem as experiencing reward and non-reward in the same place would create interference with regard to the rats' ability to link its previous choice with a good or bad outcome. However, the actual learning mechanism at work in this experiment remains quite elusive, and one can only speculate as to how the differential goal box group were so successful in learning the task with a 10 second delay of reinforcement.

Putting It All Together – The Task at Hand: In much of the literature reviewed here, rats have shown considerable sophistication in their representations of events and the relations among those events. In some cases detailed theoretical treatments were made available that were able to capture that sophistication. However, our understanding of the 'content' of learning and how learning can 'map' onto performance is still, in some cases, unknown (Rescorla, and Holland, 1982). The experiment by Lawrence and Homel (1961), as a case in point, raises the question of 'what is learned' in a temporally discontiguous task and how it enables certain subjects to improve acquisition in the task. The present study seeks to examine this question by looking at a possible role for the hippocampus, as indicated by the activity of hippocampal place cells, in bridging the temporal gap in a delayed reinforcement experiment akin to that of Lawrence and Homel (1961). If the hippocampus functions as a 'cognitive map' (O'Keefe and Nadel, 1978, how would the hippocampus map various compartments of the apparatus? Would there

be different place fields in the white and black goal boxes, signifying the rats' treatment of them as different places despite their similar physical location? Would similar place fields be found when the animal enters the gray goal box after taking different routes to get there (i.e., white or black choice alleys) and experiences different behavioral outcomes (reward and non-reward) in the same gray goal box? Or, as predicted by Eichenbaum et al (1999), will hippocampal activity reflect sequences of events that serve to link successive events into representations of episodes that are unique in behavioral significance, and remap the goal box according to outcome? Similarly, if the hippocampus codes' current location in relation to memories for previous episodes at those locations, how will hippocampal place cell activity be reflected in the delay area of the apparatus? The main focus of the experiment was to examine the putative effects of experience on the perception of the delay box area, i.e., entering from two alleys differing in color which lead to differential endpoints (black or white goal boxes and reward and non-reward). It is hypothesized that remapping the delay box according to color (black or white) of the choice alley will prevent interference in learning to choose the correct alley that will lead to reward. In other words, if the animal sees the black and white goal boxes as different places, it will remap a constant environment (the delay box) based on the alley it takes to get there and expected outcome (black or white goal box, reward or non-reward). Contrarily, rats that experience reward and non-reward in the same gray goal box, are expected to maintain the same map in the delay box. This could account for the improved black and white discrimination seen by the rats which experienced black and white goal boxes in the Lawrence and Homel (1961) study. Though only remapping the delay box according to alley color or the expected goal box would help the animal

solve the task, the experiment will also examine the influence of side of entry (choosing the left or right alley) on place cell activity in the delay box.

Electrophysiological techniques will also be coupled with measures of the animals' performance, or number of correct choices in order to compare any putative differential mapping strategies with acquisition of the task. This may provide evidence that if the rats in the differential endpoint group remap in the delay box as two different episodes according to the color of the choice alley they used to enter the delay area, or to the expected outcome, they would be more likely to be successful in bridging the temporal gap during the delay period and be more successful in learning the problem. Contrarily, it may also show that experiencing reward and non-reward in the same environment, and having a single map for the delay box, may lead to an impairment in acquisition of the task. This could suggest that having a single map for an environment, regardless of the route taken to get there or the expectation of reward and nonreward, may provide interference in learning an association between response and reward outcome.

Methods for Experiment 1

Subjects:

Twenty-five male Long-Evans rats (Charles River, Montreal, Quebec) weighing 75-110 grams at arrival were used in the experiment. Animals were housed individually in translucent containers (45.5cm x 25.0cm x 20.0 cm high) with wire covers. The rats were maintained at 90% of their body weight (fed Prolab rat 3000, PMI Feeds, Inc., St. Louis, MO) as indicated by the normal growth curve of the rat. Rats were fed ad lib one day a week when they were not being trained. Between experimental sessions, the

animals were housed in a colony room at 20 +/- 1° C, on a 12:12-hr light-dark cycle, with lights on at 8:00 am. Each animal's cage also contained a piece of black PVC tubing with a length of 10.5 cm and an inner diameter of 7.5 cm that served as environmental enrichment. However, this piece of tubing was removed after surgery as to prevent interference with the implanted electrode assembly.

Apparatus:

Experimental sessions were conducted in a maze based on an original design by Lawrence and Homel (1961) (see Figure 1). The entire apparatus was 188 cm in length, with walls 46 cm high. The height of the maze walls was believed to discourage the rats' access to the external visual cues of the recording room. During behavioral training, each rats' cage was placed on a separate table 40 cm in front of the start box (see Figure2). In order to initially locate pyramidal cells, electrodes were lowered while rats were on this 'holding' table. The table measured 48.3 cm x 88.9 x 43.2 cm, stood 24 cm from the floor, and had a surrounding edge 5.1 cm high.

Experimental Room and Illumination:

Light from outside the room was minimized by keeping the blinds closed at all times and covering the windows in the doors and near the ceiling with aluminum foil. Illumination was provided by a 104 cm diameter array of six 25 Watt incandescent bulbs, suspended 213 cm above the floor of the maze. This provided light to the apparatus but left the rest of the room in shadow.

Recording Equipment (Huxter, 1998):

The stereotrode was (see Figure 3) made from a pair of insulated 25 micrometer (uncoated diameter) Teflon coated Platinum-Iridium fine wires (A-M Systems, Inc., Carlsborg, WA) twisted together and used for recording (see A in Figure 3). A single strand of the same wire was used as a reference electrode (see B in Figure 3). A small flame was used to remove approximately 5mm of insulation from the ends of the wire that were to be soldered to gold plated female Amphenol pins (see D in Figure 3) (catalogue no. 19003-02, Fine Science Tools, Inc, Vancouver, B.C.). After soldering the impedance of both reference and stereotrode wires were adjusted by slicing the ends of the wire, that were to be inserted into the brain, with a sharp razor blade until the desired impedance was obtained (0.5 - 1.0 MΩ). The stereotrode and reference wires were then mounted in a glass pipette with a tip pulled to a diameter of approximately .05 mm (see C in Figure 3), such that the tip of the reference electrode and stereotrode extended beyond the endpoint of the pipette tip by about 1.5 mm and 0 .5 mm respectively. The insulation was removed from both ends of a 5.0 cm ground wire (made from 75 micrometer stainless steel wire to permit good attachment to skull screw and a female amphenol pin (see H in Figure 3).

The amphenol pins connected to the stereotrode, reference and ground wires were inserted into an amphenol strip (see E in Figure 3) that was then attached using epoxy to the side of a 3 cc syringe cut to a length of 20 mm (see F in Figure 3). The pins in the strip served as a socket for attaching the FET (Field Effect Transistor) plug during recording. The syringe formed a cylindrical shield large enough to be lowered over the microdrive during surgery. The shield served to protect the wires of the electrode. A

smaller ring of plastic was attached to the shield from the cap of a 26 gauge needle cover (see G in Figure 3), which provided an anchor point, and strain relief for the field effect transistor connection.

The microdrives were constructed from specially modified machine screws (model MX-080-12, Small Parts Inc., Miami Lakes, FL.) with the head of the screw and approximately a quarter of the upper threads ground to a smooth surface, and a new slot made in the bottom of the screw (see I in Figure 3). The smooth head of the screw was coated with a thin layer of petroleum jelly and fixed in dental cement alongside a steel post of the same length, cut from an 18 gauge needle (see J and L in Figure 3). When this had dried the other end of the post and screw were also coated with Vaseline and fixed in dental cement (see K in Figure 3). This was done in order to create a base in which the head of the screw could turn, and a top stage that could be lowered by inserting a jeweler's screwdriver into the slot made in the foot of the screw. As the screw was turned counterclockwise, the top stage was lowered by turning on the threads of the screw and sliding down the smooth post. A brass cylinder (see M in Figure 3) was then attached to the base of the microdrive so that it was parallel to the screw and post, and extended approximately 1.5 mm below the base of the microdrive. This cylinder is approximately the size of the hole in the rats' skull during surgery. The cylinder accommodates the electrode so that when the cylinder touches the surface of the skull the electrode would be protected and there would be enough space between the skull and the base of the microdrive to pour in a stabilizing layer of dental cement. The space between the cylinder and the hole in the skull was also coated with a layer of sterile petroleum jelly that prevents the dental cement from seeping into the hole in the skull and blocking the glass

pipette. The pipette containing the stereotrode was then mounted to the top of the stage of a microdrive so that the tip passed through the brass cylinder and extended an additional 2 mm beyond the end of it. One full turn of the screw lowered the electrode by 0.33 mm. By keeping careful track of the number of turns of the screw, it was possible to measure the depth of the electrode in the brain (CA1 was approximately 2.6 mm, while CA3 was approximately 3.2 mm deep, relative to brain surface; at stereotaxic co-ordinates 3.8mm AP and 2.5mm ML relative to bregma).

Stereotrode and reference signals from the animal's brain traveled via independent unity-gain FETs mounted in the FET plug and a 3.96m length of hearing–aid wire (model VP3, Plastics One, Roanoke, VA) to a mercury swivel (Josef Biela Idea Development) centered 165cm above the floor of the maze. From there the signal from each recording tip of the stereotrode was sent to a separate Grass differential amplifier (RP5107E), where the reference signal was subtracted out, and the differential signal was amplified 20,000 times and band-pass filtered between 600HZ and 3000 Hz. The amplified signals from the FET were then fed into a digital storage oscilliscope (model 400, Gould Electronics, Valley View, OH), an audio analyzer (FHC, Brunswick ME, 04011), and an A/D converter in order to perform data collection analysis on a 386 Pentium processor using Discovery V5.1 (DataWave, Longmont, CO).

The animal's position during the recording sessions, as indicated by a red light emitting diode (LED) attached to the FET plug, was monitored using a video camera (series 3500, Computar) suspended 127cm above the maze and a video tracker (DataWave, Longmont, CO). The Discovery program sampled position 10 times per

second and thus allowed the experimenter to relate the position of the rat to the firing of hippocampal cells.

Cell Identification:

Cells were distinguished primarily via the relative amplitudes of their spikes on the two individual stereotrode wires. The cells were identified and isolated by 'cluster cutting' using Discovery (Brainwave systems). Cluster cutting was based primarily on the spike height and spike width of each spike from the two stereotrode channels using the interactive graphics software of Discovery. Different combinations of the parameter pairs were projected as two-dimensional scatter plots as points derived from single cells tend to form recognizable clusters. Clusters from pyramidal cells usually take an oval shape as opposed to noise and artifacts which tend to form diagonal lines. The spikes within a cluster are enclosed in a polygon drawn by using a computer mouse. The data points were then projected into new two dimensional plots in which the earlier partitions of the data were preserved by color coding the points lying within the polygon boundaries. This process was repeated until a multidimensional set of boundaries was established that best provided separation of spike waveform clusters.

Behavioral Training

Behavioral training in the experiment took place in five phases (see Table 1); Pretraining, correction factor and regular trials, introduction of small barrier to the black choice alley, the removal of the delay box, and the introduction of swinging doors to the choice alleys.

Phase 1 – Pretraining:

In order for the rats to become accustomed to rice as a food reward, they were taken from their cages in the colony room and placed on a Table with a linear metal track (96cm long x 15cm wide x 2.5cm high). The rats were trained to shuttle from one end of the linear track to the other to obtain a rice reward. If the rats would not shuttle, they were removed from the track and put back in their cage. After a brief interval, they were returned to the track. This was repeated for a maximum of three times or until the rat shuttled to the other end of the track and ate the rice. This was done for 12 days at which point all of the rats were shuttling reliably.

Phase2 - Correction Factor and Regular Trials:

The rats were assigned to one of three main groups. Each group was further sub-divided into two sub-groups (see Table 2). One group (Black and white goal box condition) was given both black and white goal boxes according to their previous alley choices (a black goal for choosing the white alley and vice versa). The rats in the second group (Gray goal box condition) were only given one gray goal box regardless of which alley they had previously chosen. The subjects in both groups were only reinforced for entering one of the two alleys. Half of the B/W rats were assigned the white alley as correct (n=5) while the remaining rats were assigned the black alley as correct (n=5). Similarly, half of the rats in the Gray goal box group were assigned the white alley as correct (n=5) while the remaining rats were assigned the black alley as correct (n=5). The third principle group in the study (n=5) experienced no delay in the delay box, and was permitted to enter the goal area immediately after leaving the choice alley. Two of these rats were assigned black as their correct alley while the remaining three were assigned the

white alley as correct. This 'no long delay group' was also given one gray goal box regardless of which alley they had previously chosen.

The rats were individually housed in the colony room on a rack that could hold 5 rats per shelf.

The rats were randomly assigned to a particular condition and were trained in random order. It was believed that this would discourage the rats from using scent cues and force them to rely on other intra-maze cues such as the color of the alleys.

The behavioral training procedure of the present experiment mimics that of Lawrence and Homel (1961). Rats from each condition (B/W or Gray goal box groups) were brought into the experimental room in squads of five. The 5 rats were trained in a random order. Five trials a day were given for each rat with an inter-trial interval of at least 10 minutes. When the rats were placed in the start box of the apparatus, they were placed facing away from the choice alleys (to avoid being pointed in the direction of a particular alley by the experimenter). A choice was defined as the rats' entering an alley and touching (either with its nose or paw) the barrier on the other side of the alley. Once the rat did this, the opposite alley was barred off in case the animal suddenly decided to run back out of the alley and tried to get into the other alley. The appropriate goal box was then inserted into the goal area and reinforcement was distributed in the goal box (before the animal entered) if the correct alley had been chosen. If an animal made a correct choice, the barrier between the choice alleys and the delay area was removed and the rat was held in the delay box for 10 seconds. The entrance to the correct goal box was then raised and the rat was permitted to enter and received a rice reward. The entrance door to the goal box was then lowered and the rat was kept in the goal box for

approximately 30 seconds while the animal ate the rice reward. The experimenter then recorded the animal's alley choice and put the animal back in its home cage. The choice area was then cleaned with warm water in case the previous animal left a urine or boli in the choice alleys, and a new rat was placed in the apparatus. If an animal made an incorrect choice, it was held in the delay compartment for 10 seconds. The entrance door to the incorrect goal box was opened and the animal had 10 seconds to enter. The animal was kept in the incorrect goal box for 30 seconds. The animal was then taken out of the goal box and returned to the start box where it was permitted to make another choice.

If the animal did not enter the incorrect goal box within 10 seconds after the entrance door was opened, it would be returned to the start box where it would make another choice. If an animal did not choose an alley within ten minutes, it was returned to its home cage and another animal was trained. After the other animals had made their 5 correct choices, the previous rat was returned to the start box for another opportunity to choose an alley.

During the first 15 trials, or the first three days of training, a correction factor was applied in which each animal was permitted two errors per trial with the third choice forced to the correct side by blocking off the incorrect alley. Thereafter, the animal would only be forced to the correct alley if 10 incorrect choices had been made during a trial. Criterion for the rats was set at 18 correct choices out of 20.

Phase 3 – Small Barrier:

At trial 136 two small barriers (23cm long X 8cm high X 2cm wide) placed 23cm apart were added to the floor of the black alley. These were added to alter the motor responses required to pass through each alley (i.e., walking through the white alley and

having to jump in and out of the black alley) and to make the differences between the two alleys more salient.

Phase 4 – Delay Removed:

At trial 156, the animals were still not showing signs of coming close to reaching criterion. In order to speed up acquisition of the task, the delay box was removed entirely and the appropriate goal box was placed in the delay box area. However, the animal was delayed in the choice alley for ten seconds.

Phase 5 – Swinging Doors Introduced:

At trial 206, swinging doors were introduced to the entrance areas in front of the choice alleys (43cm high x 13 cm long x 0.76cm thick). The delay box was still removed during this phase of the experiment but the ten-second delay took place in the choice alleys. The swinging doors were similar to the balsa doors used by Lawrence and Homel (1961) that the animal would have to push open in order to gain access to the choice alley and delay area. However, unlike Lawrence and Homel's experiment where the animal would crawl under the doors, the doors in the present study were pushed open from the left side and closed behind the animal (by action of rubber bands attached to the doors). Due to the presence of the barriers in the black alley, the animals would have to push at a higher point on the door (10 cm as opposed to 5cm on the white alley door) in order to get inside the alley.

Surgery:

Three rats from the black correct group were operated on before they met criterion (Subjects 2,7, and 20). The rest of the rats were only operated on after they met criterion. Once the rats met criterion, and weighed approximately 375-400 grams, they

underwent surgical implantation of the stereotrode and microdrive apparatus in order to record single cells from the CA1 and CA3 regions of hippocampus. All surgical procedures were conducted under sterile conditions. Rats were anaesthetized with 1.5 ml / 100g b.w. Avertin, administered i.p. in two doses: an initial 1 ml/100g b.w., followed by a 0.5ml / 100g b.w. dose given 5 minutes after the first injection. The second dose was accompanied by a 0.3 ml i.p. injection of atropine sulphate (0.6mg/ml), to alleviate respiratory problems while the animal was under anesthetic. Approximately ten minutes after the initial dose, and after the animal showed no signs of tail pinch reflex, the head area was shaved and the animal was placed in the stereotaxic apparatus. An incision was made, the skin and fascia were retracted exposing the skull from approximately 3 mm anterior to bregma to approximately 3 mm posterior to lambda.

A hand drill was used to make five small holes to accommodate the jeweler's screws that anchored the dental cement and electrode assembly to the skull. A hole was drilled at co-ordinates 3.8 mm posterior and 2.5 mm lateral to bregma. The dura matter beneath the hole in the skull was then removed using a hooked 26 gauge needle, and the stereotrode was lowered until the brass cylinder attached to the bottom of the microdrive covered the hole in the skull and the tip of the electrode was in the brain. The area between the brass cylinder and the hole in the skull was then coated with a layer of sterile Vaseline in order to keep dental cement from seeping into the hole in the skull and blocking the glass pipette delivering the electrode. At this point, the tip of the electrode was at a depth of approximately 1.5 -2.0 mm below brain surface. The microdrive was then fixed in place using dental cement, and the ground wire was soldered to one of the posterior skull screws after an application of a small amount of 85% orthophosphoric

acid to the screw. The microdrive shield was then lowered over the microdrive and fixed in place using dental cement.

Flowers of sulphur were applied around the incision as a topical antibiotic, and the rat was given a 0.25 ml subcutaneous injection of Chloramphenol (25mg/kg, 10mg/.25ml). The bedding in the rats' home cage was replaced with paper towels`, and acetaminophen in a flavored solution was added as an analgesic to the rats' drinking water, at a rate of 1mg/100g b.w./ml. The rat was then removed from the stereotaxic device, replaced in its home cage, and allowed to recover under a heat lamp. On the following day, the rat was returned to the colony room and given at least four days to fully recover. During this time, the rat had ad lib access to regular food pellets, mash, and rice.

Recording Procedure

After each animal had recovered from surgery, screening and recording from the apparatus was begun. Rats were screened individually in the experimental room. A screening session typically began by hooking the electrode up to the FET plug and placing the animal on the holding table on which the animal was free to move around. While the animal was moving around the screening table, the experimenter listened for any individual burst firing units (complex spikes) with waveforms at least three times as large as the noise level of the recording that may have had a spatial correlate. If no place cells were found on the screening table further screening took place in the experimental apparatus. If no place cells were found the microdrive was lowered in order to place the electrode deeper into the cell layer (of CA1 or CA3 depending on the estimated depth of the electrode). If potential place cells were found, they were marked and separated

according to their firing properties compared across both tips of the recording electrode (cluster cutting) using interactive graphics software (Discovery). Once the cells were separated, the animal was placed in the apparatus in order to find cells that may have had spatial correlates in the delay box or in the goal box (the 10 second delay was reintroduced before entry to the goal box during recording sessions). Recording trials required the animals to be kept in the delay or goal boxes from five to seven minutes. In an attempt to compensate for any adverse effects that this may have had on the choice behavior of the subjects, two different behavioral protocols were put in place for recording place cells in either the delay or goal boxes. The two protocols allowed the experimenter to record the choice behavior of a particular animal and to record cell activity in the delay box when the animal entered from both colored alleys from both directions, and in each of the different colored goal boxes. The protocols also allowed for behavioral trials after each two recording sessions that gave the animal a free choice in which it was not forced through either alley and given no prolonged delay in the delay box. The experimenter would not proceed with the next recording session until the animal made a correct choice.

Delay box:

After screening and cluster cutting on the screening table, the animal was placed in the start box of the maze and allowed to make a choice. Following a choice the animal was then held in the delay area for approximately 5 minutes in order to see if any of the previously marked cells found on the screening table had fields in the delay area. Cells were often re-cut at this point to further differentiate any cells which had similar firing properties. After 5 minutes, the animal was allowed into the appropriate goal box if no

cells or place fields had been found in the delay box. If no place fields were found in the goal box, the electrode was lowered and the screening process was continued the following day

If a place field was found in the delay area, another trial was carried out where the animal was permitted another choice and held in the delay box for the usual ten second delay, allowed into the appropriate goal box, and returned to the holding Table. A correct choice had to be made at this stage before a recording session was begun.

Once a correct choice had been made, the animal was placed back in the start box. After a choice was made the animal was held in the delay area for a recording session of 5-7 minutes. This long delay was necessary in order to record the firing rate of the place cell in its field and to get a good sampling of the cell activity in the rest of the delay area. After two consecutive recording sessions, the animal was permitted to make a choice and was held in the delay area for the usual ten seconds. This was done in order to reduce any detrimental effects the prolonged delay during the recording sessions may have had on the choice behavior. A correct choice was necessary before the commencement of the next pair of recording sessions.

According to the experimental protocol, there were 5 recording sessions in which the animal had to move through the choice alleys in each combination of color and direction with one repeat of the first trial (e.g., White left, Black right, White right, Black left, and White left). Frequently it was necessary to force the animal through the incorrect alley by blocking off the correct alley. However, if the animal did not first try to get into the correct alley (by pawing or biting at the blocked alley) the trial was reported as an incorrect choice.

Delay Box Probe Trials and Rotations:

For one subject (#17), three recording sessions were added that investigated the effect of changing the color of the delay box on the firing rate of place cells. Changing the color of the delay box was accomplished by switching or turning one or all four of the panels in the delay box. In one recording session, only the color of the entrance door was changed. The door was originally gray and was changed to half white (23 cm) and half black (23 cm). In the other two recording sessions, the entrance door to the delay box was still half black and half white while the remaining sides were black, white and uncolored (plain wood).

One recording session was devoted to examining the effect of rotation on the firing rate of place cells that had fields in the delay box. The entire apparatus was first rotated 45 degrees and then 135 degrees relative to its original position. The firing rate of the cells in both of these rotation trials were then compared to the firing rate of the cells in a normal trial.

In the recording sessions involving changes of the color of the delay box and rotation of the apparatus, the usual protocol for recording from the delay box was still in place. There still had to be a minimum of 5 recording sessions after the animal had moved through the choice alleys in each combination of color and direction with a repeat of the first trial. The recording sessions were also of the same duration of 5-7minutes.

Goal Box:

If no place fields were found on the initial screening of the delay box, the experimenter would look for place fields in the goal area once the animal had left the

delay box and had entered the appropriate colored goal box (or both goal boxes in the case of the B/W group). The rat was kept in the goal area for 5 minutes in order to see if any of the previously marked cells had spatial firing correlates. If no place fields were found, the animal was returned to the screening table and the electrode was lowered.

If a place cell was found in the goal box during this initial screening, the animal was returned to the screening table. As in the delay box recording procedure, a correct choice was necessary before a recording session could begin. Once a correct choice had been made, the experimenter prepared for a recording session by moving the apparatus 61 cm in the direction of the South wall (see Figure 2.0) in order to put the goal box in a position directly under the camera (as the camera above the apparatus was in a fixed position).

As in the delay box recording protocol, there had to be 5 recording sessions in the appropriate colored goal boxes after the animal had moved through the choice alleys in each combination of color and direction with a repeat of the first trial (e.g., White left, Black right, White right, Black left, and White left). Again, it was usually necessary to force the animal through the incorrect alley by blocking off the correct alley. However, if the animal did not first try to get into the correct alley (by pawing or biting at the blocked alley) the trial was reported as an incorrect choice.

If a place field had been found in the gray goal box, the experimenter recorded the firing of the cell in its field after the rat had entered the goal box by taking different routes through each of the choice alleys. The rats were kept in the goal box for 5-7 minutes. Unlike delay box recording sessions, there were no prolonged delays in the delay box during goal box recording sessions. Therefore, there was no need to balance

out the effects of prolonged delays by giving the animals extra behavioral trials that allowed a free choice.

Goal Box Probe Trials:

Trials were added during the recording sessions of subjects 5, 18, and 17 that investigated the effect of exposing the animal to a goal box, with which it had had no previous experience, on the firing rate of place cells. For subject 5 the experimenter replaced the black or white goal boxes with the gray goal box to see if changing the color of the goal box would affect the firing of the place cell. For subjects 17 and 18 the experimenter replaced the gray goal box with either the black and white goal boxes. If a change was found in the firing rate of the place cell after changing the color of the goal box, the experimenter verified that the place cell returned to its original firing pattern in the previous familiar goal box.

Data Analysis

Choice Behavior) A mixed analysis of variance (ANOVA) was carried out in order to analyze the number of correct choices made by subjects from each group for each of the different phases of the experiment. The analysis examined the influence of the goal box condition (black and white goal box vs. gray goal box condition), correct alley color (white vs. black), and repeated measures over blocks of trials on behavioral performance. The number of trials in a block was either 10, 15 or 20 trials. This variation was necessary owing to the low number of trials in some phases and owing to the uneven number of trials in some phases. The purpose of analysis by phase was to test for learning in each phase. It was hypothesized that having a black and white goal box (differential endpoint), and having a black alley as correct would lead to more correct

choices. The two groups that did not experience a long delay were not included in this analysis as they were only used to indicate whether or not learning could occur in the absence of a delay.

The total number of correct choices for each subject across phases were also analyzed by dividing the total number of correct choices by the total number of trials and converting the numbers to a percentage of correct choices. This was done as some animals were trained for more trials than others (due to days where animals were recovering from surgeries etc.). A two-way (2 x 3) analysis of variance (ANOVA) was then carried out over all phases. The analysis examined the influence of correct alley color (white vs. black), and the goal box condition (black and white goal box vs. gray goal box vs. no long delay) on the percentage of correct choices across all phases of behavioral training.

Trials to criterion) The number of trials to criterion for subjects in each group were analyzed by a two-way (2 x 3) analysis of variance (ANOVA). The analysis examined the influence of the correct alley color (white vs. black), and the goal box condition (black and white goal box vs. gray goal box vs. no long delay condition) on how many trials it took to reach criterion of 18/20 correct choices. In addition, a two-way (2 x 3) analysis of variance (ANOVA) was carried out to examine the influence of the correct alley color (white vs. black), and the goal box condition (black and white goal box vs. gray goal box vs. no long delay condition) on how many trials it took each subject from each group to reach criterion in the final phase of the experiment.

Place cell analysis) A computer program (created by Huxter, 1998; Appendix A) displayed firing rate maps based on the positions in the maze at which individual cells fired. The area of the apparatus in which the recording was taking place was divided into bins with each bin representing an area on the surface of the apparatus. Each recording session involved the calculation of the number of spikes recorded from a given cell in each bin (S), the amount of time the animal spent in each bin (T), and the firing rate for that cell in each bin (S/T). The resulting firing rate maps from each trial in a particular recording session were then compared to determine the influence of color and direction of choice alley on the firing rate of place cells in both the delay and goal boxes. In addition, the effect of experiencing different colored goal boxes on the firing rate of a place cell was also examined. Statistical analysis was accomplished by examining the firing rate of a particular cell (S/T) recorded in each of the bins that the recording environment was divided into. The firing rate patterns for each trial during a recording session were then compared using a cross correlational analysis.

Correlation coefficients that compared delay box place cell activity after entering the delay box from: 1) Alleys of same direction/same color (e.g., WL and WL); 2) Alleys of same direction different color (WR/BR and WL/BL); 3) Alleys of same color different direction (WR/WL and BR/BL); and 4) Alleys of opposite color and direction (WR/BL and WL/BR) were calculated and tabulated in a correlation matrix where individual correlation coefficients of the place cells' firing rate were separated according to color and position of choice alleys for all subjects regardless of group. The correlation coefficients that compared the firing rate of place cells in the delay box after it had been entered from each of the 4 conditions were then averaged. These averaged correlation

coefficients were analyzed in a two-way (2 X 2) analysis of variance (ANOVA) that was carried out in order to examine any putative differences in the firing rate of the place cells in each recording session based on changes in color (black or white) and side (left or right) of the choice alley used to enter the delay area.

While the previous analysis examined the effect of entering the delay box from alleys of differing side and color using data collected from animals of different groups, two new two-way (2 x 2) analyses of variance were carried out in order to analyze the effect of goal box condition on the firing rate of place cells in the delay box. This was done by separating the averaged correlation coefficients for each subject according to goal box condition (black and white goal box group and gray goal box group). The correlation coefficients comparing place cell activity in the delay box when the rat was about to enter the black and the white goal boxes (only for subjects in the black and white goal box group) were also analyzed by carrying out a one-way analysis of variance (ANOVA). This tested whether or not the color of the expected goal box affected place cells in the delay box.

Correlation coefficients were also averaged for firing rates of place cells found in each goal box. However, these were not used in any further statistical analyses.

Results Experiment 1

Behavioral Results:

Twenty-two out of 25 subjects (84%)in the experiment met the designated criterion of 18 correct choices out of 20 within 235 trials. Of the three rats that did not reach criterion, one (Subject 20, Gray goal box condition and black alley as correct) died before criterion had been reached. The remaining two rats that did not reach criterion

were subject 7 (Gray goal box condition with black alley as correct) and subject 2 (Black white goal box condition with black alley as correct). It should be noted that these two subjects were only one correct choice from criterion (17/20 correct choices) when training had ended (Figure 4.0 shows the performance of all groups through all 235 trials in the experiment). Table 3.0 shows the mean number of correct choices (out of five) for the animals in each of the experimental conditions in each phase of the experiment. In general, having no delay or having a black alley as the correct choice significantly improved the number of correct choices. With regard to the subjects with the white alley as correct, having differential goal boxes appeared to lead to a higher overall percentage of correct choices in comparison to those subjects that had a single gray goal box. Analyses were carried out on each of the behavioral training phases in order to see if experiencing a long delay in the delay box, having differential goal boxes, or having a black alley as correct affected the number of correct choices.

Phase 2) Data from phase 2 were evaluated by a mixed 2 x 2 x 7 analysis of variance (ANOVA) across goal box condition (black and white goal box vs. gray goal box condition), correct alley color (white vs. black), and repeated measures over blocks of trials (first block consisting of 15 trials while the remaining six blocks consisted of 20 trials). There was no significant effect of goal box type. However, there was a significant effect of correct alley color; $F(1, 16) = 55.59$, $p < .05$. There was also a significant block effect; $F(6, 96) = 5.50$, $p < .05$. A significant interaction was found between block and correct alley color; $F(6, 96) = 2.28$, $p < .05$. A linear trend analysis revealed a significant increase in the number of correct choices over blocks of behavioral trials; $F(1, 16) =$

23.15, $p < .05$. A significant linear trend was also found with the animals in both the black and white goal box group and the gray goal box group that had the white alley as correct ($F(1, 8) = 13.52$, $p < .05$) and the black alley as correct ($F(1, 8) = 9.81$, $p < .05$) over blocks of trials (see Table 4.0 for complete F-Table and Table 5.0 for means and standard errors). As suggested by Table 5.0, subjects with the black alley as correct made significantly more correct choices over trials than subjects with the white alley as correct. The findings also indicate that the rats in all groups were improving, with regard to the number of correct choices, throughout phase 2 of the experiment.

Phase 3) Data from phase 3 were evaluated by a mixed 2 x 2 x 2 analysis of variance (ANOVA) across goal box condition (black and white goal box vs. gray goal box condition), correct alley color (white vs. black), and repeated measures over blocks of trials (2 blocks of 10 trials). There was no significant effect of goal box type. However, there was a significant effect of correct alley color; $F(1, 16) = 21.25$, $p < .05$. There was also a significant block effect; $F(1, 16) = 4.79$, $p < .05$ (see Table 6.0 for complete F-Table). As can be seen from Table 7.0 the significant effect of alley reflected more correct choices by the black alley group. The effect of block reflects more correct choices in the second than the first block.

Phase 4) Data from phase 4 were evaluated by a mixed 2 x 2 x 5 analysis of variance (ANOVA) across goal box condition (black and white goal box vs. gray goal box condition), correct alley color (white vs. black), and repeated measures over blocks of trials (5 blocks of 10 trials). There was no significant effect of goal box type. However,

there was a significant effect of correct alley color; $F(1, 15) = 25.50$, $p < .05$. There was also a significant interaction between the goal box condition and the correct alley color; $F(1, 15) = 12.40$, $p < .05$. The gray goal box group with the black alley correct made significantly more correct choices than the gray goal box group with the white alley as correct; $F(1, 15) = 4.76$, $p < .05$. The black and white goal box group with the white alley as correct appeared to make as many correct choices as the two groups with the black alley as correct. There was also a significant block effect; $F(4, 60) = 6.10$, $p < .05$. (See Table 8.0 for complete F-Table). As can be seen from Table 9.0 the effect of block reflects more correct choices over blocks of trials.

Phase 5) After the introduction of swinging doors in the fifth phase of acquisition, the majority of subjects were reliably making correct choices. A mixed analysis of variance did not reveal any significant differences between groups with regard to the number of correct choices. Of the 17 rats that met criterion (excluding the group with no long delay), 16 rats (94%)met criterion in phase 5 of the experiment. The number of trials to criterion (18/20) in phase 5 were analyzed for each rat in each group by a 2 X 2 analysis of variance (ANOVA) across goal box condition (black and white goal box vs. gray goal box condition), and correct alley color (white vs. black). No significant differences were found between the different groups with regard to the number of correct choices to criterion in phase 5 (see Table 10.0 for complete F-Table and Table 11.0 for means). This suggests that the introduction of swinging doors to the choice alleys may have encouraged the rats in each group to make more correct choices and thus reach criterion in the final phase of the experiment.

Trials to criterion) A two-way analysis of variance (ANOVA) (2 X 3) analyzed the overall number of trials to criterion for subjects in all six groups. The analysis examined the influence of the correct alley color (white vs. black), and goal box condition (black and white goal box vs. gray goal box vs. no long delay condition), on how many trials it took to reach criterion. A significant effect of alley color was found; $F(1, 16) = 30.82$, $p < .05$. No significant differences were found between the three groups that had the white alley as correct (black and white goal box, gray goal box, and the no long delay group). However, significant differences were found with regard to the three groups that had the black alley as correct; $F(2, 16) = 14.15$, $p < .05$. The group without the long delay (M=56) met criterion in significantly fewer trials than the black and white goal box group (M=218) and the gray goal box group (M=172); $F(2, 16) = 22.93$, $p < .05$. No significant differences were found between the two black and white goal box groups with the black (M=218) and white (M=217) alleys as correct. The gray goal box group with the black alley as correct (M=172) met criterion in significantly fewer trials than the gray goal box group with the white alley as correct (M=222); $F(1, 16) = 6.34$, $p < .05$. A significant difference was found with regard to the two groups that did not experience a long delay. The group with the black alley as correct (M=56) met criterion in significantly fewer trials than the white alley as correct group (M=213); $F(1, 16) = 38.83$, $p < .05$ (See Table 12 for complete F-Table and Table 13.0 for Table of means). A significant interaction was found between the correct alley color and goal box condition; $F(2, 16) = 12.82$, $p < .05$. These results suggest that having the black alley as correct in the gray and no long delay goal box groups led to significantly fewer trials to criterion when compared to their

white alley as correct counterparts. The two groups in the black and white goal box group met criterion in a similar number of trials regardless of correct alley color.

Correct choices across phases) An overall percentage of the total number of correct choices for each animal was obtained (see Figure 5.0) as some rats had experienced more trials than others (due to recovery from surgery). A two-way analysis of variance (ANOVA) (2 x 3) examined the percentage of correct choices for subjects in each of the 6 groups. The analysis examined the influence of the correct alley color (white vs. black), and goal box condition (black and white goal box vs. gray goal box vs. no long delay condition), on the overall percentage of correct choices. A significant effect of alley color was found; $F(1, 19) = 46.29$, $p < .05$. No significant differences were found between the three groups that had the black alley as correct (black and white goal box, gray goal box, and the no long delay group). However, significant differences were found with regard to the three groups that had the white alley as correct. The group without the long delay (M=59.3%) and the black and white goal box group (M=51.2%) made a significantly greater percentage of correct choices than the gray goal box group (M=39.5%); $F(2, 19) = 5.49$, $p < .05$. A significant effect of goal box condition was also found; $F(2, 19) = 6.71$, $p < .05$. With regard to the black and white goal box group, the group with the black alley correct (M=69.4%) made a significantly higher percentage of correct choices than the group with the white alley as correct (M=51.2%) ; $F(1, 19) = 11.47$, $p < .05$. The gray goal box group with the black alley as correct (M=69.8%) made a significantly greater percentage of correct choice than the gray goal box group with the white alley as correct (M=39.5%); $F(1, 19) = 31.91$, $p < .05$. A significant difference was found with regard to

the two groups that did not experience a long delay. The group with the black alley as correct (M=84.6%) made a significantly higher percentage of correct choices than the group with the white alley as correct (M=59.3%); $F(1, 19) = 10.66$, $p < .05$. There was no significant interaction between correct alley color and goal box condition; $F(2, 19) = 1.29$, $p > .05$ (See Table 14.0 for complete F-Table). As suggested by Table 15.0, the groups with the black alley as correct consistently made a higher percentage of correct choices than their white alley as correct counterparts. The results also imply that when the white alley is correct, experiencing reward and non-reward in two different goal boxes, as in the black and white goal box group, or not experiencing a long delay before reward or non-reward, can lead to a significantly greater percentage of correct choices compared to the gray goal box group that experienced reward and non-reward in the same box.

Electrophysiological Results:

Much of the behavioral data suggests that having a black alley as correct or experiencing reward and non-reward in different goal boxes, such as in the black and white goal box group, can significantly improve behavioral performance. Yet, what does this suggest with regard to how the subjects solved the problem of bridging the temporal gap between the choice point and reward and non-reward? Does the hippocampus distinguish between the different goal boxes? Is the delay box perceived or processed as different places contingent upon where it is entered from or does the expectancy of entering a black or a white goal box or a place of reward or non-reward determine the rat's location? The present hypothesis states that the black and white goal box group would treat the delay box as different places contingent either upon which alley it uses to

enter the delay box (based on color or side) or the color of the expected goal box (black or white place). Would treating the delay box or goal boxes as different places correlate with improved behavioral performance by the black and white goal box group?

The recording setup was used to examine the effects of different entry points (i.e., the black or white choice alleys entered from the left or right side) on the firing rate of place cells in the delay box. This was done by examining the correlation coefficients of the place cell's firing rate when the animal entered the delay box from alleys of the same color and the same side, alleys of different color and the same side, alleys of the same color and different sides, and alleys of different color and different sides. Statistical analysis could reveal if the color of the alley, or the side the alley was on (left or right) was more influential in determining activity of place cells in the delay box. Similarly, analysis could reveal if expecting a black, white, or gray goal box would affect the firing rate of place cells in the delay box by separately examining the place cell activity of animals from each goal box condition. It was also possible to ask if different color goal boxes were seen by the rats as different spaces as hypothesized by Lawrence and Homel (1961), or as the same space, and whether specific training with goal boxes influenced differentiation.

Delay Box: Table 16.0 shows the group information and behavioral performance during recording sessions (in which the delay was re-introduced) for the 6 animals (8 place cells) in which place fields were found in the delay box. All 6 animals had previously met criterion of 18/20 correct choices. The correlation coefficients of the place cells were averaged according to sessions that compared cell activity from the delay box after it had been entered from alleys of the same color and direction , the same direction, the same

color, and opposite colors and directions. Correlation coefficients from multiple cells and multiple days of recording from individual animals were also averaged (see Table 17.0 for averaged correlation coefficients for each animal).

A two-way analysis of variance was carried out to examine the averaged correlation coefficients of all 6 rats from which place cells were found in the delay box. The results of the analysis showed no significant effect of side ($F(1, 5) = .02$, $p > .05$) or color ($F(1, 5) = .02$, $p > .05$) of the choice alley on the firing rate of place cells from 6 rats (8 Place cells): 1) Same color and same side (M=.37); 2) Different color and same side (M=.36); 3) Same color and different side (M=.36); 4) Different color and different side (M=.37) (see Table 18.0 for complete F-Table and Table 19.0 for means and standard errors). These results suggest that the cognitive map remains stable across trials of left/right, black/white alternations (see Figure 7a)

In order to test for a possible effect of goal box condition on the firing rate of place cells in the delay box a second two-way analysis of variance was carried out to examine the averaged correlation coefficients of each of the subjects in the black and white goal box group and the gray goal box group. With regard to the black and white goal box group (n=2), the results of the analysis showed no significant effect of side ($F(1, 1) = 1.35$, $p > .05$) or color ($F(1, 1) = .61$, $p > .05$) of the choice alley on the firing rate of place cells: 1) Same color and same side (M=.42); 2) Different color and same side (M=.36); 3) Same color and different side (M=.34); 4) Different color and different side (M=.37). This suggests that the cognitive map remains stable in the delay box across trials of left/right, black/white alternations (see Figure 7a). With regard to the gray goal box group (n=4), the results of the analysis showed no significant effect of side ($F(1, 3) =$

1.91, $p > .05$) or color ($F(1,3) = .01$, $p > .05$) of the choice alley on the firing rate of place cells: 1) Same color and same side (M=.35); 2) Different color and same side (M=.35); 3) Same color and different side (M=.37); 4) Different color and different side (M=.37) (see Table 20.0 for complete F-Table and Table 21.0 for means and standard errors).

A one-way analysis of variance was carried out to examine the averaged correlation coefficients of each of the 2 subjects in the black and white goal box group in which place cells were found in the delay box. The analysis compared the correlation coefficients of place cell activity when the animal was about to enter the black goal box and the white goal box in order to see if the animal remapped the delay box in expectation of goal boxes of different color. The results of the analysis showed that there were no significant differences with regard to place cell activity when the rat was about to enter the black goal box (M=.34) or the white goal box (M=.34); $F(1, 4) = .0034$ $p > .05$. These findings suggest that the cognitive map in the delay box remains stable regardless of which goal box the animal is about to enter (see Figure 7b).

Figure 6.0 illustrates firing rate maps from 5 recording sessions in the delay box: Trial 1) Black alley on the right; Trial 2) White alley on the left; Trial 3) White alley on the right; Trial 4) Black alley on the left; Trial 5) White alley on the left. The firing rate maps illustrate the firing of two place cells from CA1 in the hippocampus that had adjacent place fields in the delay box of the apparatus. The lighter the color of the rate map, the higher the rate of firing for the place cell. The place fields appear to remain stable regardless of which side the animal enters the delay box from.

Goal Box: Table 22.0 shows the group information and behavioral performance during recording sessions for the 3 animals in which place fields were found in the black or white goal box. All 3 animals had previously met criterion of 18/20 correct choices. Rats with Black/White goal boxes all showed evidence of remapping (as indicated by changes in firing rate or rotation of place field) in accordance with the change of goal box color. Table 23.0 illustrates the averaged correlation coefficients for the firing rates of place cells in each goal box for each subject (ns*=not significantly correlated). Figure 8.0 provides an example of firing rate maps from 4 recording sessions in the black and white goal boxes: Trial 1) Black alley on the left (White goal); Trial 2) White alley on the right (Black goal); Trial 3) Black alley on the right (White goal); Trial4) White alley on the left (Black goal). The firing rate maps illustrate the firing of a place cell from CA1 in the hippocampus that had a place field in the white goal box of the apparatus. The lighter the color of the rate map, the higher the rate of firing for the place cell. These results suggest that the cognitive map changes according to the color of the goal box. In other words, the rat may have treated the white and black goal boxes as different places.

Table 24.0 illustrates the group information and behavioral performance during recording sessions for the 2 animals in which place fields were found in the gray goal box. Both animals had previously met criterion of 18/20 correct choices. These two rats, which had only experienced Gray goal boxes, either were unaffected by goal box color changes (subject 17) or showed a change in place field when exposed to a white goal box (subject 18). Table 25.0 illustrates the averaged correlation coefficients for the firing rates of place cells in the gray goal box. Figure 9.0 illustrates a series of firing rate maps from 7 recording sessions with subject 18: Trial 1) White alley on the left (gray goal box);

Trial 2) Black alley on the right (gray goal box); Trial 3) White alley on the right (gray goal box); Trial 4) White alley on the left (black goal box); Trial 5) White alley on the left (gray goal box); Trial 6) White alley on the right (white goal box); Trial 7) Black alley on the left (gray goal box). The firing rate maps illustrate the activity of a place cell from CA3 in the hippocampus that fired throughout the gray goal box. The lighter the color of the rate map, the higher the rate of firing for the place cell. These results suggest that the cognitive map changes according to the color of the goal box. The rat may have seen the white and gray goal boxes as different places. However, the firing rate of the place cell in the gray and black goal boxes are not significantly different. This may suggest that the rat sees the black and gray goal boxes as more similar than the gray and white goal boxes.

Probe Trials: Several probe trials were carried out with subject 17 to examine the influence of rotating the apparatus (Figure 10.0) and changing the color of the delay box (Figure 11.0) on the firing rate of a place cell in the delay box. Figure 10.0 illustrates firing rate maps from 7 recording sessions in the delay box with subject 17: Trial 1) Black alley on the right; Trial 2) White alley on the left; Trial 3) White alley on the right; Trial 4) Black alley on the left; Trial 5) Black alley on the right (45° counter clockwise rotation); Trial 6) Black alley on the right (135° counter clockwise rotation); Trial 7) White alley on the left (returned to original angle). The firing rate maps illustrate the firing of two place cells from CA1 in the hippocampus that had place fields in the delay box of the apparatus. The lighter the color of the rate map, the higher the rate of firing for the place cell. The place fields appear to remain stable regardless of rotation or from

which way the animal enters the delay box. However, the firing rate of both cells did show a marked increase after the 135° CCW rotation

Figure 11.0 demonstrates firing rate maps from 3 recording sessions in the delay box from subject 17: Trial 1) White alley on the left; Trial 2) Black alley on the right (color of all walls changed); Trial 3) White alley on the left (color of walls returned to normal). The firing rate maps illustrate firing of two place cells from CA1 in the hippocampus that had adjacent place fields in the delay box of the apparatus. The lighter the color of the rate map, the higher the rate of firing for the place cell. The place fields appear to remain stable despite changing the color of all of the walls in the delay box in trial 2. This suggests that color may not be the only determinant of place cell firing in the apparatus. Color may primarily become important in a place only if consistently associated with different outcomes (e.g. reward and non-reward in the goal box).

Discussion – Experiment 1

The present study hypothesized that better performance by subjects in the black and white goal box group, in comparison to those in the gray goal box group, could be accounted for by a remapping process in the delay box. The study proposed a putative role for the hippocampus in bridging the temporal gap between the choice point and reward by 'remapping' the delay box in accordance with the color of the previously chosen alley or expected goal box.

The results of Experiment 1, as well as those of Lawrence and Homel (1961), indicate that experiencing reward and non-reward at differential endpoints (black and white goal boxes) can significantly improve learning over a long delay. Having the black alley as correct and the addition of swinging doors in the final phase of the experiment

also seemed to improve choice performance for animals in experiment 1. Treating the delay box, as well as the different goal boxes, as different places would prevent interference as it would separate the routes that would lead to reward or non-reward. Taking the same route to reward and non-reward, which is believed to be the case with the gray goal box rats, could lead to interference in making the necessary associations in the task and could account for the poor performance of this group in Lawrence and Homel's study and in Experiment 1(gray goal box group with white alley as correct).

Contrary to the hypothesis, place cell activity remained stable in the delay box for both subjects in the black and white goal box group and the gray goal box group. There was no remapping of the delay box. However, remapping did occur in goal boxes of different color.

Group differences during phases of acquisition:

During the first three phases of acquisition (phases 2 to 4) there were three consistent findings: 1) There was never an effect of goal box type; 2) There was always an effect of correct alley color; and 3) There was always a block effect where the number of correct choices would increase significantly over trials. In phase 2 of the experiment, there was a significant interaction between alley color and block effect. This effect reflected the better performance over trials by the rats with the black alley as correct relative to the rats with the white alley as correct.

A significant interaction was found between goal box type and correct alley color in phase 4 of the experiment, in which the gray goal box group with the black alley as correct made significantly more correct choices than subjects in the gray goal box group

with the white alley as correct. In contrast, rats in the black and white goal box group with both the white and black alley as correct were performing at the same level.

Though no differences were found between groups in phase 5 of the experiment, in which swinging doors were introduced to both the black and white alleys, 94% of the subjects that met criterion did so in phase 5. This finding indicates that the swinging doors improved performance.

Rats with the black alley as correct performed better than rats with the white alley as correct:

In terms of performance across trials, subjects that had the black alley as correct made a significantly greater overall percentage of correct choices than the subjects with the white alley as correct. The group that did not experience a long delay in the goal box and had the black alley as correct also met criterion in significantly fewer trials than subjects in any other group. As the subjects in the no long delay condition with the black alley as correct made a greater percentage of correct choices than subjects in the no long delay condition with the white alley as correct, having both the black alley as correct and not experiencing a long delay between choice point and reinforcement were probably necessary for improved behavioral performance.

The better performance of rats that had the black alley as correct may be attributed to a predisposition by naïve rats to have a natural bias for choosing small dark places (Rossi and Reid, 1976). Does this predisposition, as reflected in a preference for black, affect the place cell activity of the rat in the delay box or goal boxes? Alternatively, is the choice so automated that it does not affect place cell activity? Rescorla and Holland (1982) have suggested that under some training conditions instrumental behaviour becomes automatized so as to become independent of its goal. It may be that instrumental responding to the black alley is not determined by anticipation

of a goal and the animals choose the black alley irrespective of reward or non-reward and are merely choosing a preferred brightness that happens to bring them to the correct place.

Having the black alley as correct did not affect the activity of hippocampal place cells in the delay and goal box areas. Place cell activity with the rats that had the black alley as correct behaved similarly to rats that had the white alley as correct. Mapping processes therefore can not account for the improved performance of rats with the black alley as correct.

Black and white goal boxes improve performance:

The results of experiment 1 did, in part, match those of the original experiment of Lawrence and Homel (1961). The subjects in the experiment that had the white alley as correct and were in the black and white goal box group made a significantly greater overall percentage of correct choices than subjects in the gray goal box condition that had the white alley as correct. The observation of improved learning with subjects that experienced reward and non-reward in differential endpoints, black and white goal boxes, may be explained in terms of a learning advantage. It has often been suggested that learning over a long delay can be facilitated by the simple presentation of a salient event in close temporal proximity to a behavioral choice (Lett, 1975; Lieberman, McIntosh & Thomas, 1979; Rawlins, 1985; Revusky, 1971). Though the choice point and reward or non-reward are separated by approximately ten seconds, the perception of the different goal boxes as different places may be considered a significant event that could help bridge the temporal gap. Even after a long ten-second delay, enough elements of the trace memory for the correct alley choice were present so that the salient event of entering two

different places (white or black goal box) caused better rehearsal and thus better memory of the choice response.

Rawlins (1985) proposed that the hippocampus acts as an intermediate-term, high capacity memory buffer, in which items of all kinds are registered and maintained over relatively long periods. Such an intermediate store would allow items to be associated with each other even though they might be presented or spaced widely apart in time; it would also enable simultaneous storage of a number of items, thus enabling lists of stimuli to be maintained. In this regard the hippocampus might function to permit the rehearsal and serve to increase the time that an item (or chosen alley) can be retained in a short-term memory store over a ten second delay. The black and white goal boxes may be seen as different places and serve as significant events which can cue rehearsal of the rats' previous choice.

As predicted, black and white goal boxes were seen as different places while the gray goal box was always seen as the same place regardless of whether reward was present or not or which route was taken to get there.

This finding raises the question that if the black and white goal boxes were seen as different places, was the delay box also seen as different places according to the color of the alley used to enter it or according to the expected goal box that the animal was about to enter? Parsing the delay box into two different places according to the route taken to differential endpoints could be an effective means of bridging the temporal gap between choice point and reward. In contrast, taking the same route to the same gray goal box where the rat sometimes receives reward and sometimes does not, would most likely

lead to interference – which is suggested by the poor choice performance of subjects in the gray goal box group that had the white alley as correct.

Place cell activity was constant in the delay box. The rats in both the black and white goal box group and the gray goal box group did not remap the delay box in accordance to the color of the alley used to enter the delay box or the expected goal box. The better behavioral performance of the black and white goal box group in comparison to the gray goal box group (that had the white alley as correct) can therefore not be attributed to a parsing of the delay box into different routes taken to differential endpoints.

The presence of doors in the choice alleys improves performance:

The sudden improvement in phase 5 of the experiment when swinging doors were introduced, like those used in the original experiment (Lawrence and Homel, 1961), suggests that some aspect of the presence of doors was able to influence performance. The doors may serve to improve performance by 'slowing down' the choice process so that the animals make fewer spurious decisions. An experiment that supports this hypothesis was carried out by Cohen and Laroche (1973) in an experiment where choice behavior was improved by the introduction of doors at the choice point of their apparatus. The rats learned to go to the correct arm for reinforcement in a plus maze with the arm 180 degrees from the start point blocked off so the apparatus was more like a T-maze. The rats were then required to perform a reversal task. The reversal involved starting the rats from the previously unused arm while the old start arm was blocked off. For the rats that had learned a place hypothesis there was no 'real reversal' as the rats would still go

to the same place as defined by extra-maze cues. However, for the rats that had learned a habit or orientation response there would be a reversal of which arm would lead to reinforcement. Hippocampal rats in both groups had trouble with this new reversal task. However, the presence of doors at the choice point were found to eliminate the deficit in the performance of the hippocampal rats in the reversal task. This is in accordance with the theory of behavioral inhibition (Gray, 1982) and the observation that hippocampal animals are prone to perseveration, a condition in which animals continue to respond in certain situations where responding is no longer beneficial or even aversive. According to Gray, hippocampal animals are unable to stop and reevaluate the situation and, in the case of reversal, are 'stuck' in old response patterns. In the present study, the introduction of swinging doors may serve the same role as in Cohen and LaRoche (1973) by forcing the animals to slow down the decision process. The presence of the swinging doors may thus serve to prevent spurious choices with normal animals as well as hippocampal animals, thus improving the rats' likelihood of making a correct choice.

Place cell activity remains stable in the delay box:

Contrary to the experimental hypothesis, firing rate and place fields remained constant in the delay box regardless of which alley was used to enter the delay box, or the expectation of which goal box was about to be entered. Therefore, the cognitive map was constant in the delay box. This observation seems to support O'Keefe and Nadel's (1978) view of the hippocampus as a cognitive map and not as a temporary memory store as suggested by Rawlins (1983), or Eichenbaum (Eichenbaum, Dudchenko, Wood, Shapiro and Tanila, 1999; Otto and Eichenbaum, 1992; Rawlins, 1985; Shapiro and Eichenbaum,

1999; Wallenstein, Eichenbaum and Hasselmo, 1998; Wood, Dudchenko and Eichenbaum, 1999).

Observations of color manipulations in the delay box also suggested color changes in this box did not alter mapping as indexed by place field stability, in contrast to what was typically observed in the goal boxes. When the color of the delay box was altered, either just one wall or the entire box, the place fields would remain stable. This seems to be contrary to what was observed when the animals entered goal boxes of different color. Although the finding was consistent over several trials, the manipulation was only carried out with one rat. The same manipulation would have to be carried out with several animals before any real conclusion could be drawn.

Manipulations were also carried out with regard to the orientation of the apparatus. Place cells were recorded from a single rat while the delay box was rotated 45 and 135 degrees respectively, in relation to the usual apparatus orientation. The firing rate of both cells with adjacent place fields showed marked increases during the 135 degree rotation. It is difficult to speculate how this may have related to the subjects' choice behavior as the animal was already prone to incorrect choices after the reintroduction of the delay. Cheng (cited in Gallistel, 1990) performed an experiment testing the reliance of rats on the geometry of a rectangular test environment when searching for a food reward. Cheng found that when the apparatus was rotated beyond 120 degrees the animals in the experiment were more likely to confuse corners of equal angles when looking for a food cache. Perhaps moving the box in a different direction, or a mismatch between vestibular and extra-maze cues created some confusion for the rat, resulting in the increased firing rate of the two place cells. Again, this manipulation was carried out

with only one rat and further testing with more subjects would be necessary before any reliable conclusions could be drawn.

Remapping occurs in goal boxes of different color:

Black and white goal boxes were seen as different places, as were gray goal boxes and novel white goal boxes. These findings confirm the hypothesis of the present study and of Lawrence and Homel (1961) that the treatment of black and white goal boxes as different places could help improve performance as seen in the greater number of correct choices in the black and white goal boxes of the present study and of Lawrence and Homel (1961).

Place fields in the gray goal box were stable no matter what route was taken, which alley the rat chose, or if there was rice or no rice present. However, gray and black goal boxes were treated as similar. That is to say, the correlation between place cell activity in the black and gray goal boxes was stronger than the correlation between place cell activity in the gray and white goal boxes.

The remapping of goal boxes of different color raise some important questions in terms of what constitutes a place, and the influence of non-geometrical properties such as color on hippocampal place cells. Behavioral outcome has also been found to be an important determinant of hippocampal activity (Otto and Eichenbaum, 1992). It may be that the black and white goal boxes were seen as different places due to behavioral outcome. These results suggest that maybe Eichenbaum has the right idea in suggesting that outcome, or items of behavioral significance such as goal box color may be represented by the hippocampus as opposed to the rats' position on a cognitive map (O'Keefe and Nadel, 1978).

Problems in interpreting experiment 1:

In experiment 1 it was hypothesized that in order to prevent interference in learning a black/white alley discrimination, the rat would remap the delay box according to the color of the alley it had used to enter the delay box or according to the color of the expected goal box that it was about to enter. However, the removal of the delay in experiment 1 in order to improve acquisition may have eliminated the need to remap the delay box. This may have made the task too easy and decreased the need to bridge a temporal gap between the choice point and reward and non-reward. This may have discouraged the hypothesized remapping process in the delay box as measured by hippocampal place cell activity. In addition, the number of place cells found from animals in the black and white goal box group was quite low (n=2). Therefore, a second experiment was carried out with an altered training procedure in order to improve the choice discrimination in acquisition and to maintain use of the delay box.

The second experiment attempted to more closely adhere to elements of the original pretraining procedure of Lawrence and Homel. No subjects with the black alley as correct were included in experiment 2 as the results of experiment 1 suggest black preferences could mask learning as subjects with the black alley as correct may have merely been choosing an alley of preferred brightness that happened to get them to the correct goal box. The swinging doors were also placed in the alleys at the very start of behavioral training in experiment 2 as the late introduction of the doors in experiment 1 appeared to have an effect on the choice behavior and therefore may have also altered the place cell activity in the delay box.

Experiment 2

In Experiment 1 it was hypothesized that to prevent interference in learning a black/white alley discrimination, the rat would remap the delay box according to the color of the alley it had used to enter the delay box or according to the color of the expected goal box that it was about to enter. However, the removal of the delay in experiment 1 in order to improve acquisition may have eliminated the need to remap the delay box. This may have made the task too easy by lessening the need to bridge a temporal gap between the choice point and reward and non-reward. In addition, the number of place cells found from animals in the black and white goal box group was quite low (n=2). Therefore, a second experiment was carried out with an altered training procedure in order to improve the choice discrimination while maintaining use of the delay box. Experiment 2 also focused on the black and white goal box group with the white alley as correct. Due to the improved behavioral performance of the black and white goal box group in experiment 1 and of Lawrence and Homel (1961), this was the group that was hypothesized to be most likely to remap in the delay box. It was again hypothesized that a delay of reinforcement would create a need for an animal to have separate maps in the delay box in order to disentangle the route to the appropriate goal box in accordance with the alley it had just chosen.

The second experiment attempted to more closely adhere to elements of the original pretraining procedure of Lawrence and Homel. No subjects with the black alley as correct were included in experiment 2 as the results of experiment 1 suggest black preferences could promote a black choice solution. In Experiment 1 the pre-training procedure was carried out outside the maze and consisted of shuttling for rice. In

experiment 2, a pre-training procedure took place inside the maze, as in the pre-training procedure of Lawrence and Homel, to habituate the rat to the actual experimental apparatus (see Table 26.0 for the principle differences in method between experiment 1 and 2). In addition, the swinging doors were put in place in the choice alleys at the very start of the experiment.

Methods

Subjects:

Ten male Long-Evans rats (Charles River, Montreal, Quebec) weighing 75-110g at arrival were used in the experiment. Housing and feeding conditions were the same as in experiment one. Rats were maintained at 90% of their body weight and fed ad lib for one day a week when they were not being trained.

Apparatus and experimental room:

The apparatus and experimental room were the same as those described in experiment 1 (Figures 1 and 2). The barrier in the black alley and the swinging balsa doors that were added to the choice alleys in the latter stages of experiment 1 were present at the start of experiment 2.

Behavioral Training

Group assignment:

The ten rats in the experiment were divided into 2 groups: 1) Group 1 (n=7) experienced reward and non-reward in black and white goal boxes (differential endpoints); 2) Group 2 (n=3) experienced reward and non-reward in the same gray goal box. Both groups had the white alley as correct.

Phase 1 – Pretraining:

Pre-training occurred over 6 days and consisted of the subjects being forced through the apparatus with reward on each trial. The first two days of pre-training consisted of encouraging the rats to explore and move through the apparatus. The animals were rewarded in each of the goal boxes appropriate for their group (i.e., black and white or gray). The animal traveled through each color on each side (i.e., white left and black right on the first day and white right and black left on the second day). The door of the alley in which the animal was to be forced through was removed, while the door of the opposite alley was blocked off. Each rat was given 10-15 minutes to shuttle from the start box, through to the goal box (no delay was imposed at this point and the door to the goal box was left open) where the animal always experienced rice reward. The black and white goal box group received rice in both the white and black goal boxes according to the choice alley they had been forced through (i.e., forced through white alley and received rice reward in black goal box) while the gray goal box group received rice reward in the same gray goal box regardless of which alley it had been forced through. This continued on the following two days of pre-training and was carried out for all possible pairings of right/left choices in the black/white choice compartments (i.e., white alley on the right, black alley on the left, white alley on the left, and the black alley on the right).

On the final two days of pretraining, consisting of four trials per day, the swinging doors were placed in the entrance of each of the choice alleys. For the first two trials on each day one door was blocked while the other was left open. The rat was allowed to enter the through the open door, travel through the delay area and experience reward in

the appropriate colored goal box. For the final two trials each day, the swinging door in the alley the rat was moving through was left partially open. For all trials following pretraining, the door was closed and the animal had to push it open in order to gain entry to the choice alley.

Phase 2 – Correction Factor and Regular Trials:

The rats were brought into the experimental room in squads of five. One group of five rats was trained for ten trials (10 correct choices) on alternating days while the remaining five rats were trained for five trials (5 correct choices). In other words, if a group experienced ten trials on one day, they would experience five on the following day. As in the previous experiment, individual rats were trained in a random order. When the rats were placed in the start box of the apparatus, they were placed facing away from the choice alleys. A choice was defined as entering an alley after pushing the swinging door open. Once the rat did this it would be trapped inside the alley. The appropriate goal box was then inserted into the goal area and reinforcement was distributed in the goal box (before the animal entered) if the correct alley had been chosen. When the animal made a correct choice, the barrier between the choice alleys and the delay area was removed and it was held in the delay box for 10 seconds. The entrance to the correct goal box was then raised and the rat was permitted to enter and receive a rice reward (if the choice had been incorrect the rat would receive no rice reward). The entrance door to the goal box was lowered and the rat was kept in the goal box for approximately 30 seconds. Once the rat had spent 30 seconds in the goal box it was taken from the goal box and placed on the holding table for approximately 30 seconds and another trial was begun. Continuing with another trial after a correct choice was made, was unlike the training procedure of

Lawrence and Homel (1961). Once a correct choice had been made Lawrence and Homel would put the subject back in its home cage and another rat would begin a trial. The previous rat would not receive another trial until all other rats had made a correct choice. This would take a minimum of 10 minutes. It was believed that removing this 10 minute period between trials in experiment 2 could improve choice performance while it was maintaining the delay that was critical to the experimental hypothesis.

If the animal made an incorrect choice, it would be removed from the goal box after 30 seconds and returned to the start box so it could make another choice. This was continued until the rat had made a correct choice, at which point the rat was placed on the holding table for 30 seconds and a new trial was begun. After the subject had made 5 correct choices in total (1 correct choice per trial in 5 trials) the rat was placed on the screening table for 30 seconds and returned to its home cage. The choice alleys were then cleaned with warm water, and after trial 75 the experimenter also cleaned the start area of the apparatus. After the choice alleys and the start area were cleaned, a new rat was placed in the apparatus.

As in the procedure of Lawrence and Homel, if the animal did not enter the incorrect goal box within 10 seconds after the entrance door was opened, it was returned to the start box where it would make another choice. If an animal would not choose an alley, it was returned to its home cage and another animal was trained. After the other animals had made their 5 correct choices, the previous rat was returned to the start box for another opportunity to choose an alley.

During the first 15 trials a correction factor was applied in which each animal was permitted two errors per trial with the third choice forced to the correct side by blocking

off the incorrect alley. Thereafter, no restriction was placed on the number of incorrect choices per trial until a correct choice was made. Criterion for the rats was set at 18 correct choices out of 20.

Recording Procedure

After all animals had met criterion (with the exception of subject 27) they were operated on and implanted with the same chronic recording electrode as in experiment 1 and with the same surgery protocol. Following recovery, all animals that had received chronic implantations would have to meet a new criterion of 7 correct choices out of ten before the screening process was begun. This was to guarantee that the subjects' ability to correctly choose an alley had not been compromised by the surgery. The recording procedure was also the same as in experiment 1.

Data Analysis

Choice Behavior) A one-way analysis of variance (ANOVA) was carried out to examine the influence of the goal box condition (black and white goal box vs. gray goal box condition) on the average number of correct choices for each animal from each goal box condition. In addition, a mixed analysis of variance (ANOVA) was carried out in order to analyze the number of correct choices made by subjects from each group over blocks of trials up to trial 175 when the first animal met criterion. The analysis examined the influence of the goal box condition (black and white goal box vs. gray goal box condition) and repeated measures over blocks of trials (consisting of 15 trials in the first block or 20 trials in the remaining 8 blocks) on the number of correct choices for each subject in each goal box condition.

The number of trials to criterion for subjects in each group were analyzed with a one-way analysis of variance (ANOVA). The analysis examined the influence of the goal box condition (black and white goal box vs. gray goal box) on how many trials it took to reach criterion of 18/20 correct choices.

Place cell analysis) The place cell analysis for experiment 2 was the same as experiment.

Results

Behavioral Results:

Nine out of 10 subjects in the experiment reached the designated criterion of 18 correct choices out of 20. Subject 27 (black and white goal box with white alley correct) was the only subject that did not reach criterion. The number of correct trials to criterion ranged from 170 to 260. The acquisition curve in Figure 12.0 illustrates the mean number of correct choices (out of five) for the subjects in each of the two experimental conditions: 1) Black and white goal box condition with white alley as correct; 2) Gray goal box condition with the white alley as correct. There was no significant difference in the mean number of correct choices for both groups though the black and white goal box group did reach criterion in fewer trials. Figure 12.0 also illustrates the marked decrease in the average number of correct choices for the gray goal box group once the start box was wiped down between subjects after trial 75.

Correct choices) A one-way analysis of variance (ANOVA) revealed no significant differences for the mean number of correct choices between the black and white goal box group (n=7) with the white alley as correct (M=2.64) and the gray goal box group (n=3)

with the white alley as correct (M=2.60); $F(1, 8) = .032$, $p > .05$ (see Table 27.0 for complete F-Table and Table 28.0 for means and standard errors).

The number of correct choices to trial 175 were evaluated by a mixed 2 X 9 analysis of variance (ANOVA) across goal box condition (black and white goal box vs. gray goal box condition) and repeated measures over blocks of trials (the first block consisted of 15 trials while the remaining 8 consisted of 20 trials). There was no significant effect of goal box type. However, there was a significant block effect; $F(8, 64) = 6.27$, $p < .05$. There was also a significant interaction between the goal box condition and block effect; $F(8, 64) = 2.19$, $p < .05$ (see Table 29.0 for complete F-Table and Table 30.0 for means and standard errors). The nine blocks of trials were multiplied by the appropriate contrast numbers (Keppel, 1991) and a mixed analysis of variance was carried out between the first and final four blocks of trials revealing a significant linear trend over blocks of trials; $F(1, 8) = 10.85$, $p <.05$. Linear trends were also examined separately for subjects in the black and white goal box group and the gray goal box group by carrying out two separate t-tests comparing the number of correct choices in the first four blocks of trials and the last four blocks of trials. A significant linear trend was found for the black and white goal box group ($t(6) = -4.47$, $p < .05$) but not for the gray goal box group ($t(2) = -1.04$, $p > .05$). The nine blocks of trials were multiplied by the appropriate contrast numbers (Keppel, 1991) and a mixed analysis of variance was carried out between the first five blocks of trials and the final four blocks of trials revealing an overall quadratic trend; $F(1, 8) = 45.34$, $p < .05$. Quadratic trends were also examined separately for subjects in the black and white goal box group and the gray goal box group by carrying out two separate t-tests comparing the number of correct choices

in the first five blocks of trials and the last four blocks of trials. A significant quadratic trend was found for the black and white goal box group ($t(6) = -7.41$, $p < .05$) but not for the gray goal box group ($t(2) = -3.03$, $p > .05$). As suggested by Figure 13.0, the number of correct choices did not increase significantly over the 9 blocks of trials (trial 1 –175) for the subjects in the gray goal box group while the number of correct choices did increase significantly over blocks of trials for the black and white goal box group.

The lack of a significant linear or quadratic trend with the subjects in the gray goal box group may be due to the drop in the number of correct choices after trial 75 when the experimenter started to clean the start box of the apparatus. A two-way (2 x 2) analysis of variance (ANOVA) was carried out to analyze the average number of correct choices for one block of 20 trials before and one block of 20 trials after the cleaning of the start box in trial 75 for subjects in both goal box conditions (black and white goal box condition vs. gray goal box condition). No significant effect of goal box condition or blocks of trials was found. Yet, there was a significant interaction of goal box condition and blocks of trials; $F(1, 8) = 8.64$, $p < .05$. The cleaning of the start box did not appear to interrupt learning for subjects in the black and white goal box group over blocks of trials with regard to the number of correct choices 20 trials before ($M=2.07$) and 20 trials after ($M=2.36$) trial 75. However, as suggested by Figure 14.0, the subjects in the gray goal box condition were affected by the cleaning of the start box as indicated by the mean number of correct choices 20 trials before ($M=3.00$) and 20 trials after ($M=1.25$) trial 75 (see Table 31.0 for complete F-Table and Table 32.0 for means and standard errors). The sharp decline in the number of correct choices for subjects in the gray goal box group after the cleaning of the start box may indicate a reliance on olfactory cues to solve the

discrimination problem. Yet, the sharp contrast between the performance of the gray goal box group and the black and white goal box group after trial 75 could also be due to the small number of subjects (n=3) in the gray goal box group.

Trials to criterion) A one-way analysis of variance (ANOVA) analyzed the number of trials to criterion for subjects in each group. The analysis examined the influence of the goal box condition (black and white goal box vs. gray goal box), on how many trials it took to reach criterion. No significant differences were found for the number of trials to criterion between the black and white goal box (M=221.67) and the gray goal box group (M=244.33) (see Table 33.0 for complete F-Table and Table 34.0 for means and standard errors).

Experiment 2 failed to replicate the results of Lawrence and Homel in that there were no significant differences between the rats in the black and white and gray goal box groups. Subjects in the gray goal box group appeared to perform as well as rats in the black and white goal box group, contrary to the findings of Lawrence and Homel. This may suggest that some aspect of the procedure in experiment 2 enabled the subjects in the gray goal box group to bridge the temporal gap between the choice point and the experience of reward or non-reward in the goal boxes.

Electrophysiology Results:

Experiment 2 focused on the black and white goal box group with the white alley as correct because of the low number of place cells found from this group in experiment 1. Due to the improved behavioral performance of the black and white goal box group in experiment 1 and of Lawrence and Homel (1961), this was the group that was

hypothesized to be the most likely to remap in the delay box. It was again hypothesized that a delay of reinforcement would create a need for an animal to have separate maps in the delay box in order to disentangle the route to the appropriate goal box in accordance with the alley it had just chosen. This was done by comparing the correlation coefficients of the place cell's firing rate, when the animal came from the same alley on the same side, different alleys on the same side, the same alley on different sides, and different alleys on different sides. Statistical analysis could then reveal if the color of the alley or the side the alley was on (left or right) was more influential in determining how the delay box was mapped. In contrast to experiment 1, no analysis was carried out to examine the effect of goal box color or expected endpoint on place cell activity in the delay box as no place cells were obtained from animals in the gray goal box group. Therefore comparison of both groups with regard to place cell activity based on expected goal box color was impossible.

Statistical analysis could also reveal if place fields would either disappear or rotate depending on the color of the goal box that the animal was in. In other words, if different goal boxes would be seen as different places according to properties like color as opposed to their physical space in the room.

Delay Box: Table 35.0 shows the group information and behavioral performance during recording sessions for the 3 animals in which place fields were found in the delay box. All 3 animals had previously met criterion of 18/20 correct choices. The correlation coefficients of the place cells (n=4) found in each animal and for each section (same alley on the same side, different alleys on the same side, the same alley on different sides,

and different alleys on different sides) were averaged, as were multiple cells and multiple days of recording from individual animals (see Table 36.0).

A two-way analysis of variance showed no significant effect of side ($F(1, 2) = .81$, $p > .05$) or color ($F(1, 2) = .77$, $p > .05$) of the choice alley on the firing rate of place cells from 3 rats in the delay box (4 Place cells); 1) Same color (M=.42); 2) Different color (M=.43); 3) Same side (M=.41); 4) Different side (M=.44). Figure15(a) shows the means of the averaged correlation coefficients for the firing rates of place cells in each of the recording sessions. These results suggest that entering the delay box from different sides and through differently colored alleys does not significantly alter the firing rate of the place cell (see Table 37.0 for complete F-Table and Table 38.0 for means and standard errors). This may further suggest that the remapping the delay box may not be necessary in order to bridge the temporal gap between the choice alley and reward.

A one-way analysis of variance was carried out to examine the averaged correlation coefficients of each of the 3 subjects in the black and white goal box group in which place cells were found in the delay box. The analysis compared the correlation coefficients of place cell activity when the animal was about to enter the black goal box and the white goal box in order to see if the animal remapped the delay box in expectation of goal boxes of different color. The results of the analysis showed no significant differences between place cell activity when the rat was about to enter the black goal box (M=.42) or the white goal box (M=.52); $F(1, 11) = 1.46$ $p > .05$ (see Figure 15(b)).

Figure 16.0 illustrates firing rate maps from 5 recording sessions in the delay box with subject 31: Trial 1) White alley on the left; Trial 2) Black alley on the right; Trial 3)

White alley on the right; Trial 4) Black alley on the left; Trial 5) White alley on the left. The firing rate maps illustrate the firing of a place cell from CA1 in the hippocampus that had a place field in the delay box of the apparatus. The lighter the color of the rate map, the higher the rate of firing for the place cell. The place fields appear to remain stable regardless of which way the animal enters the delay box. This suggests that the animal does not remap in the delay box according to the choice alley that it used to enter the delay box These results may also suggest that the animal does not use separate maps in the delay box to bridge the temporal gap between the correct choice alley and reward.

Goal Box: Table 39.0 illustrates the group information and behavioral performance during recording sessions for the 2 animals in which place fields were found in the black and white goal box (subject 26), and the gray goal box (subject 33). Both animals had previously met criterion of 18/20 correct choices. Subject 26, which was in the black/white goal box condition, showed evidence of remapping (as indicated by a rotation of place field) in accordance with the change of goal box color. Subject 33, which was in the gray goal box condition, also showed evidence of remapping when a place field that normally occurred in the gray goal box failed to occur in the white goal box. Tables 40.0 shows the averaged correlation coefficients for the firing rates of place cells in the black and white goal boxes for subject 26 and the averaged correlation coefficients for the firing rates of the place cell in the gray and white goal boxes for subject 33.

Figure 17.0 shows the firing rate maps from 6 recording sessions in the goal box with subject 26: Trial 1) White alley on the right (Black goal); Trial 2) Black alley on the

left (white goal); Trial 3) White alley on the left (Black goal); Trial 4)Black alley on the right (white goal); Trial 5) White alley on the right (Black goal); Trial 6) Black alley on the left (white goal). The firing rate maps illustrate the burst-firing of a place cell from CA3 in the hippocampus that had two different place fields in both the white and black goal boxes of the apparatus. The lighter the color of the rate map, the higher the rate of firing for the place cell. These results suggest that the cognitive map changes according to the color of the goal box. In other words, the rat may have seen the white and black goal boxes as different places. Figure 18.0 illustrates firing rate maps from 6 recording sessions with subject 33: Trial1) White alley on the right (gray goal box); Trial 2) Black alley on the left (gray goal box); Trial 3) White alley on the left (gray goal box); Trial 4) Black alley on the right (gray goal box); Trial 5) White alley on the right (white goal box); Trial 6) White alley on the right (gray goal box). The firing rate maps illustrate the burst-firing of a place cell from CA3 in the hippocampus that fired along the right wall of the gray goal box of the apparatus. The lighter the color of the rate map, the higher the rate of firing for the place cell. The place field disappears in the white goal box and the firing rate of the cell drops markedly. These results suggest that the rat may have treated the gray and white goal boxes as different places.

Discussion

Experiment 2 did not replicate the behavioral results of experiment 1while keeping the delay box intact and training subjects that only had the white alley as correct. It was believed that this would produce a better test of the hypothesis that different goal boxes would improve performance by causing a remapping of the delay box. However, no differences were observed between the black and white goal box group and the gray

goal box group in experiment 2, a finding that is in conflict with the results of experiment 1 and Lawrence and Homel (1961). In accordance with the findings of Experiment 1, place cell activity in the delay box was stable indicating that animals did not remap the delay box according to the initial alley choice. In addition, differential place cell activity was found in black and white goal boxes, indicating a remapping process that correlated with goal box color.

Failure to replicate behavioral results of experiment 1:

The results of Experiment 2 did not replicate those of the original experiment of Lawrence and Homel (1961) or Experiment 1. The subjects in the experiment that had the white alley as correct and were in the black and white goal box group did not make a significantly greater number of correct choices than subjects in the gray goal box condition that had the white alley as correct. Both groups appeared to perform equally as well. Certain elements of the training procedure in experiment 2 may have allowed the animals in the gray goal box condition to perform at the same level as the animals in the black and white goal box condition. Two factors of experiment 2 that may have produced comparable performance in the two groups may have been inter-trial interval and the presence of olfactory cues.

Potential influences of inter-trial interval:

The most obvious difference between the procedures of experiment 1 and experiment 2 was the difference in the inter-trial interval. It was shortened from at least 10 minutes in experiment 1 to approximately 30 seconds in experiment 2. One could

speculate that the shortened inter-trial interval allowed some aspects of the trace memory of reward or non-reward to become associated with olfactory cues in the start box over trials through a process of redintegration (Capaldi, 1967). This means that if stimuli have occurred in a reasonably close temporal proximity in the past, presentation of some of these stimuli will tend to produce recall of the absent stimuli. Thus, the stimuli in the start box, having previously become associated with the stimuli in the goal box, produce recall of whether or not reward was obtained in the goal box on the preceding trial. Hypothetically, this could allow the animal to better remember which alley leads to reward and encourage the rat to choose the correct alley.

Potential influences of olfactory cues in the start box:

The more likely explanation for the performance of the rats in the gray goal box condition is that a combination of scent cues in the start box and a low inter-trial interval somehow helped these rats bridge the temporal gap between the choice point and reward. The abrupt and significant decline in the number of correct choices by the animals in the gray goal box group after the start box was cleaned (at trial 75) is a clear indication of this. It is interesting to note that subjects in the gray goal box condition were the only ones to be affected by this change.

One possible explanation has been proposed by Save, Nerad and Poucet (2000). Their research found that olfactory information can be used to compensate for the lack of visuospatial information. Save et al. discovered this fact by performing an experiment that assessed the effects of removing visual and/or olfactory cues on place field stability. Overall, the results of Save et al. show that place fields were unstable when olfactory

cues were eliminated between recording sessions. In contrast, place fields were stable when olfactory cues were still available. Previous research has suggested that in the absence of visual cues or the removal of external controlling cues, place fields remained stable; presumably through the use of idiothetic cues utilized by the hippocampus (O'Keefe and Speakman, 1987; McNaughton et al, 1996; Quirk, Muller, and Kubie, 1990). Alternatively, these results could be interpreted in terms of the use of background cues or local cues (such as olfactory marks deposited by the rat itself) rather than through the use of path integration. Idiothetic cues alone support place field stability. Instead, place field stability seems to require a combination of olfactory and idiothetic cues (Save et al., 2000).

Perhaps the rats use of idiothetic information alone to get its bearings in the apparatus of the present study would be more difficult than in an environment such as a radial maze that would be richer in terms of the number of available geometrical cues. The apparatus in the present study has few, if any, available cues, apart from black and white goal boxes in one group, which the animal might use to orient itself. Perhaps this lack of visuospatial information caused the animals in the gray goal box group to compensate by relying more heavily on olfactory cues, such as those in the start box, to orient itself before it made a choice between the white or black alleys. The removal of these olfactory cues after trial 75 probably caused the animals in the gray goal box condition to rely on an arbitrary angular frame of reference provided by some other source of spatial information, leading to a significant decline in the number of correct choices. This observation is somewhat consistent with behavioral results showing that rats are able to form a spatial representation based on local olfactory cues (Lavenex and

Schenk, 1995). Yet, to date nothing is known about how a rat manages to discriminate places only on the basis of self-deposited olfactory marks and how it uses them appropriately. Perhaps the most puzzling question is why the animals in the black and white goal box group were not also affected by the cleaning of the start box? One could speculate that the subjects in the black and white goal box group did somehow have an advantage over the gray goal box group. Perhaps the added information these subjects obtained from receiving reward and non-reward in differential black and white endpoints somehow prevented the subjects in the black and white goal box group from an overreliance on scent cues in the start box. Perhaps the black and white goal boxes gave these subjects enough visuospatial information to prevent this overreliance.

The processes underlying the tentative interaction between visual and olfactory cues are not known. One could imagine that reliability of olfactory cues depends on their initial association with some visual reference framework. An interaction between an olfactory-based representation and the path integration system may be necessary to cope with the absence of adequate visual cues and allow the rat to keep track of its position between places with different scent marks (Lavenex and Schenk, 1995; Lavenex and Schenk, 1998). How this complex interaction takes place is unknown, particularly how the rats in the gray goal box group are able to make use of the olfactory cues in the start box while the black and white alleys are randomly switched from left to right. It would be interesting to see how the start box of the apparatus was represented at the place cell level before and after the cleaning had taken place.

Place cell activity remains stable in the delay box:

As in experiment 1, firing rate and fields of place cells again remained constant in the delay box regardless of which alley was used to enter the delay box, or which goal box the rat was about to enter. Remapping did not occur. In accordance with the results of experiment 1, the observation of stable place fields in experiment 2 seems to support O'Keefe and Nadel's (1978) view of the hippocampus as a cognitive map and not as a temporary memory store as suggested by Rawlins (1983) or Eichenbaum (Eichenbaum, Dudchenko, Wood, Shapiro and Tanila, 1999; Otto and Eichenbaum, 1992; Rawlins, 1985; Shapiro and Eichenbaum, 1999; Wallenstein, Eichenbaum and Hasselmo, 1998; Wood, Dudchenko and Eichenbaum, 1999).

Remapping occurs in goal boxes of different color:

Another observation in accordance with experiment 1 was that the black and white goal boxes were again seen as different places. The place cell activity in the gray goal box was also stable regardless of what route or particular choice alley was taken, or if there was rice in the goal box. Gray goal boxes and novel white goal boxes were seen as different places. As black and white, and gray and white goal boxes were in the same physical location in the experimental room, O'Keefe and Nadel's (1978) hypothesis of the hippocampus functioning as a cognitive map appears to fall short in interpreting this finding. The general model of place cell activity, in terms of the cells' representation of Cartesian co-ordinates in a cognitive map, simply suggests that 'place cells are driven by inputs sensitive to the distance and allocentric direction of boundaries ("boundary vectors") in the environment, with several such inputs combining to produce location

specificity' (Hartley et al, 2000, p. 369). However, it is not clear what exactly constitutes a boundary. O'Keefe and Burgess (1996) suggest that walls which impede movement are particularly powerful cues in determining the location of peak response for most place cells while Muller and Kubie (1987) observed that the introduction of a barrier to an environment is sufficient to affect place cell firing patterns. Place fields have also been found to maintain their positions relative to the edges of a raised holding platform as it was moved in a laboratory, suggesting that a drop that impedes movement also constitutes a boundary. The literature concerning inputs that may influence place cell activity generally does not consider non-geometrical properties, such as color of environment, as potential boundaries. In general, boundaries are solely treated as those features of an environment that impede movement. The results of the present study as well as those of Bostock, Muller and Kubie (1991) suggest that the color of relevant cues in the environment may also be sufficient to drive the activity of place cells.

The behavioral results found in experiment 2 when the start area was cleaned, suggest that maybe there could be another explanation for the remapping of the different colored goal boxes. Perhaps color and outcome may not be the only possible determinants of place in the goal box area. Olfactory cues in each goal box may also be powerful determinants of place. After receiving a reward, rats excrete an odor that differs from one they would excrete if they were not rewarded. It may be that these odors make the black and white goal boxes more discriminable. However, if both odors were deposited in the gray goal box, as rats experienced both reward and non-reward in the same gray goal box, the odors could lead to interference (Lavenex and Schenk, 1998).

General Discussion

An overview of experiments 1 and 2:

Did the present study succeed in replicating the findings of Lawrence and Homel (1961)? Experiment 1 did somewhat reflect the findings of Lawrence and Homel, with regard to the greater overall percentage of correct choices being made by the subjects in the black and white goal box group (with the white alley as correct) compared to subjects in the gray goal box group (with the white alley as correct). However, experiment 2 did not replicate the results of Lawrence and Homel. Both the black and white goal box group and the gray goal box group, which both had the white alley as correct, performed at the same level with regard to the number of correct choices. As the experimental hypothesis hinged on the presence of differences in the performance of the two groups, the place cell data found from these animals may not be able to accurately test the proposed hypothesis. Yet, there was one observed difference between both groups in experiment 2. The animals in the gray goal box group began the behavioral trials making more correct choices than the animals in the black and white goal box group. After cleaning the start box and thus removing any potential olfactory cues, the number of correct choices made by the animals in the gray goal box group dropped significantly. One could speculate that the olfactory cues in the start box allowed the rats in the gray goal box group to use some sort of associative chaining mechanism which helped them recall the set of associations that lead to food reward in the goal box. A reliance on scent cues could thus have overcome their lack of visuospatial information. The animals in the black and white goal box group, on the other hand, did not demonstrate an overreliance on scent cues. The rats in the black and white goal box group may have been

compensated by experiencing reward and nonreward in differential black and white goal boxes. Yet, the original experiment of Lawrence and Homel (1961) did not mention in their procedure any type of strict cleaning procedure. Therefore, the same type of olfactory cues must have existed in their experiment as well, although they did not observe the same improved performance with the rats in the gray goal box condition. Certain conditions present in experiment 2 may have made utilization of these olfactory cues possible.

Procedural factors, which may have influenced experimental findings:

A variety of factors seem to be important to generating the outcome observed by Lawrence and Homel (1961). At least two of these factors have become evident in experiments 1 and 2 – inter-trial interval and the presence of swinging doors. In experiment 2, the most obvious procedural difference with Lawrence and Homel (1961) is the shortening of the inter-trial interval from approximately 10 minutes to approximately 30 seconds. Perhaps this inter-trial interval was the critical factor that allowed the gray goal box rats in experiment 2 to use olfactory cues present in the start box. Through the previously mentioned process of redintegration, these olfactory cues may have allowed the gray goal box rats to recall whether or not reward was obtained in the goal box on the preceding trial.

The introduction of swinging doors in experiment 1 also seemed to improve the performance of animals in all of the experimental groups. The presence of the doors may have served to inhibit spurious choices by forcing the animals to delay the choice. This suggests that delaying the animal's choice is beneficial for normal subjects.

Place cell data in both the delay and goal boxes:

The cell data in both experiments was the same. The animals did not remap the delay box according to the chosen alley, or according to the expected goal box. However, as predicted, black and white goal boxes were seen as different places and gray goal boxes were seen as the same place regardless of the presence of rice reward. It appears that both the theories of O'Keefe and Nadel, and Eichenbaum are in part supported by these findings. With regard to hippocampal place cell activity, the present study hypothesized that the delay box would be remapped in accordance with the chosen alley or the expected goal box, a theory more in line with Eichenbaums's theory of hippocampal function as encoding conjunctions of cues according to their temporal order, similarity, or spatial arrangement, as well as relations of cues to their significance and responses made to them; in other words, any relationship worth remembering (Eichenbaum et al., 1999). However, the results of both of the present studies show that the cognitive map in the delay area remains stable; a finding more in keeping with O'Keefe's notion of the hippocampus as a cognitive map, computing the more abstract concept of place or location (O'Keefe, 1999).

In contrast to the findings of place cell activity in the delay box, black and white goal boxes were seen as different places, despite their similar physical location with regard to the distal cues in the experimental environment. This finding is at odds with the cognitive map theory of O'Keefe (1999) and is more in keeping with that of Eichenbaum et al (1999) and the findings of Wible et al (1986) as hippocmapal cell activity correlated more with non-spatial features such as color of the goal box than physical location. It may also be that the behavioral outcome of reward or non-reward over repeated trials

increased the mnemonic significance of goal box color and thus served to differentiate the two goal boxes. However, it is important to note that the presence of reward did not influence place cell activity as the cognitive maps remained stable in the gray goal box if there was rice present or not. Perhaps in earlier behavioral trials rats in the black and white goal box condition valenced the different goal boxes as 'good' or 'bad ' places depending on whether or not reward was present. After prolonged exposure to both goal boxes, and differential outcomes, both the black and white goal boxes became distinct and thus elicited hippocampal remapping.

Bostock, Muller and Kubie (1991) have reported similar findings. Bostock et al recorded from place cells in a cylindrical arena, first with a white cue card, and then with a black cue card, removing the animal from the environment after each session. Both the white and black cards had control over place cell activity as rotation of either card generally caused an equal rotation of place fields. When the black cue card was substituted for the white cue card, place cells showed a time-variant alteration in their spatial firing patterns. During initial exposure of the novel black cue card the spatial firing patterns of the majority of place cells were similar to those observed during trials presenting the white cue card. With more experience in subsequent presentations of the black cue card, the spatial firing patterns associated with the 2 cue cards became distinct from each other. Once the differentiation of firing patterns had occurred in a given rat, all place cells subsequently recorded from the rat had different firing patterns in the presence of the white and black cards. The findings of Bostock et al (1991), as well as the present study, may indicate an experience-dependent modification of place cell firing patterns

mediated in both cases by exposure to salient stimuli – black and white cues in the case of Bostock et al., and black and white goal boxes in the case of the present study.

It would be interesting to examine the factors that made the different goal boxes distinct. Was it the properties of the box – color or smell (Bostock, Muller and Kubie, 1991; Lavenex and Schenk, 1998)? Or was it due to behavioral experience or outcome (Bostock, Muller and Kubie, 1991; Otto and Eichenbaum, 1992; Sharp, Blair, Etkin, and Tzanetos, 1995; Skaggs and McNaughton, 1998)? While recording from animals that had reached criterion in a similar study, the stability of place cells in two different goal boxes with the same color could reveal the importance of olfactory cues in determining place cell activity in the goal box. If the place cells for a particular box remained stable when the rat was exposed to a novel goal box of the same color, then the most obvious determinant of place would be color. Likewise, an experiment could be performed to determine the role of experience in determining place. Place cells could be recorded from the goal box from naive rats that had never been in the goal box. Such a study could determine the level of experience necessary to differentiate the two boxes. Similarly, place cells could be recorded from animals that had been rewarded no matter what alley they had chosen and thus were rewarded in both goal boxes. This could determine if different goal boxes would still be considered different places if there was no difference in behavioral outcome.

The hippocampus and the bridging of temporal gaps:

Apart from remapping in the goal box, both of the present experiments failed to shed light on how the hippocampus might bridge the temporal gap between choice point

and reward vis á vis place cell activity in the delay box. Due to alterations in the experimental procedure in experiment 1 and a failure to find behavioral differences in experiment 2 it is hard to form reliable conclusions regarding place cell activity in the delay box. The observations of both experiments 1 and 2 could make sense in terms of observations of hippocampal activity in other experiments involving delayed reinforcement. These studies have found that the hippocampus is not involved in active processes which maintain a representation of a 'to be remembered' cue, such as an odor or tone, during a memory delay (Foster et al., 1986; Otto and Eichenbaum, 1992). That is to say, hippocampal cells do not behave differently by changing firing rate or firing in different places in order to remember items of behavioral relevance, such as alley choice, over delays. However, these studies examined hippocampal activity in terms of whether firing rates increased preferentially during trials with specific stimulus and response combinations, not in terms of 'place code' as in the present study. Nonetheless, the findings were the same; the hippocampus does not maintain behaviorally relevant task parameters during the delay.

A distinction should be drawn between bridging a delay by active processes (memories maintained by continuously active reverberating neuronal loops) and a reinstantiation of memory after the delay (Otto and Eichenbaum, 1992; Redish, 1999). Differential hippocampal activity in both black and white goal boxes in the present study, in terms of remapping, may assist in recalling the previously chosen alley. It is possible that the differences in place cell activity in the goal box triggered in the rat a recognition that a problem to be solved existed. In the case of experiments 1 and 2 the different color goal boxes experienced by rats in the black and white goal box group indicated that color

was a relevant dimension. This may have caused the rats in the black and white goal box group to be more likely to 'attend' to the colors at the choice point. This may be a potential explanation of the improved performance of the black and white goal box rats in the original experiment of Lawrence and Homel (1961) and experiment 1.

Hippocampal lesions have been found to affect delay tasks or tasks that involve a context-switch, where the animal has to reinstate the context after the delay where a behaviorally relevant cue must be recalled at the time of reward (Redish, 1999). This may be what the hippocampus is doing in terms of the navigating the apparatus of the present study: on reentry into the goal box, the animal must self-localize to find its position in that environment in order to determine its current contextual state. This can also be seen as an instance of 'memory recall'. This recall process could help rats in the black and white goal box group learn the relevance of color in the task and thus improve performance (Redish, 1999).

The cortex and 'active' memory representations:

With regard to 'active' memory representations, data from other studies indicate that neither hippocampal nor parahippocampal neurons sustain stimulus-evoked responses across a memory delay, in contrast to the findings from neocortical areas. Sakurai (1990a; 1990b) observed that hippocampal neurons did not fire preferentially during the delay phase of an auditory-cued continuous delayed non-matching to sample task but that they did fire differentially depending on match or mismatch comparisons. Sakurai (1990a; 1990b) found that cells in the auditory cortex and prefrontal cortex did show signs of maintaining an active representation of the sample cue during the memory

delay. It would be interesting to see if cortical cells would maintain active representations during the delay in the present apparatus that would behave differentially according to which door the rat had used to enter the delay box or according to the expected goal box color. In order for the rat to solve the black/white discrimination over the 10 second delay, there would logically have to be some physiological process that maintained a continuously active memory of the color of the previously chosen alley. Solution of this complex cognitive process may necessitate simultaneous recording of both cortical and hippocampal cell activity.

Concluding remarks:

It may be that the hippocampus does not maintain either active or passive memory representations. Instead, hippocampal activity may reflect processing of the comparison between current and previous cues relevant in remembering behaviorally significant information over a delay, such as which alley had been chosen while getting a reward in the current goal box. Such task parameters would be maintained during the delay in the neocortex and/or parahippocampal areas. The predictive value derived from outcomes of such processing may constitute the hippocampal contribution to memory of items of behavioral significance over a delay (Otto and Eichenbaum, 1992).

The principle aim of the present study was to examine hippocampal place cell activity in terms of solving a complex, inherently non-spatial task. It is hoped that the results of the experiment point out that that it is futile to debate whether or not non-spatial information, such as the outcome of the last trial, reaches the hippocampus. Of course it does. What is important in attempting to unwrap the enigma that is hippocampal function

is to consider exactly what the hippocampus does with these inputs. Sharp, for example, suggests that 'the hippocampus is always signaling non-spatial aspects of the situation, but does so using an obligatory spatial code' (cited in Nadel and Eichenbaum, 1999, pp. 344). Attempting to understand the empirical rules that regulate spatial as well as non-spatial alterations of hippocampal firing fields will enhance our understanding of hippocampal function. The findings of the present study have raised important points about the role of the hippocampus in learning over a delay. Several questions have also been raised throughout the experiment and it is hoped that these can be resolved through further experimentation. Perhaps the eventual decision about the theories of hippocampal function discussed here will be resolved by that old arbitrator, research.

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Figures and Tables

Figure 1.0: The experimental apparatus (Discrimination Box). The first two black bars in the start box represent swinging doors, while the rest represent removable panels. From the gray start box, rats chose between entering either a white or black compartment by pushing through the swinging doors. The position of the white and black choice alleys was changed on a pre-determined quasi-random basis. The back doors of the choice alleys were also removable and could be adjusted when the choice box was rotated between trials. The entrance doors were the same color as the choice compartment (black or white) while the back side of the exit doors were gray, matching the color of the delay area. The rat was then permitted to move to the delay area by removing the rear door of the choice compartment. The rat was held in the delay area for 10 seconds, at which point they were permitted to enter the goal box area (white, black, or gray depending on condition and previous choice) and the doors were reinserted after each transition. The three goal boxes made for the apparatus, white, black and gray, were capable of being quickly inserted into and removed from the goal area.

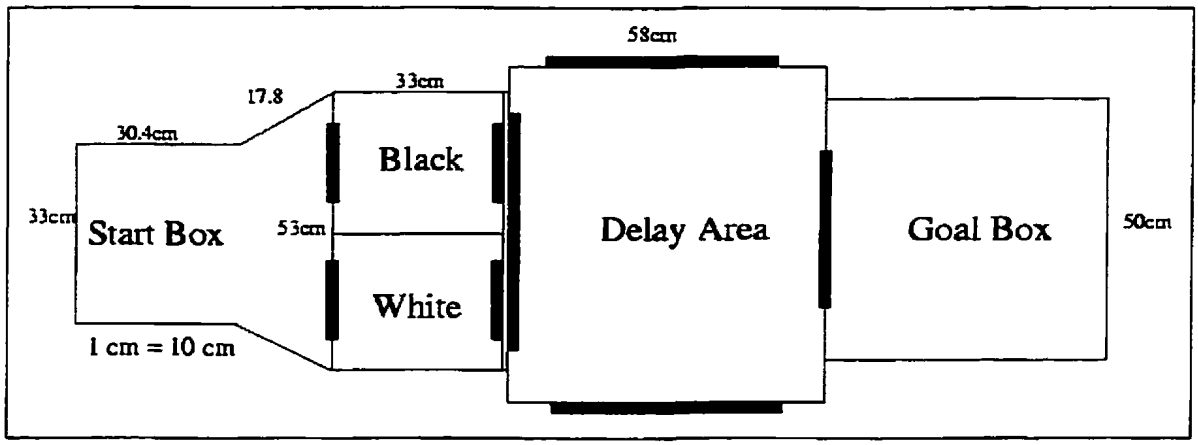


Figure1.0 - Discrimination Box

Figure 2.0: The experimental room. Experimental sessions were conducted in a 5.27m X 4.52m room (Figure 2) with a large window on the west wall, a sink in the northeast corner. Tables and recording equipment in the southwest corner, and an animal holding rack in the southwest corner.

Figure 2.0 - Experimental room

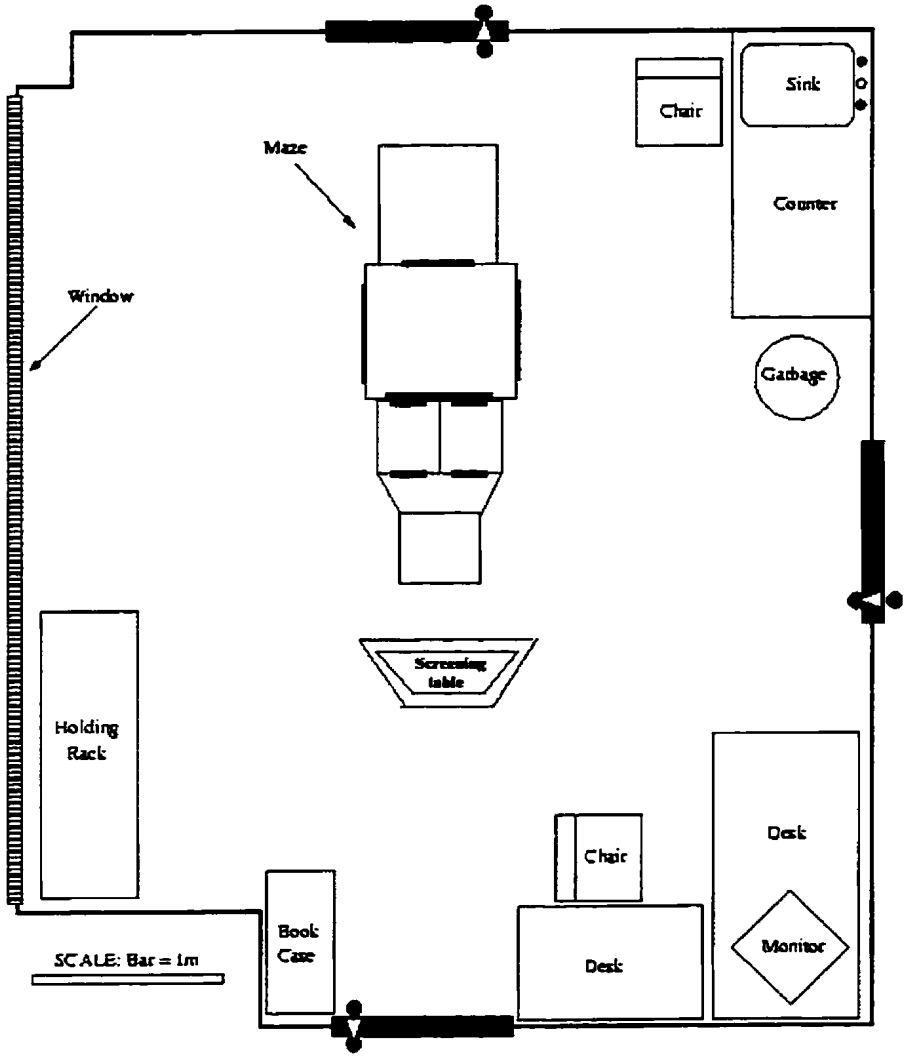


Figure 3.0: The microdrive and electrode: A) Stereotrode; B) Reference electrode; C) Glass pipette; D) Stereotrode and reference wires soldered to female amphenol pins; E) FET socket; F) Microdrive shield; G) FET anchor; H) Ground wire and skull screw; I) Machined screw used in microdrive; J) Microdrive post; K) Top part of microdrive; L) Base of microdrive; M)Brass cannula

Figure 3.0 - Stereotrode recording apparatus

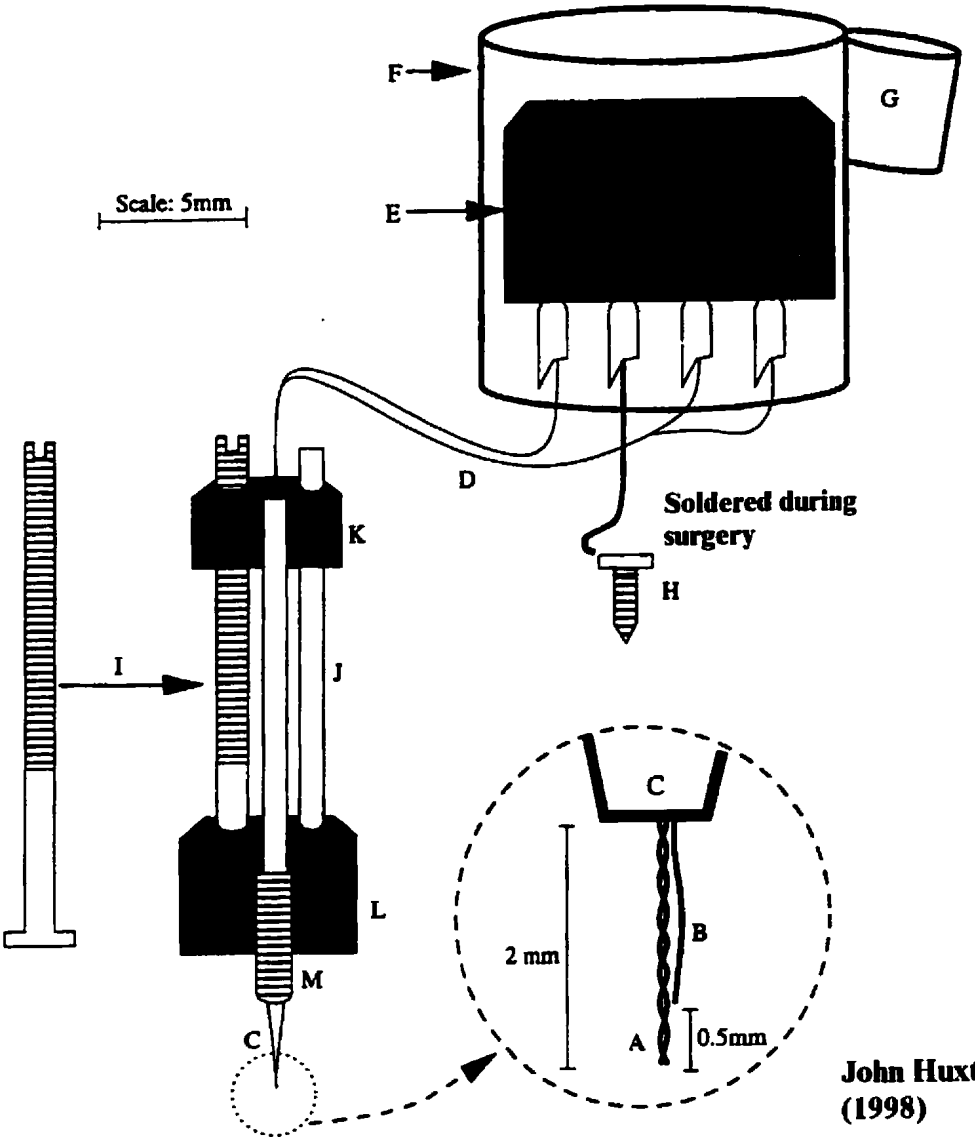


Figure 4.0: The Acquisition curve (trials 5 to 235) illustrates the mean number of correct choices out of five trials for the subjects in each of the six experimental conditions: 1) Black and white goal box group. A) Black alley as correct alley (n=5). B) White alley as correct alley (n=5); 2) Gray goal box group. C) Black alley as correct (n=5). D) White alley as correct (n=5); 3) No delay group. E) Black alley as correct (n=2). F) White alley as correct (n=3).

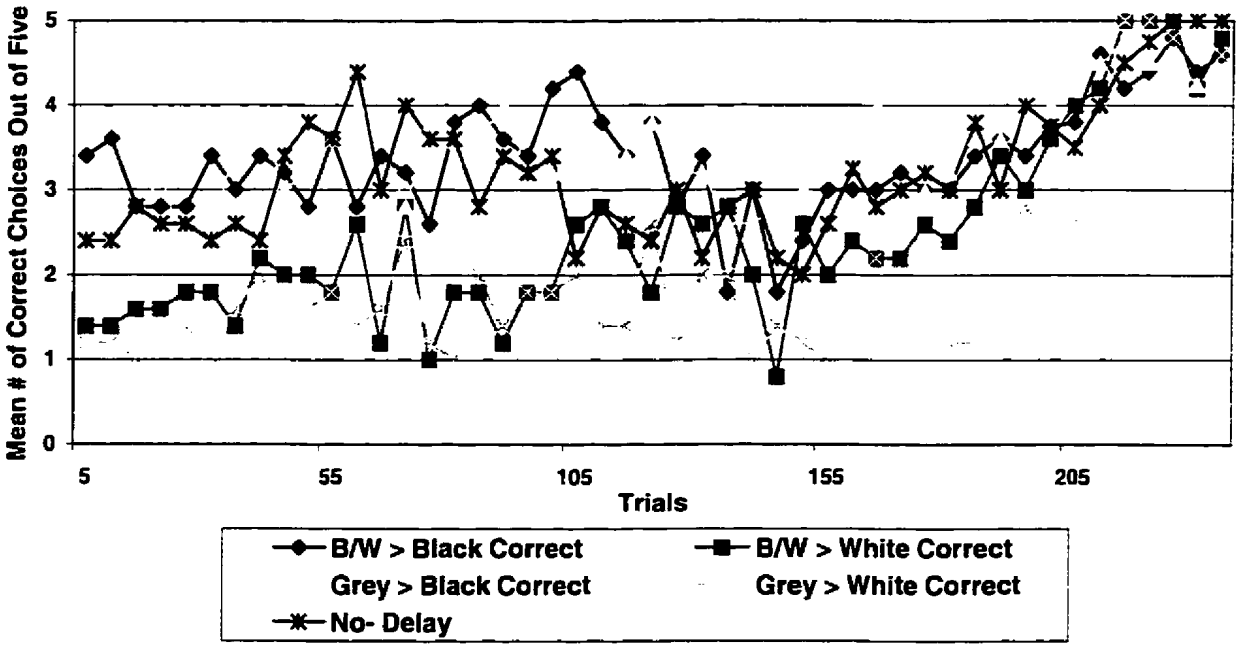
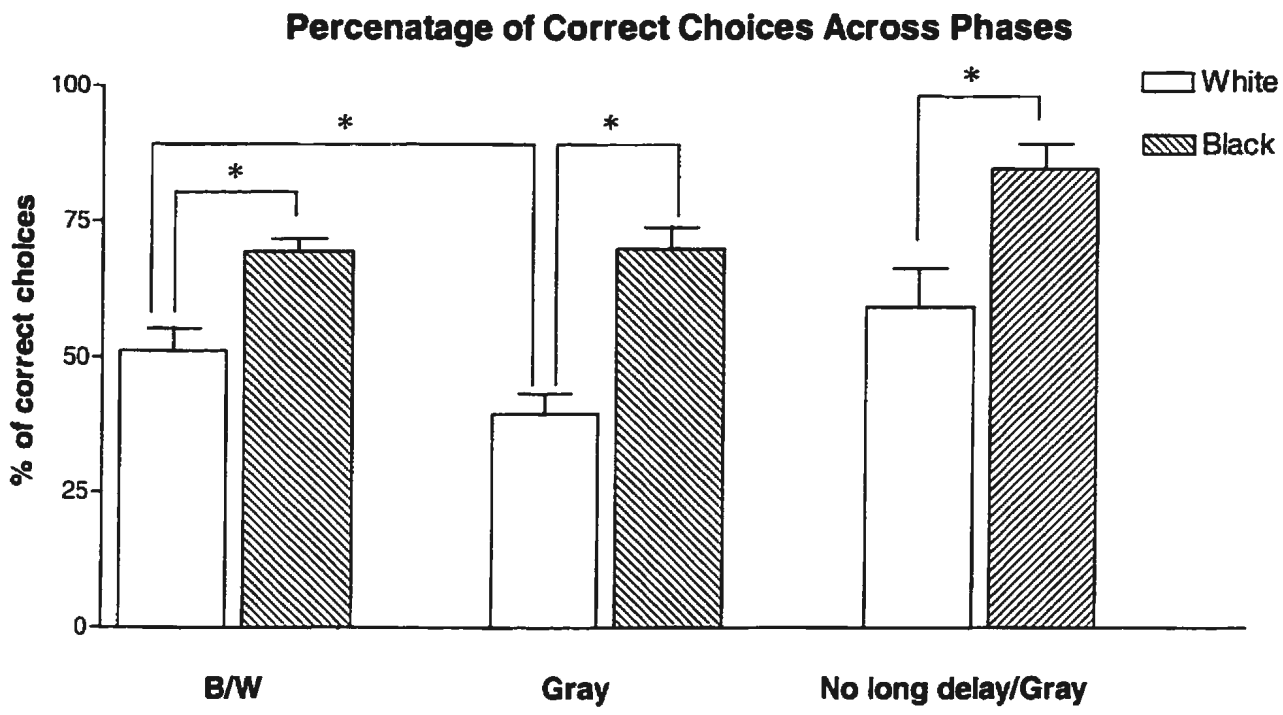

Mean # of Correct Choices Out of Five
0
1
2
3
4
5
5
55
105
155
205
Trials
B/W > Black Correct
B/W > White Correct
Grey > Black Correct
Grey > White Correct
No- Delay

Figure 4.0

Figure 5.0: An examination of the percentage of correct choices for each group revealed a significant difference between groups ($p < .05$). The groups having the black alley as correct and the group that had no delay made more correct choices than the groups that had the white alley as correct. The group that experienced differential endpoints and had the white alley as correct (the black/white goal box group) made a significantly greater percentage of correct choices across phases than the group that only experienced the gray goal box ($p < .05$).



* = Significant differences

Figure 5.0

Figure 6.0: Two adjacent place fields from Subject 17. The firing rate and location of firing remain stable across trials. This suggests that Subject 17 did not remap the delay box area according to the side or color of the alley that it came from.

Figure 6.0

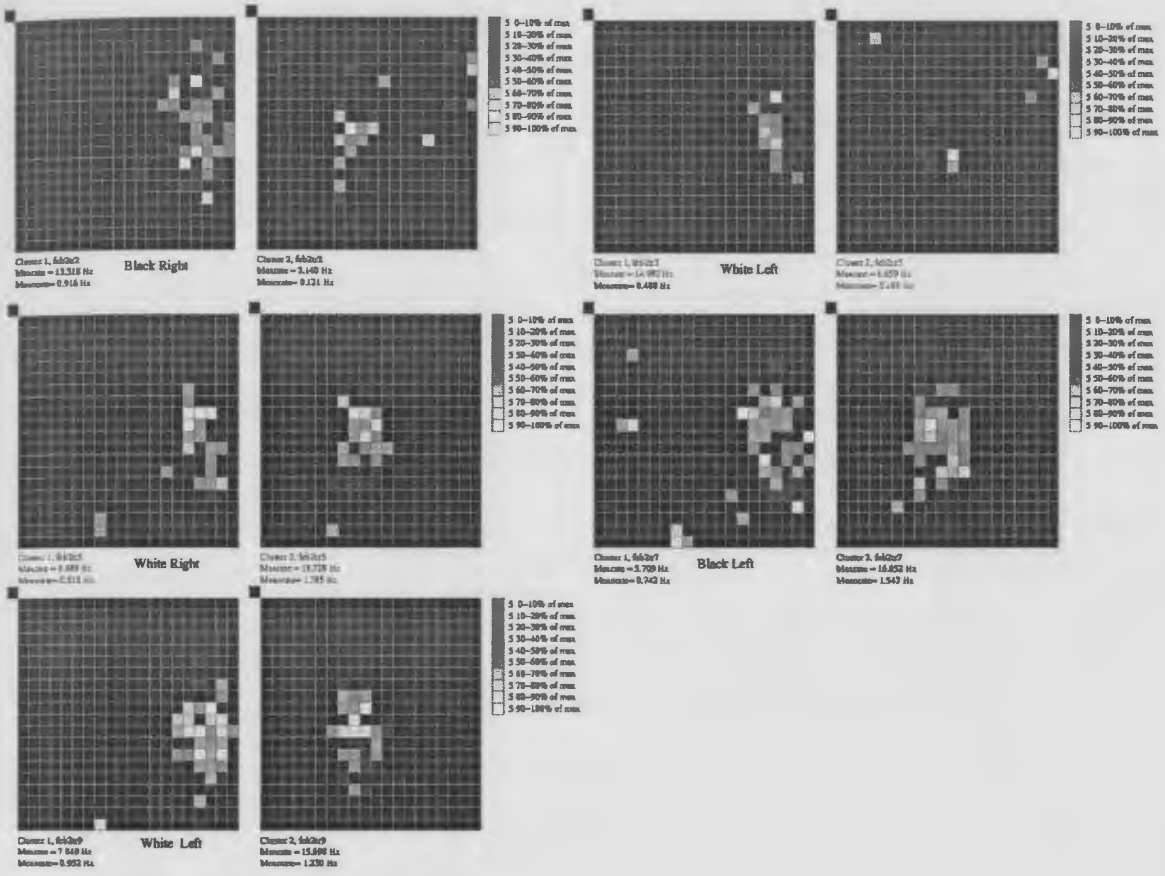


Figure 7(a): The averaged correlation coefficients for the firing rates of place cells in each of the recording sessions suggest that entering the delay box from different sides and through differently colored alleys does not alter the firing rate of the place cell. This may further suggest that the cognitive map remains stable in the delay box despite entering it through different routes that would lead to areas of reward and non-reward.

Figure 7(a) Mean correlation of firing rate for place cells found in the delay box in Experiment 1

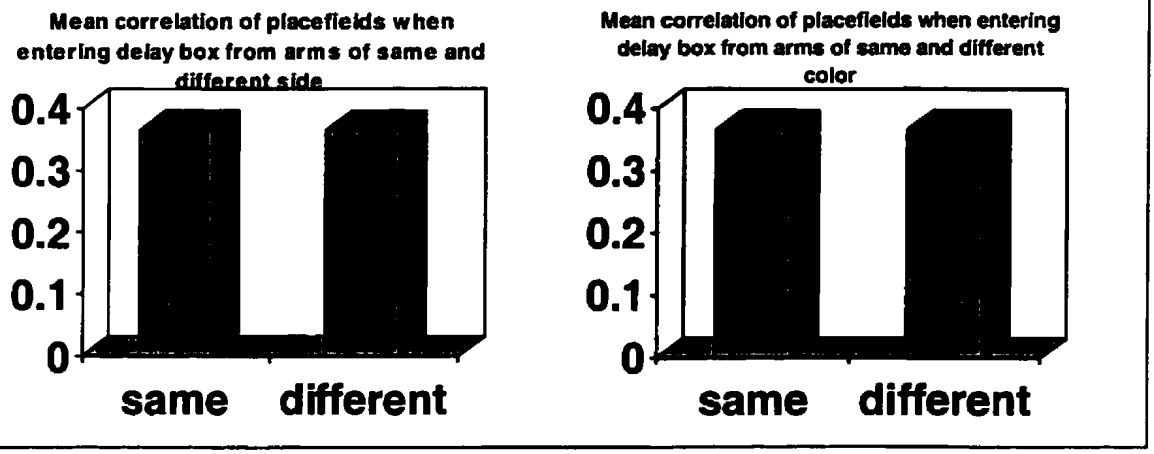


Figure 7(b): The averaged correlation coefficients for the firing rates of place cells in the delay box before the rat enters the black goal box or the white goal box. This may suggest that the cognitive map remains stable in the delay box despite entering it through different routes that would lead to areas of reward and non-reward (the black and white goal boxes). In addition, the results may suggest that 'expectation' of a black or white goal box does not influence the cognitive map in the delay box.

Figure 7(b)

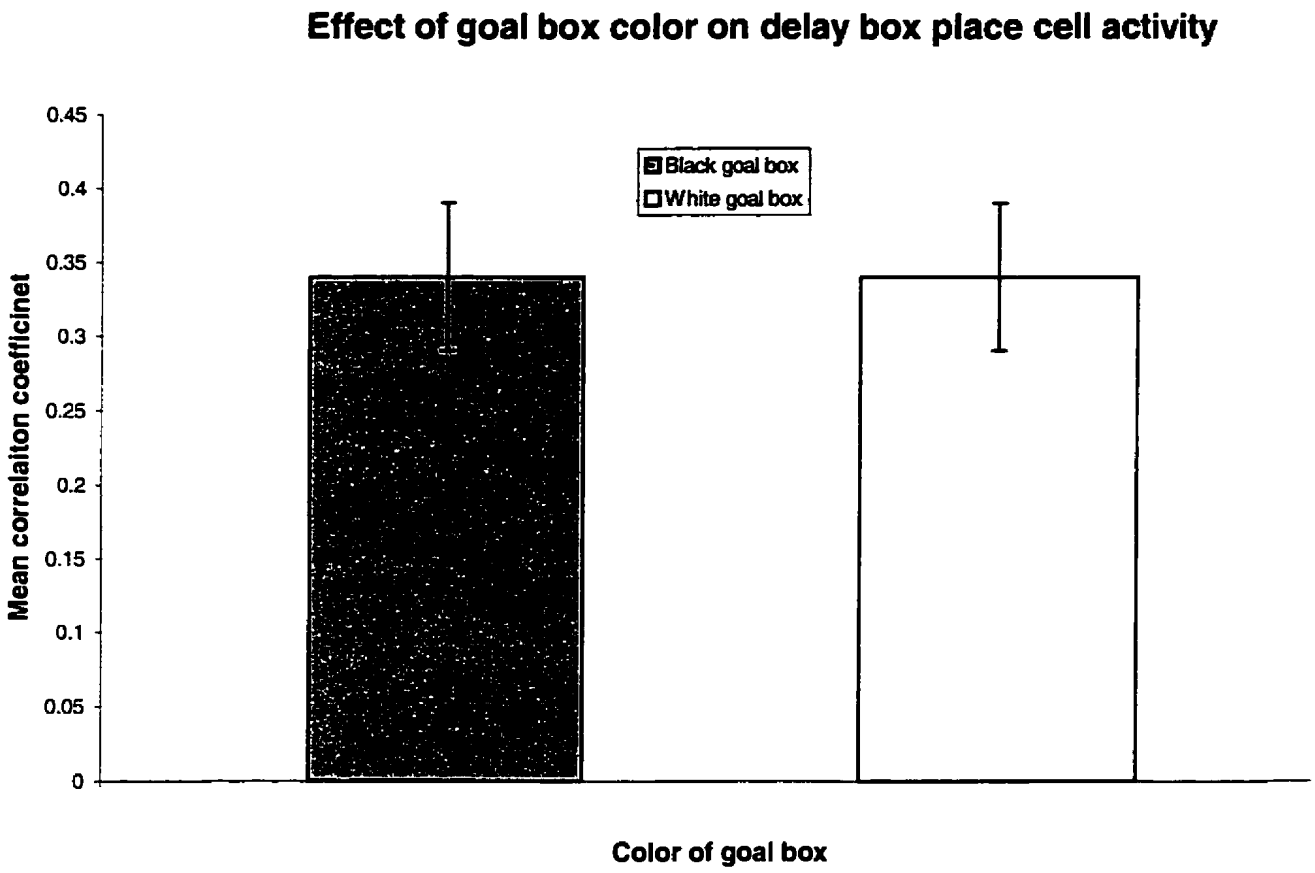
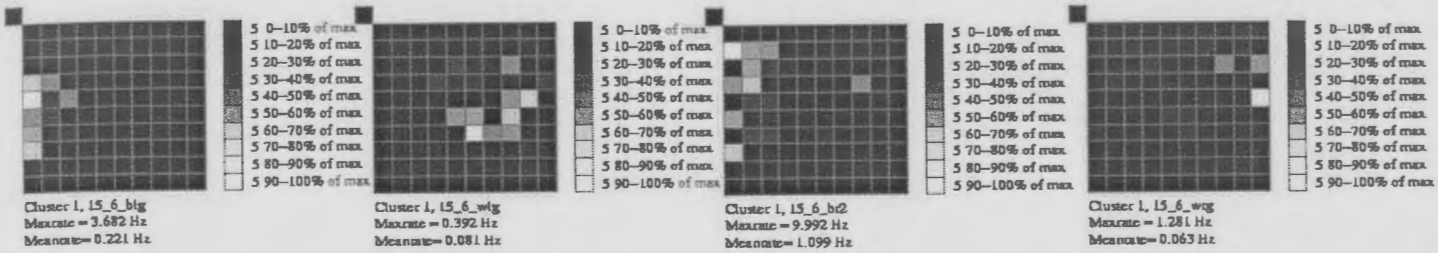

Effect of goal box color on delay box place cell activity
Mean correlaiton coefficinet
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Black goal box
White goal box
Color of goal box

Figure 8.0: A place cell that has a field in the white goal box but does not fire significantly in the black goal box. This suggests that the hippocampus processes the different colored goal boxes differently.

Figure 8.0



White goal box **Black goal box** **White goal box** **Black goal box**

Figure 9.0: Firing rate maps that demonstrate a place cell in the gray goal box with subject 18. The firing rate drops off significantly when the animal is in the white goal box. This suggests that remapping occurs in the white goal box. Yet, the firing rate does not drop off significantly in the black goal box suggesting that the black and gray goal boxes are more alike than the gray and white goal boxes.

Figure 9.0

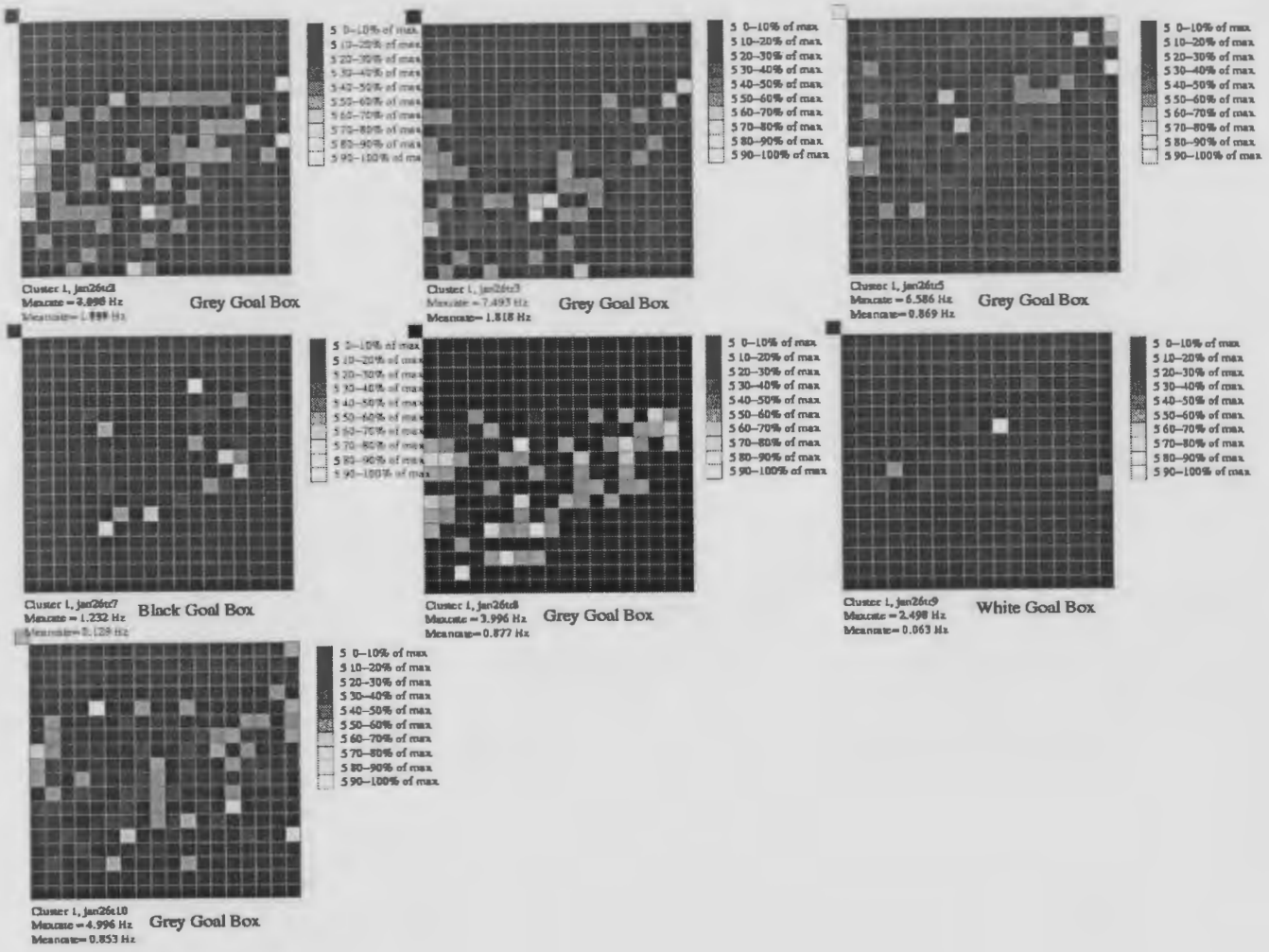

Grey Goal Box
Grey Goal Box
Grey Goal Box
Black Goal Box
Grey Goal Box
White Goal Box
Grey Goal Box
5 0–10% of max
5 10–20% of max
5 20–30% of max
5 30–40% of max
5 40–50% of max
5 50–60% of max
5 60–70% of max
5 70–80% of max
5 80–90% of max
5 90–100% of max

Figure 10.0:. The place fields of both place cells remain stable in the delay box across trials regardless of which colored alley or which side subject 17 came from. The place fields also remain stbale when the apparatus is in a rotated location. However, the firing rate of both place cells showed a marked increase during the 135° rotation.

Figure 10.0:

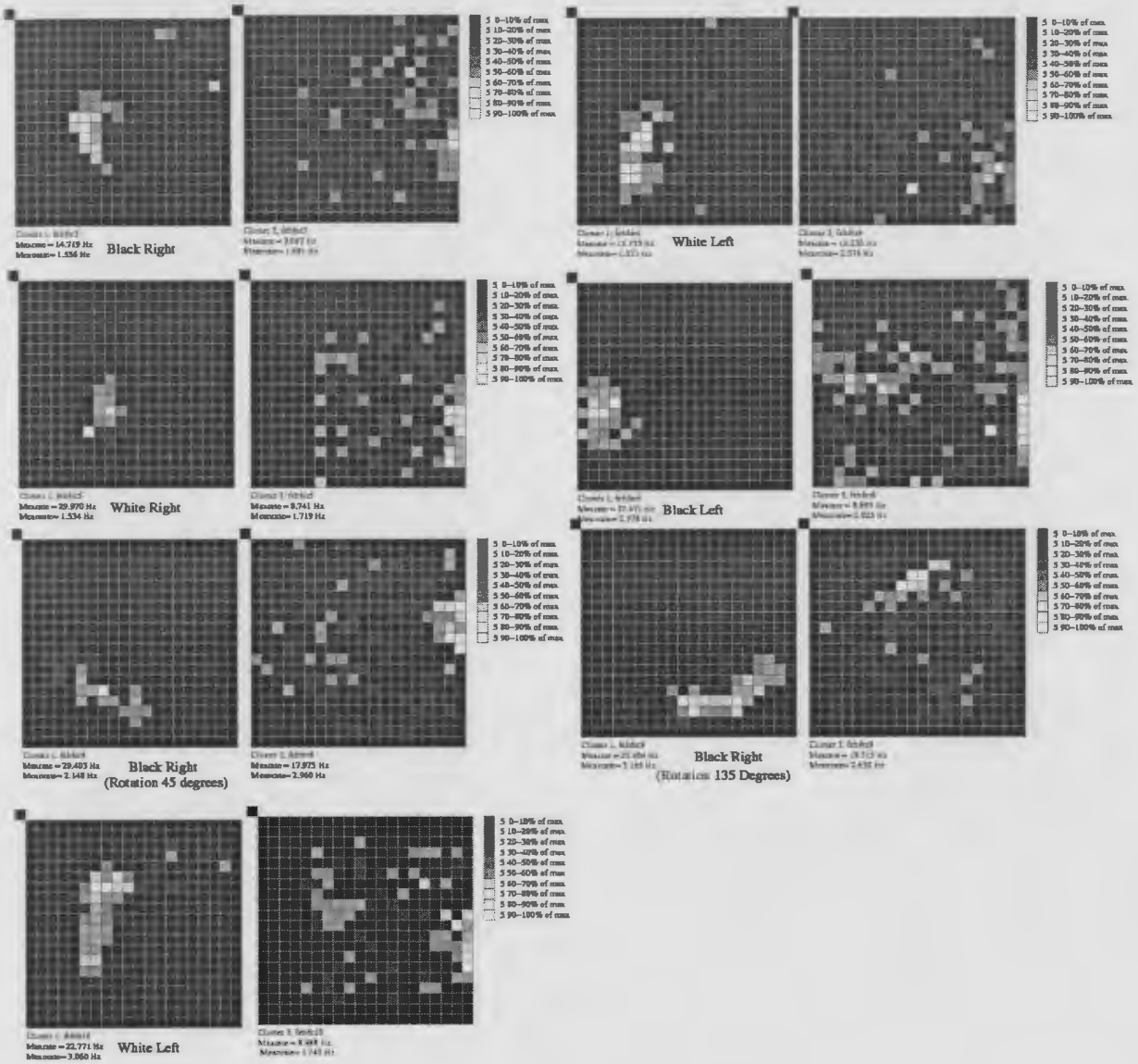
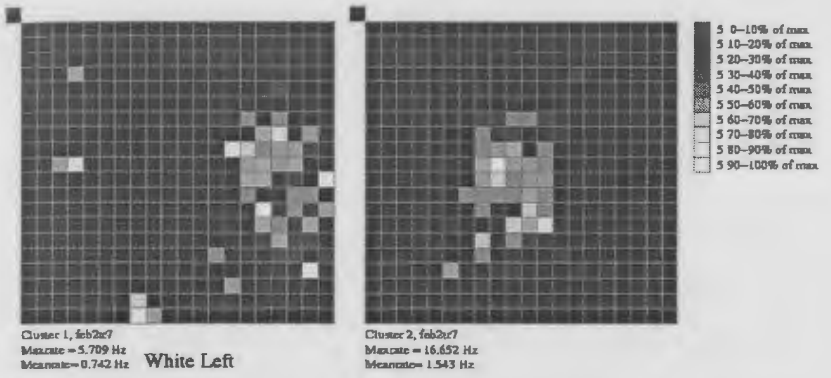
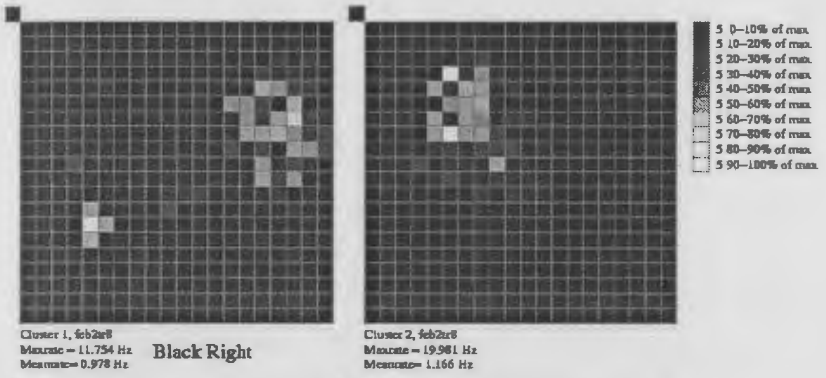

Black Right
White Left
White Right
Black Left
Black Right
(Rotation 45 degrees)
Black Right
(Rotation 135 Degrees)
White Left

Figure 11.0: Firing rate and location of firing of two place cells remain stable after altering the color of the walls in the delay box. This suggests that color may not alter place representations in all boxes in the apparatus.

Figure 11.0

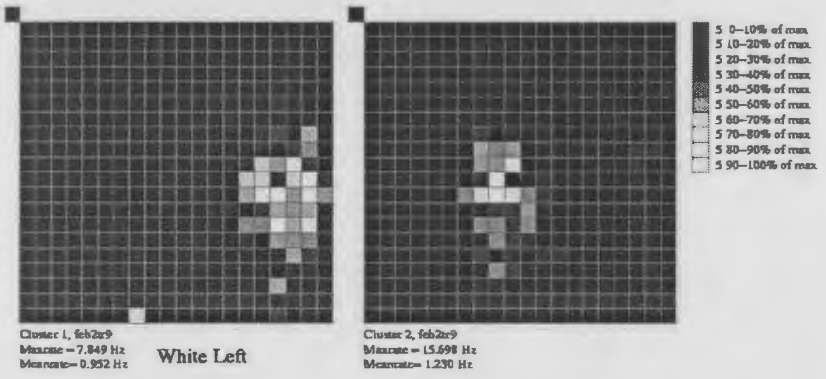


White Left



Black Right

Color of all doors were changed



White Left

Figure 12.0: Acquisition curve for the mean number of correct choices out of five trials for each animal in each experimental condition. No significant differences were found for the mean number of correct choices for each group. However, the 6 animals in the black and white goal box group that did reach criterion did so in fewer trials than the 3 animals in the gray goal box group. The fact that the rats in the gray goal box groups were able to perform as well as the rats in the black and white goal box group is contrary to the findings of Lawrence and Homel.

Figure 12.0

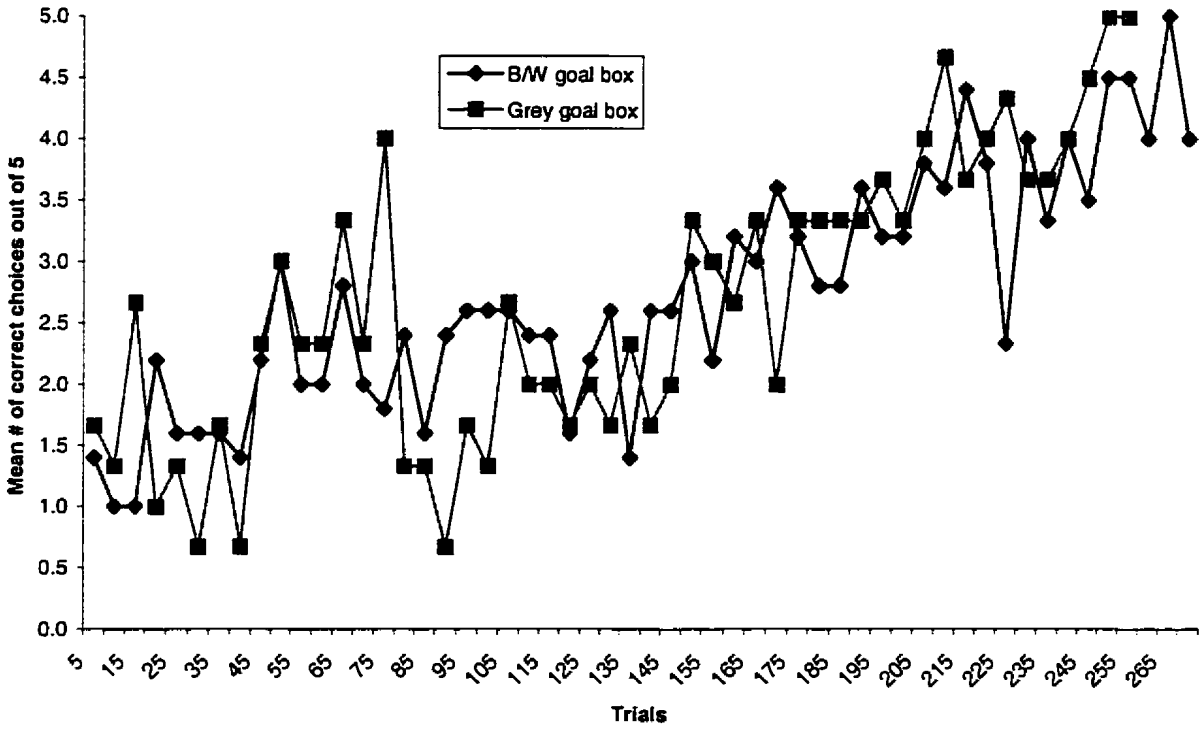

B/W goal box
Grey goal box
Mean # of correct choices out of 5
Trials
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5
15
25
35
45
55
65
75
85
95
105
115
125
135
145
155
165
175
185
195
205
215
225
235
245
255
265

Figure 13.0: Acquisition curve for the mean number of correct choices out of five trials for reach animal in each experimental condition until trial 175 at which point the first animal met criterion. No significant effect of goal box type. However, significant differences were found with regard to the number of correct choices made over blocks of trials. A significant interaction was also found between the goal box condition and block effect. When analyzed separately, linear and quadratic trends were found for the subjects in the black and white goal box group but not for subjects in the gray goal box group. The lack of a linear or quadratic trend for the gray goal box group may be due to the effect of cleaning the start box at trial 75. Yet, it may also be due to the small number of subjects (n=3) in the gray goal box group.

Figure 13.0

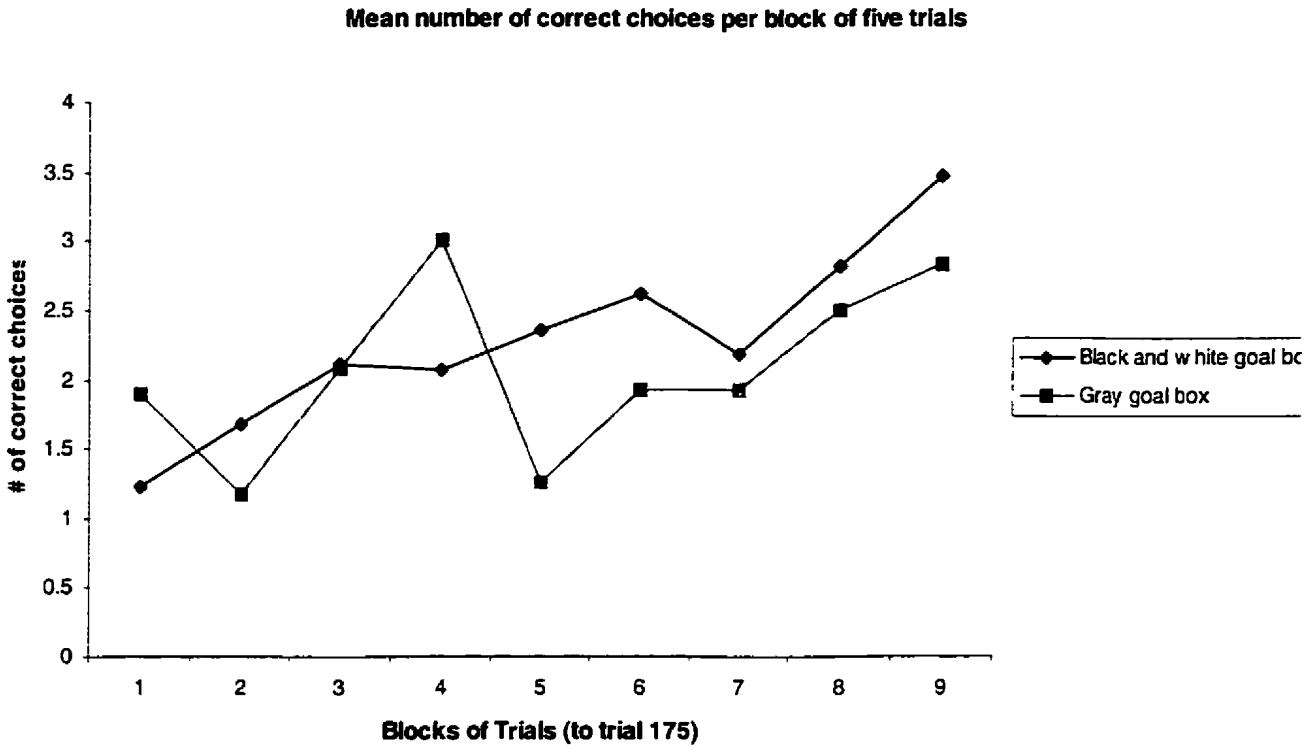

Mean number of correct choices per block of five trials
# of correct choices
4
3.5
3
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
7
8
9
Blocks of Trials (to trial 175)
Black and white goal box
Gray goal box

Figure 14.0: Mean number of correct choices 20 trials before and 20 trials after cleaning the start box of the apparatus for subjects in both groups. The results of the analysis suggest that subjects in the gray goal box group were affected by the cleaning of the start box.

Figure 14.0

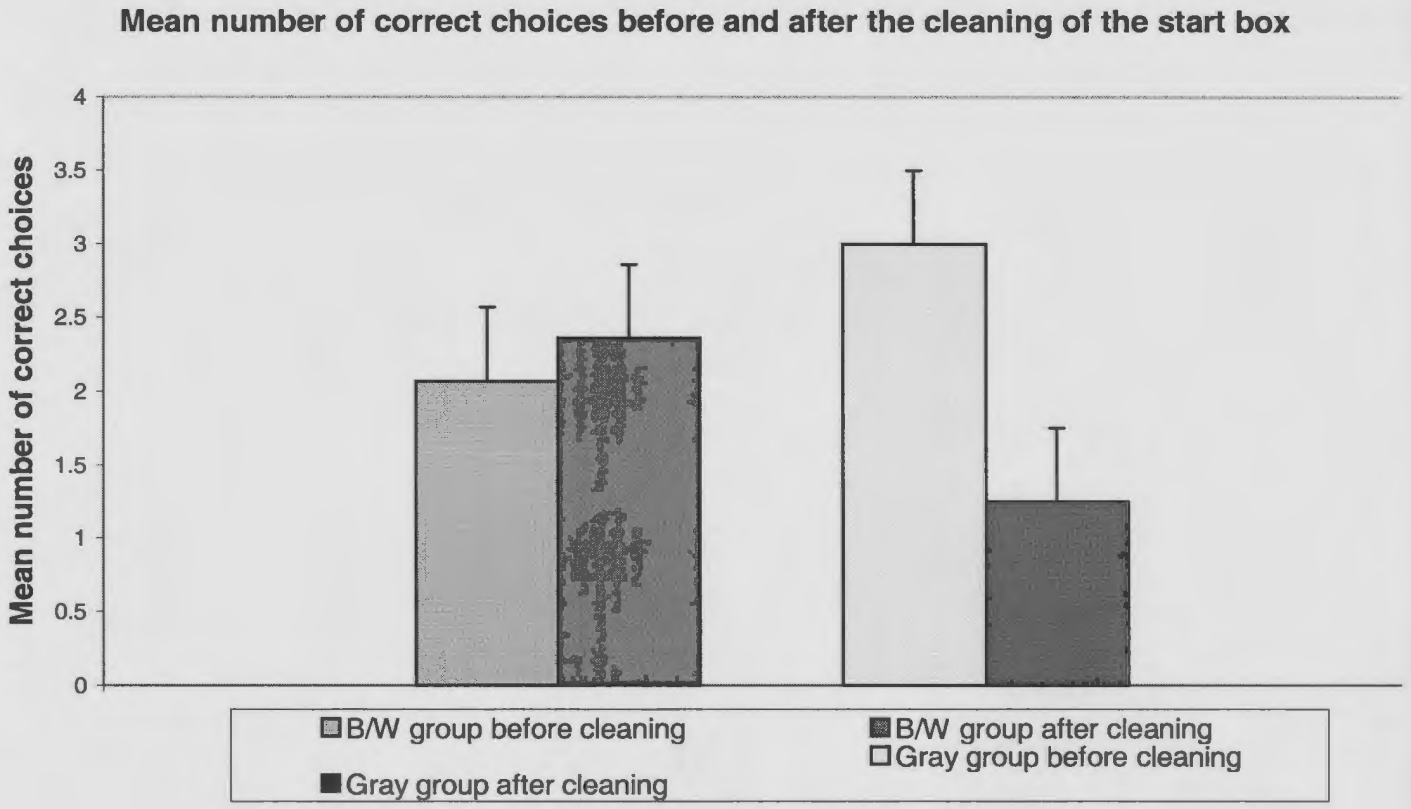

Mean number of correct choices before and after the cleaning of the start box
Mean number of correct choices
4
3.5
3
2.5
2
1.5
1
0.5
0
B/W group before cleaning
B/W group after cleaning
Gray group before cleaning
Gray group after cleaning

Figure 15(a): Figure 15.0 (a) illustrates the means of the averaged correlation coefficients for firing rates of each of the place cells with place correlates in the delay box. These results suggest that the firing rate of each place cell remains stable when entering the delay box from choice arms of the same color, different color, the same side or different sides. This suggests that remapping the delay box may not be necessary in order to bridge the temporal gap between the choice alleys and reward or non-reward.

Figure 15(a):

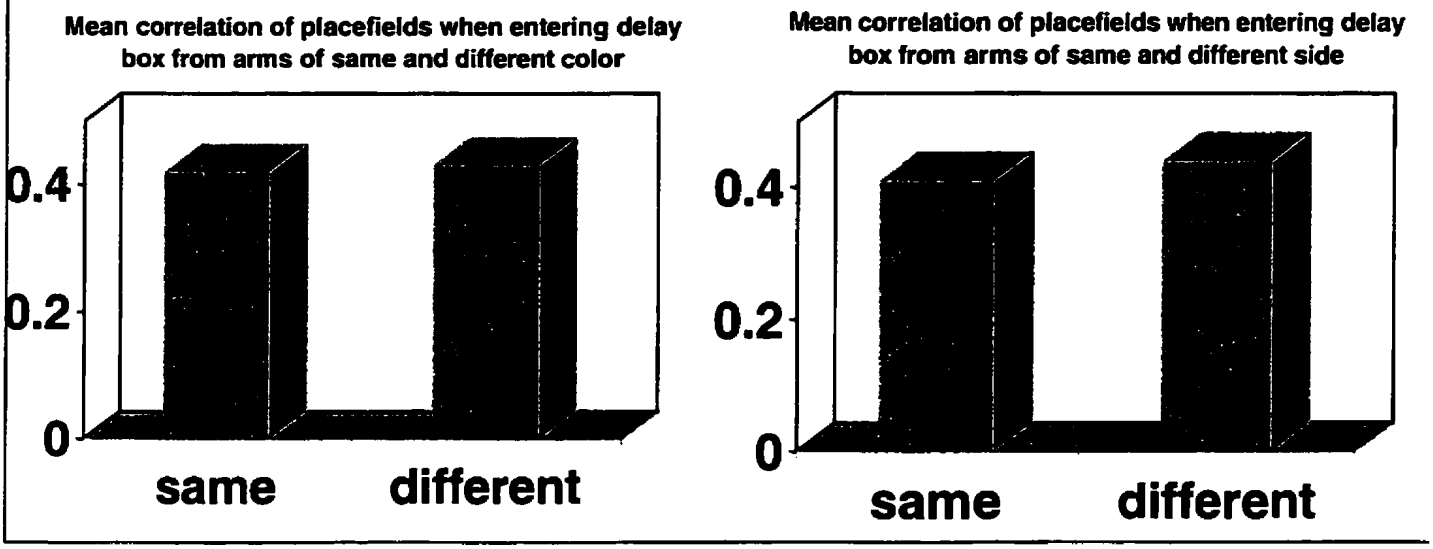

Mean correlation of placefields when entering delay box from arms of same and different color
0.4
0.2
0
same
different
Mean correlation of placefields when entering delay box from arms of same and different side
0.4
0.2
0
same
different

Figure 15(b): The averaged correlation coefficients for the firing rates of place cells in the delay box before the rat enters the black goal box or the white goal box. This may suggest that the cognitive map remains stable in the delay box despite entering it through different routes that would lead to areas of reward and non-reward (the black and white goal boxes). In addition, the results may suggest that 'expectation' of a black or white goal box does not influence the cognitive map in the delay box.

Figure 15(b)

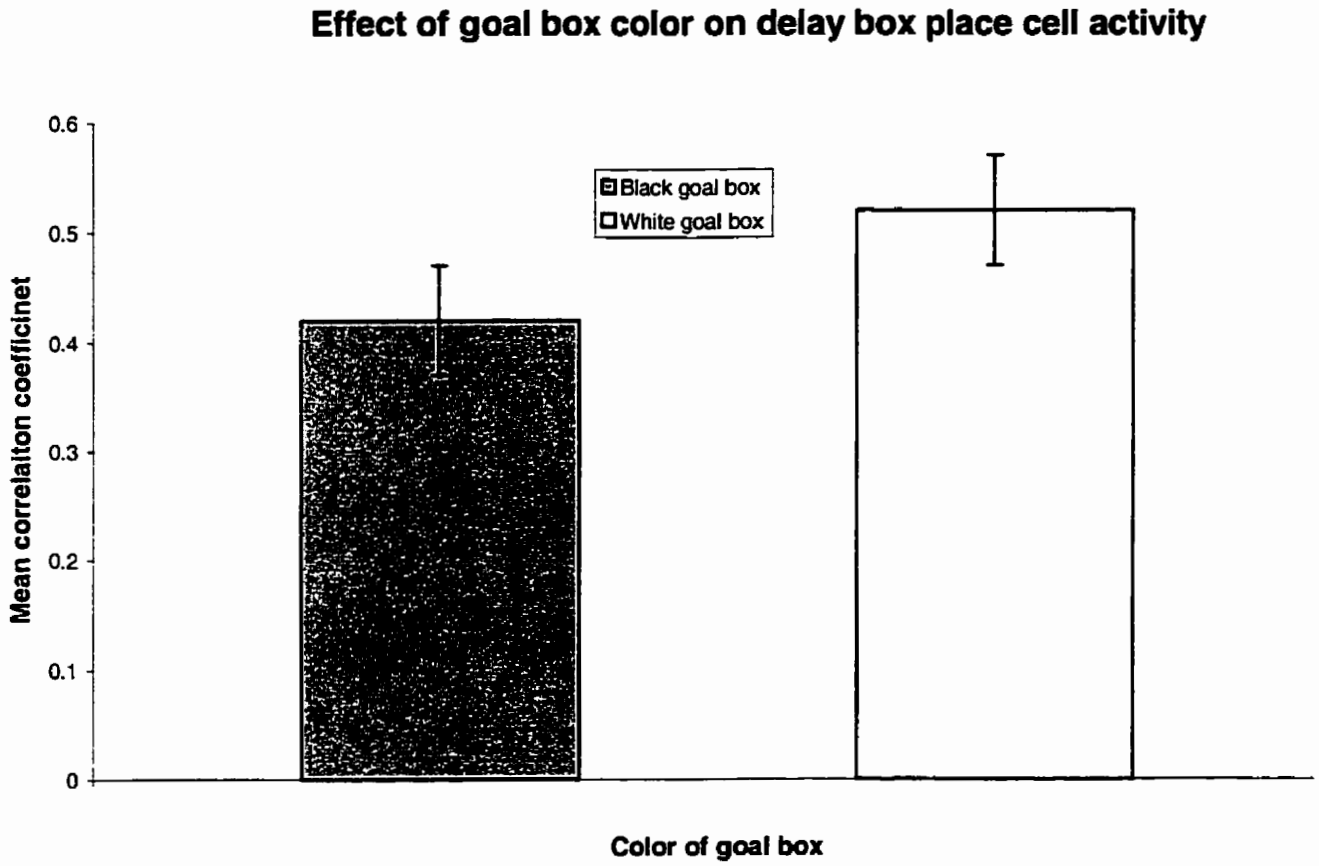

Effect of goal box color on delay box place cell activity
Mean correlalton coefficinet
0.6
0.5
0.4
0.3
0.2
0.1
0
Black goal box
White goal box
Color of goal box

Figure 16.0: An example of a place cell from subject 31 maintaining its firing rate and place field. in the delay box despite entering it from different colored alleys from different sides which lead to differential endpoints. This suggests that remapping the delay box is not necessary in order to bridge the temporal gap between the correct choice alley and reward in the goal box.

Figure16.0

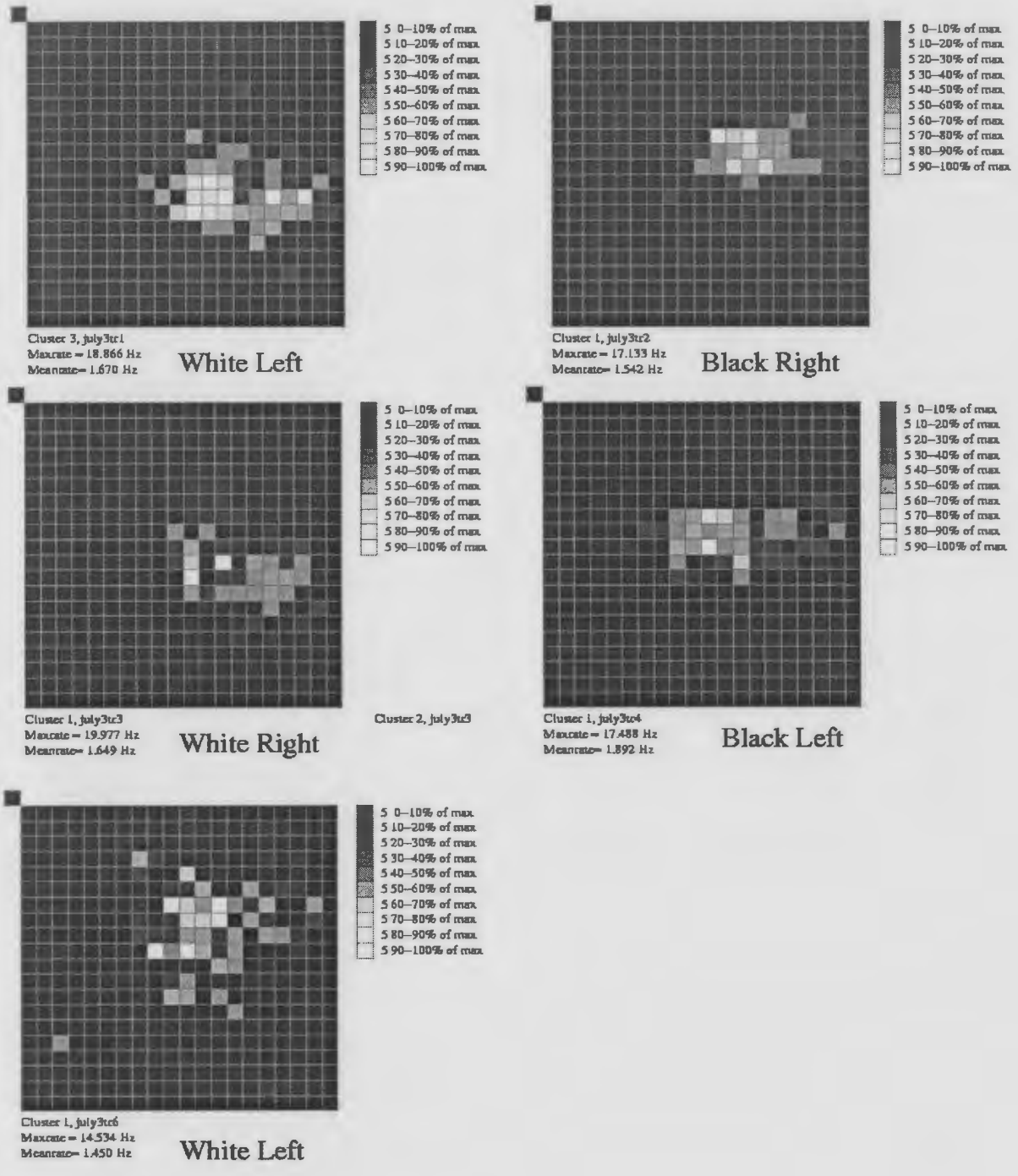

5 0–10% of max
5 10–20% of max
5 20–30% of max
5 30–40% of max
5 40–50% of max
5 50–60% of max
5 60–70% of max
5 70–80% of max
5 80–90% of max
5 90–100% of max
Cluster 3, july3tr1
Maxrate = 18.866 Hz
Meanrate= 1.670 Hz
White Left
Cluster 1, july3tr2
Maxrate = 17.133 Hz
Meanrate= 1.542 Hz
Black Right
Cluster 1, july3tr3
Maxrate = 19.977 Hz
Meanrate= 1.649 Hz
White Right
Cluster 2, july3tr3
Cluster 1, july3tr4
Maxrate = 17.488 Hz
Meanrate= 1.892 Hz
Black Left
Cluster 1, july3tr6
Maxrate = 14.534 Hz
Meanrate= 1.450 Hz
White Left

Figure 17.0: Figure 17.0 illustrates firing rate maps from a place cell from CA3 from Subject 26 that has two different place fields in each of the black and white goal boxes (as indicated by change in location of the place field). This suggests that the black and white goal boxes are seen as different places irrespective of their physical location in the room.

Figure 17.0

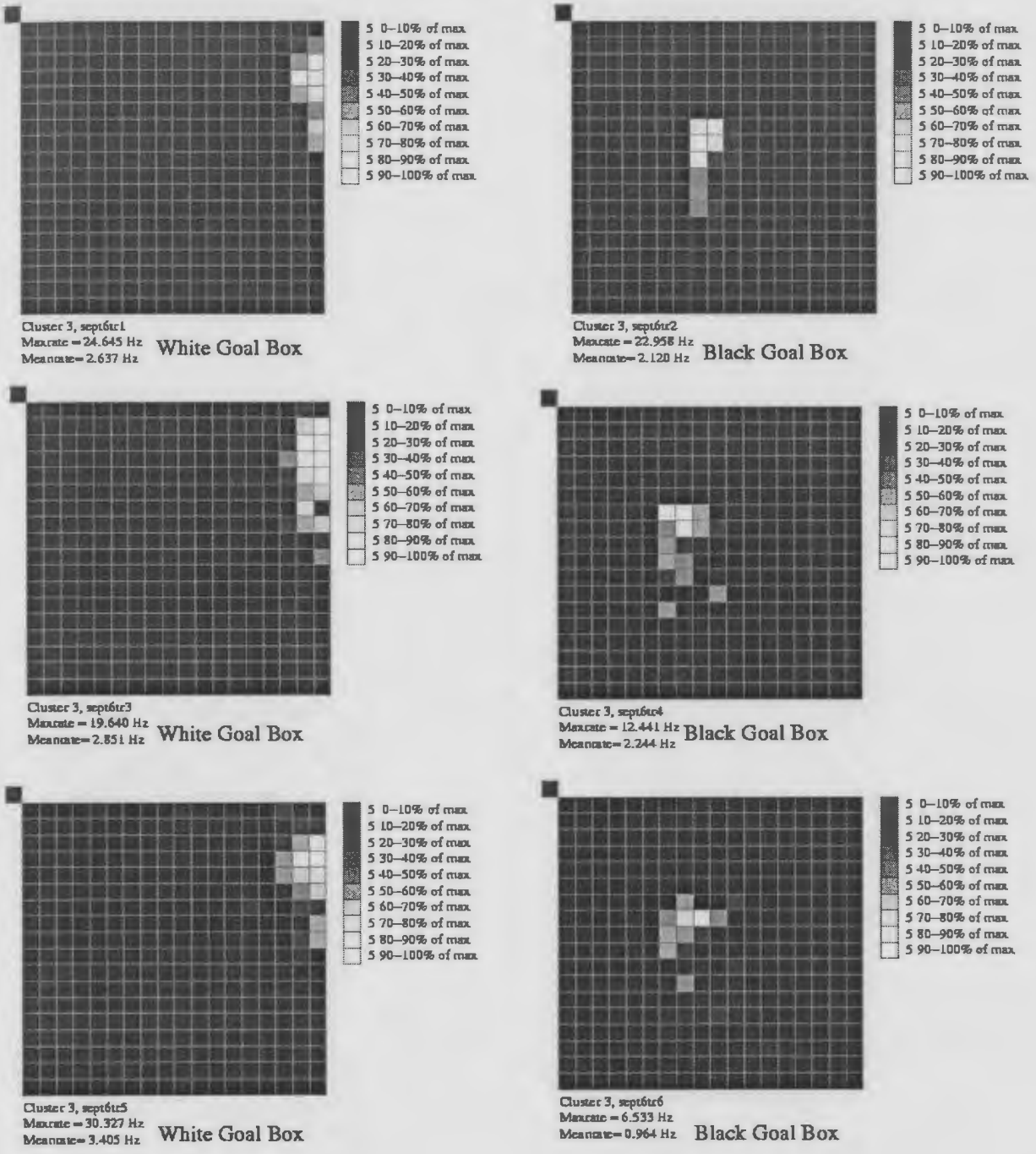

5 0–10% of max
5 10–20% of max
5 20–30% of max
5 30–40% of max
5 40–50% of max
5 50–60% of max
5 60–70% of max
5 70–80% of max
5 80–90% of max
5 90–100% of max
Cluster 3, sept6tr1
Maxrate = 24.645 Hz
Meanrate= 2.637 Hz
White Goal Box
Cluster 3, sept6tr2
Maxrate = 22.958 Hz
Meanrate= 2.120 Hz
Black Goal Box
Cluster 3, sept6tr3
Maxrate = 19.640 Hz
Meanrate= 2.851 Hz
White Goal Box
Cluster 3, sept6tr4
Maxrate = 12.441 Hz
Meanrate= 2.244 Hz
Black Goal Box
Cluster 3, sept6tr5
Maxrate = 30.327 Hz
Meanrate= 3.405 Hz
White Goal Box
Cluster 3, sept6tr6
Maxrate = 6.533 Hz
Meanrate= 0.964 Hz
Black Goal Box

Figure 18.0: Figure 18.0 demonstrates a series of firing rate maps from subject 33 that show a stable place field along the right wall of the gray goal box. A change in the firing rate and location of cell firing was found when Subject 33, which had only ever been exposed to the Gray goal box, entered the white goal box. This suggests that the gray and white goal boxes were seen as distinctly different places.

Figure 18.0

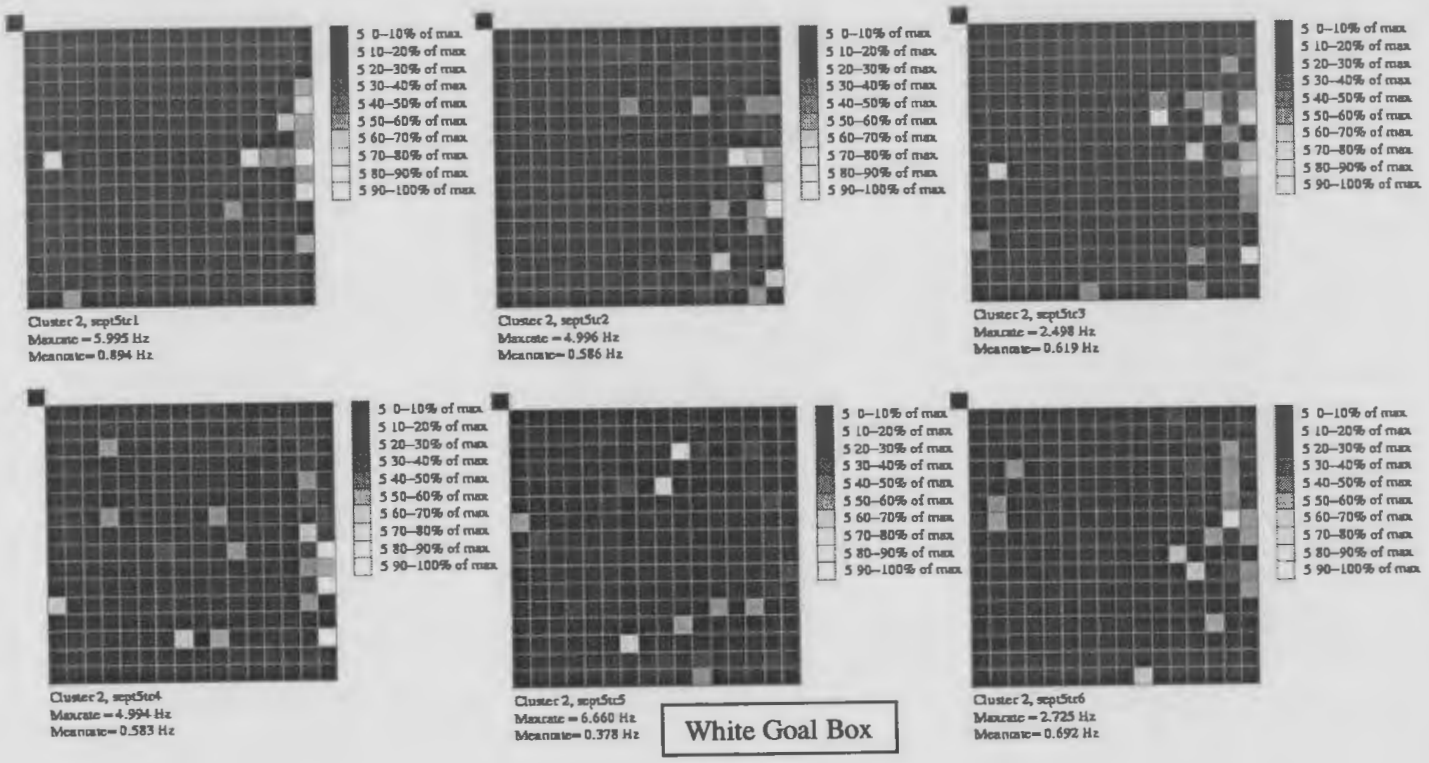

5 0–10% of max
5 10–20% of max
5 20–30% of max
5 30–40% of max
5 40–50% of max
5 50–60% of max
5 60–70% of max
5 70–80% of max
5 80–90% of max
5 90–100% of max
Cluster 2, sept5tr1
Maxrate = 5.995 Hz
Meanrate= 0.894 Hz
Cluster 2, sept5tr2
Maxrate = 4.996 Hz
Meanrate= 0.586 Hz
Cluster 2, sept5tr3
Maxrate = 2.498 Hz
Meanrate= 0.619 Hz
Cluster 2, sept5tr4
Maxrate = 4.994 Hz
Meanrate= 0.583 Hz
Cluster 2, sept5tr5
Maxrate = 6.660 Hz
Meanrate= 0.378 Hz
White Goal Box
Cluster 2, sept5tr6
Maxrate = 2.725 Hz
Meanrate= 0.692 Hz

Table 1: Phases of behavioral training in experiment 1

Phase 1) Pretraining

Phase 2) Correction factor and regular trials (trials 1-135)

Phase 3) Introduction of barrier in black choice alley (trials 136-155)

Phase 4) Delay box removed (trials 156-205)

Phase 5) Swinging doors introduced to the choice alleys (trials 206-225)

Table 2: Group Assignment

1) Black and white goal box group
   A) Black alley as correct alley (n=5); Subjects 1, 2, 3, 14, 15
   B) White alley as correct alley (n=5); Subjects 4, 5, 11, 12, 13
2) Gray goal box group
   A) Black alley as correct (n=5); Subjects 6, 7, 8, 19, 20
   B) White alley as correct (n=5); Subjects 9, 10, 16, 17, 18
3) No delay group
   A) Black alley as correct (n=2); Subjects 21, 22
   B) White alley as correct (n=3); Subjects 23, 24 25

Table 3.0: Mean number of correct choices (out of five) for subjects in each group

	B/W *Black alley*	*B/W* *White alley*	*Gray* *Black alley*	*Gray* *White alley*	*No Delay* *Black alley (n=2)*	*No Delay* *White alley(n=3)*
Phase 2 *(Trials 1-135)*	3.30	1.96	3.24	1.54	3.80	2.40
Phase 3 *(Trials 136-155)*	2.56	1.82	2.92	1.16	2.82	2.30
Phase 4 *(Trials 156-205)*	3.42	3.05	3.58 **(n=4)*	2.00	3.03 **(n=1)*	3.53
Phase 5 *(Trials 206-225)*	4.52	4.64	4.52 **(n=4)*	4.40	4.20 **(n=1)*	4.90

Table 4.0: Summary F-Table on the number of correct choices made by rats during phase 2 (* = $p < .05$)

Source	df	SS	MS	F
A) Goal Box type	1	2.00	2.00	1.35
B) Alley Color	1	82.67	82.67	55.59 (*)
A X B	1	1.04	1.04	.70
Between Error Term	16	23.80	1.49	
C) Block of Trials	6	9.03	1.50	5.50 (*)
A X C	6	2.01	.34	1.23
B X C	6	3.74	.62	2.28 (*)
A X B X C	6	1.32	.22	.81
Within Error Term	96	26.27	0.27	

Table 5.0: Summary of means and standard errors of the number of correct choices made by rats during phase 2 (* = $p < .05$)

Black and White Goal box	Block 1	Block2	Block3	Block4	Block 5	Block6
White alley correct (n=5)	1.47± .162	1.65 ± .130	2.00 ± .153	1.90 ± .114	1.65 ± .185	2.40 ± .143
Black alley correct (n=5)	3.27 ± 0697	3.00 ± ..0442	3.25 ± .140	3.00± .147	3.70 ± .103	3.95 ± .103
Gray Goal Box						
White alley correct (n=5)	1.13 ± .162	1.10 ± .0559	1.65 ± .260	1.65 ± .224	1.55 ± .231	1.65 ± .144
Black alley correct (n=5)	2.60 ± .216	2.85 ± .169	3.40 ± .122	3.55 ± .204	3.55 ± .204	3.50 ± .182
No Long Delay (Gray Goal Box)						
White alley correct (n=3)	2.11 ± .347	1.83 ± .402	2.58 ± .315	3.25 ± .250	2.67 ± .382	2.25 ± .695
Black alley correct (n=2)	3.17 ± .236	3.62 ± .530	4.38 ± .177	4.50 ± .354	4.13 ± .177	3.50 ± .354

Table 6.0: Summary F-Table on the number of correct choices made by rats during phase 3 (* = $p < .05$)

Source	df	SS	MS	F
A) Goal Box type	1	.31	.31	.43
B) Alley Color	1	15.01	15.01	21.25 (*)
A X B	1	2.76	2.76	3.90
Between Error Term	16	11.30	11.30	
C) Block of Trials	1	1.41	1.41	4.79 (*)
A X C	1	.51	.51	1.72
B X C	1	.01	.01	.02
A X B X C	1	.76	.76	2.57
Within Error Term	16	4.70	0.29	

Table 7.0: Summary of means and standard errors of the number of correct choices made by rats during phase 3

Black and White Goal box	**Block 1**	**Block2**
White alley correct (n=5)	1.40 ± .105	2.30 ± .190
Black alley correct (n=5)	2.40 ± .205	2.70 ± .143
Gray Goal Box		
White alley correct (n=5)	1.20 ± .168	1.10 ± .163
Black alley correct (n=5)	2.70 ± .190	3.10 ± .224
No Long Delay (Gray Goal Box)		
White alley correct (n=3)	2.83 ± .945	1.67 ± .382
Black alley correct (n=2)	2.25 ± .354	3.25 ± .1.06

Table 8.0: Summary F-Table on the number of correct choices made by rats during phase 4 (* = $p < .05$)

Source	df	SS	MS	F
A) Goal Box type	1	2.76	2.76	2.67
B) Alley Color	1	26.31	26.31	25.50 (*)
A X B	1	12.80	12.80	12.40 (*)
Within Error Term	15	15.48	1.03	
C) Block of Trials	4	13.71	3.43	6.10 (*)
A X C	4	1.85	.46	.83
B X C	4	4.23	1.06	1.88
A X B X C	4	2.19	.55	.98
Within Error Term	60	33.69	.56	

Table 9.0: Summary of means and standard errors of the number of correct choices made by rats during phase 4

Black and White Goal box	**Block 1**	**Block2**	**Block3**	**Block4**	**Block 5**
White alley correct (n=5)	2.30 ± .244	2.40 ± .240	2.60 ± .240	3.20 ± .265	3.80 ± .0685
Black alley correct (n=5)	3.00 ± .177	3.10 ± .163	2.90 ± .205	3.50 ± .0884	3470 ± .240
Gray Goal Box					
White alley correct (n=5)	1.80 ± .143	.90 ± .205	120 ± .258	2.60 ± .224	2.40 ± .205
Black alley correct (n=5)	3.90 ± .056	3.00 ± .234	3.40 ± .163	2.90 ± .324 (*n=4)	4.13 ± .250
No Long Delay (Gray Goal Box)					
White alley correct (n=3)	2.50 ± .615	2.17 ± .878	3.00 ± .250	3.83 ± .191	4.33 ± .289
Black alley correct (n=2)	3.00 ± .707	4.50 ± .000	4.00 ± .000	2.00 ± .000 (* n=1)	1.50 ± *

Table 10.0: Summary F-Table on the number of correct choices to criterion for rats in each goal box condition during phase 5

Source	df	SS	MS	F
A) Alley Color	1	12.43	12.43	1.57
B) Goal Box Type	1	.66	.66	.08
A X B	1	8.90	8.90	1.12
Within Error Term	15	118.75	7.92	

Table 11.0: Summary of mean number of trials to criterion for rats in each group during phase 5

Black and White Goal box	**Mean number of trials to criterion**
White alley correct (n=5)	20.00
Black alley correct (n=5)	23.00
Gray Goal Box	
White alley correct (n=5)	21.00
Black alley correct (n=4)	21.25

Table 12.0: Summary of F-Table for trials to criterion for rats in each group when phases were combined (* = $p < .05$)

Source	df	SS	MS	F
A) Alley Color	1	23522.08	23522.08	30.82 (*)
B) Goal Box Type	2	21604.00	10802.00	14.15 (*)
A X B	2	19570.03	9785.01	12.82 (*)
Within Error Term	16	12212.72	763.29	

Table 13.0: Summary of mean number of trials to criterion for rats in each group when all phases were combined

Black and White Goal box	**Mean number of trials to criterion**
White alley correct (n=5)	217
Black alley correct (n=4)	218
Gray Goal Box	
White alley correct (n=5)	222
Black alley correct (n=4)	172
No Long Delay (Gray Goal Box)	
White alley correct (n=3)	214
Black alley correct (n=2)	56

Table 14.0: Summary F-Table for percentage of correct choices for rats in each group across phases (* = $p < .05$)

Source	df	SS	MS	F
A) Alley Color	1	3342.99	3342.99	46.29 (*)
B) Goal Box Type	2	969.95	484.98	6.71 (*)
A X B	2	185.91	92.95	1.29
Within Error Term	19	1372.26	72.22	

Table 15.0: Summary of means for the percentage of correct choices for rats in each group when all phases were combined

Black and White Goal box	Mean Percentage of correct choices
White alley correct (n=5)	51.2%
Black alley correct (n=5)	69.4%
Gray Goal Box	
White alley correct (n=5)	39.5%
Black alley correct (n=5)	69.8%
No Long Delay (Gray Goal Box)	
White alley correct (n=3)	59.3%
Black alley correct (n=2)	84.6%

Table 16.0: Group information and percentage of correct choices during recording trials for subjects 6. 18. 23. 17. 5. 15

Subject	Condition	Percentage of correct choices During recording trials
6	Grey goal box Black alley correct	93%
18	Gray goal box White alley correct	68%
23	No Delay (Gray goal box) White alley correct	95%
17	Gray goal box White alley correct	46%
5	Black and white goal boxes White alley correct	69%
15	Black and white goal boxes Black alley correct	100%

Table 17.0: Averaged correlation coefficients for the firing rates of place cells found in the delay box for each animal (#=Number of comparisons in each mean correlation coefficient)

Subject	Same color alley and Same side (Same)	Same Side and different color alley (Side)	Same color alley and Different side (Color)	Different Color alley and Different Side (Opposites)
6	.23 #=1	.36 #=3	.35 #=3	.38 #=3
18	.39 #=2	.28 #=6	.34 #=6	.35 #=6
23	.42 #=1	.49 #=3	.47 #=3	.44 #=3
17(3 place cells)	.36 #=13	.29 #=22	.33 #=22	.33 #=22
5	.34 #=1	.35 #=3	.35 #=3	.34 #=3
15	.51 #=1	.37 #=3	.33 #=3	.41 #=3

Table 18.0: Summary F-Table for the analysis of correlation coefficients comparing the firing rate of place cells in the delay box after the animal had entered the delay box from alleys of the same side and color, the same side, the same color, and different sides (left and right) and different colors (white and black)

Source	df	SS	MS	F
A) Side	1	4.3 E-5	4.3 E-5	.02
Within Error Term	5	9.2 E-3	1.8 E-3	
B) Color	1	4.8 E-5	4.8 E-5	.02
Within Error Term	5	1.2 E-2	2.3 E-3	
A X B	1	1.5 E-3	1.5 E-3	.39
Within Error Term	5	2.0 E-2	3.9 E-3	

Table 19.0: Summary of means and standard errors for the correlation coefficients comparing the firing rate of place cells in the delay box after the animal had entered the delay box from alleys of the same side and color, the same side, the same color, and different sides (left and right) and different colors (white and black)

Same color alley and Same side (Same)	Same Side and different color alley (Side)	Same color alley and Different side (Color)	Different Color alley and Different Side (Opposites)
.37 ± .018	.36 ± .016	.36 ± .01	. 37 ± .008

Table 20.0: Summary F-Table for the analysis of correlation coefficients comparing the firing rate of place cells in the delay box from subjects in each goal box condition after the animal had entered the delay box from alleys of the same side and color, the same side, the same color, and different sides (left and right) and different colors (white and black). This analysis indicates if expecting different colored goal boxes or the same goal box may have effected the firing rate of place cells in the delay box

Black and white goal box condition (n=2):				
Source	df	SS	MS	F
A) Side	1	2.5 E-3	2.5 E-3	1.35
Within Error Term	1	1.9 E-3	1.9 E-3	
B) Color	1	6.8 E-4	6.8 E-4	.61
Within Error Term	1	4.2 E-4	4.2 E-4	
A X B	1	4.9 E-3	4.9 E-3	.77
Within Error Term	1	6.4 E-3	6.4 E-3	
Gray goal box condition (n=4):				
Source	df	SS	MS	F
A) Side	1	1.9 E-3	1.9 E-3	1.91
Within Error Term	3	3.0 E-3	9.9 E-4	
B) Color	1	3.6 E-5	3.6 E-5	.01
Within Error Term	3	1.1 E-2	3.5 E-3	
A X B	1	2.3 E-6	2.3 E-6	.00
Within Error Term	3	1.0 E-2	3.4 E-3	

Table 21.0: Summary of means and standard errors for the analysis of correlation coefficients comparing the firing rate of place cells in the delay box from subjects in each goal box condition after the animal had entered the delay box from alleys of the same side and color, the same side, the same color, and different sides (left and right) and different colors (white and black). This analysis indicates if expecting different colored goal boxes or the same goal box may have affected the firing rate of place cells in the delay box

	Same color alley and Same side (Same)	Same Side and different color alley (Side)	Same color alley and Different side (Color)	Different Color alley and Different Side (Opposites)
B/W goal box group	.42 ± .12	.36 ± .01	.34 ± .01	.37 ± .05
Gray goal box group	.35 ± .33	.35 ± .33	.37 ± .12	37 ± .12

Table 22.0: Group information and percentage of correct choices during recording trials for subjects in the black and white goal box group

Subject	Condition	Percentage of correct choices During recording trials
15	Black and white goal box Black alley correct	100%
5	Black and white goal box White alley correct	69%
14	Black and white goal box Black alley correct	100%

Table 23.0: Averaged correlation coefficients for the firing rates of place cells found in the goal box from animals in the black and white goal box condition (#=Number of comparisons in each mean correlation coefficient)

Subject	Black goal box	White goal box	Black and White goal boxes	Grey/white	Grey/black
15	.32 #=1	.25 #=1	-.03 ns* #=4		
5	.30 #=6	.18 #=2	.02 ns* #=12	.09 ns* #=4	.22 #=5
14	*(missing)	.20 #=2	.02 ns* #=1		

Table 24.0: Group information and percentage of correct choices during recording trials for subjects in the gray goal box group

Subject	Condition	Percentage of correct choices During recording trials
18	Gray goal box White alley correct	66%
17	Gray goal box White alley correct	43%

Table 25.0: Averaged correlation coefficients for the firing rates of place cells found in the goal box from animals in the gray goal box condition (#=Number of comparisons in each mean correlation coefficient)

Subject	Gray goal box	Gray and White goal boxes	Gray and Black goal boxes	Black and White goal boxes
18	.23 #=15	-.01 ns*#=6	.11#=6	-.02 ns*#=1
17	.38 #=15	.27 #=6	.43 #=6	.17 #=1

Table 26.0: Principle differences between experiments 1 and 2

1) Change of pre-training procedure. Pre-training was carried out inside of the apparatus as in the procedure of Lawrence and Homel.

2) The inter-trial-interval was decreased from a minimum of ten minutes, as in Lawrence and Homel's experiment, to 30 seconds.

3) Increased frequency of training per day. The subjects were trained for 5-10 trials a day in contrast to Lawrence and Homel's 5 trials a day, and 5 trials every second day in experiment 1.

4) As in Lawrence and Homel's experiment, experiment 2 consisted of only two conditions, Black/White goal box with white alley correct (n=7), and Gray goal box with white alley correct (n=3).

Table 27.0: Summary F-Table for the average number of overall correct choices per block of five trials for subjects in each group

Source	df	SS	MS	F
Between Groups	1	.0028	.0028	.0316
Within Groups	8	.7153	.0894	
Total	9	.7181		

Table 28.0: Means and standard errors for the number of correct per block of five trials for subjects in each group

	Mean number of correct trials Out of five
Black and White Goal Box Group (n=7)	2.64 ± .167
Gray Goal Box Group (n=3)	2.60 ± .114

Table 29.0: Summary F-Table for correct choices per block of five trials to trial 175 (* = significant differences)

Source	df	SS	MS	F
A) Goal Box type	1	.90	.90	.435
Within Error Term	8	10.63	1.33	
B) Block of Trials	8	20.35	2.54	6.27 (*)
A X B	8	7.12	.89	2.19 (*)
Within Error Term	64	25.96	.41	

Table 30.0: Means and standard errors for the number of correct choices (to Trial 175)

Black and White Goal box	Block 1	Block2	Block3	Block4	Block 5
White alley correct (n=7)	1.24 ± .045	1.67 ± .100	2.11 ± .092	2.07 ± .110	2.35 ± .116

	Block 6	Block7	Block8	Block9
	2.61 ± .061	2.18 ± .094	2.82 ± .127	3.46 ± .124

Gray Goal Box	Block 1	Block2	Block3	Block4	Block 5
White alley correct (n=3)	1.88 ± .360	1.17 ± .096	2.08 ± .173	3.00 ± .333	1.25 ± .250

	Block 6	Block7	Block8	Block9
	1.92 ± .316	1.92 ± .048	2.50 ± .220	2.83 ± .255

Table 31.0: Summary F-Table for the average number of correct choices per block of five trials for both groups 20 trials before and twenty trials after cleaning the start box of the apparatus (* = significant differences)

Source	df	SS	MS	F
A) Goal Box type	1	.34	.34	.451
Within Error Term	8	6.73	.84	
B) Block of Trials	1	1.74	1.74	.076
A X B	1	3.09	3.09	7.39 (*)
Within Error Term	8	3.34	.42	

Table 32.0: Summary of means and standard errors for the average number of correct choices per block of five trials for both groups 20 trials before and twenty trials after cleaning the start box of the apparatus (* = significant differences)

Mean number of correct trials
Out of five

	Before cleaning	After cleaning
Black and White Goal Box Group (n=7)	2.07 ± .13	2.36 ± .14
Gray Goal Box Group (n=3)	3.00 ± .50	1.25 ± .38

Table 33.0: Summary F-Table for the number of trials to criterion for subjects in both experimental groups

Source	df	SS	MS	F
Between Groups	1	1027.56	1027.56	1.22
Within Groups	7	5914.00	844.86	
Total	8	6941.56		

Table 34.0: Summary of means and standard errors for the number of trials to criterion for subjects in both experimental groups

	Mean # of trials to criterion
Black and White Goal Box Group (n=7)	221.67 ± 5.64
Gray Goal Box Group (n=3)	244.33 ± 4.81

Table 35.0: Group information and percentage of correct choices during recording trials for animals in which place cells were founding the delay box of the apparatus

Subject	Condition	Percentage of correct choices During recording trials
31	Black and white goal box White alley correct	86%
26 (2 place cells)	Black and white goal box White alley correct	100%
29	Black and white goal box White alley correct	83%

Table 36.0: Averaged correlation coefficients for place cells found in the delay box (#=Number of comparisons in each mean correlation coefficient). All subjects are in the black and white goal box group and have the white alley as correct

Subject	Same color and Same side (Repeats)	Different Color Same side (Direction)	Same color an Different side (Color)	Different Color Different Side (Opposites)
31	.27 #=1	.40 #=3	.46 #=3	.40 #=3
29	.43 #=1	.43 #=3	.46 #=3	.47 #=3
26	.46 #=4	.43 #=7	.43 #=7	.42 #=7

Table 37.0: Summary F-Table for the analysis of correlation coefficients comparing the firing rate of place cells in the delay box after the animal had entered the delay box from alleys of the same side and color. the same side. the same color. and different sides (left and right) and different colors (white and black)

Source	df	SS	MS	F
A) Side	1	3.5 E-3	3.5 E-3	.81
Within Error Term	2	8.5 E-3	4.3 E-3	
B) Color	1	3.6 E-4	3.6 E-4	.77
Within Error Term	2	9.5 E-4	4.7 E-4	
A X B	1	3.2 E-3	3.2 E-3	1.11
Within Error Term	2	5.8 E-3	2.9 E-3	

Table 38.0: Summary of means and standard errors for the correlation coefficients comparing the firing rate of place cells in the delay box after the animal had entered the delay box from alleys of the same side and color, the same side, the same color, and different sides (left and right) and different colors (white and black)

Same color alley and Same side (Same)	Same Side and different color alley (Side)	Same color alley and Different side (Color)	Different Color alley and Different Side (Opposites)
.39 ± .05	.43 ± .015	.45 ± .010	.43 ± .015

Table 39.0: Group information and percentage of correct choices during recording trials from animals with place cells in the goal box of the apparatus

Subject	Condition	Percentage of correct choices During recording trials
26	Black and white goal box White alley correct	100%
33	Gray goal box White alley correct	100%

Table 40.0: Averaged correlation coefficients of place cells found in the goal box for subject 26 (black and white goal box group) and subject 33 (gray goal box group) (ns*=not significantly correlated; #=number of comparisons in each mean correlation coefficient)

Subject	Black goal box	White goal box	Black and white goal boxes
26	.87 #=3	.71#=3	-.04 *ns #=9

Subject	Gray goal box	Gray and white goal box
33	.32 #=10	.027 *ns #=5

Appendix A

Firing Rate Map Program

(John Huxter, 1998)

```
#!/bin/sh

gawk '

BEGIN {
printf("\n\n\nPLACE CELL ANALYSIS PROGRAM: by J.Huxter, 1998")
while(choice!="q")      {
print"\n*****************************************************************
"
print"MAIN MENU: file = \""origfile"\", x-bins="xbintot", y-
bins="ybintot

printf("Choice ([o]ptions) ? "); getline $0 < "/dev/tty"
if($1=="1") PLACE1(origfile,basefile)
if($1=="2") PLACE2(basefile,xbintot,ybintot)
if($1=="p1") PLOT1(basefile)
if($1=="p2") PLOT2(basefile)
if($1=="p3") PLOT3(basefile,xbintot,ybintot)
if($1=="f") FILE()
if($1=="c") CLUSTERS(basefile)
if($1=="b") BINS()
if($1=="r") ROTATE(basefile)
if($1=="s") SPLIT(basefile)
if($1=="o") OPTIONS()
if($1=="ls") LS()
if($1=="q") QUIT()
                }
exit
}


function PLACE1(origfile,basefile) {
#################################################################
# eliminate commas, control-characters from discovery file
# convert numeric cluster ids to numbers (A -> 11, B -> 12)
# convert time-stamp into seconds
# calculate max/min xy coordinates
#     infile = file to read data from with following format
#           "P",timestamp,x,y
#           "E"or"S",timestamp,cluster,electrode channel
#
#     x = x coordinate from infile
#     y = y coordinate from infile
#     c = cluster number from infile
#     t = timestamp (converted to seconds from start) from infile
#     tmin = time at start of record (in ms)
#     xmin/xmax = min and max x-coordinates sampled
#     ymin/ymax = min and max y-coordinates sampled
#     tmin = time stamp at start of record
#     tpos = total number of position samples
#     ttime = time at end of record (in seconds, from start)
#     tspk = total number of spike events
#     sx[]/sy[]/st[] = x-coord/y-coord/time for each spike
#     px[]/py[]/pt[] = x-coord/y-coord/time for each position sample
#     ctot[] = total number of spikes from each cluster
#
```

```
# output =   *.sta (statistics)
#            *.spk (position & spike data)
#############################################################
print"\n    CONVERT INPUT FILE & GENERATE STATS"
print"    ----------------------------------"
if(origfile=="") {print"\[ERROR]: set input file first!";return}
infile=origfile
FS=","
z=tpos=tspk=ttime=tmin=0
xmin=ymin=999999;xmax=ymax=0
print "    * reading \""infile"\""
print "    * converting timestamp, adding xy data to spikes"
while((getline $0 < infile) > 0)     {
      z++
      if(z==1) tmin=$2
      t=($2-tmin)/10000
 if($1=="P")       {
      tpos++
      x=$3;y=$4
      if(xmin>=x && x!=0) xmin=x; if(xmax<=x) xmax=x
      if(ymin>=y && x!=0) ymin=y; if(ymax<=y) ymax=y
      px[tpos]=x;py[tpos]=y;pt[tpos]=t
            }
 if($1=="E" || $1=="S")    {
      tspk++
      c=$3; if(c=="A")c="10"; if(c=="B")c="11"
      sx[tspk]=x;sy[tspk]=y;st[tspk]=t;sc[tspk]=c;ctot[c]++
            }
                              }
close (infile)
ttime=t
xrange=xmax-xmin;yrange=ymax-ymin
print"    * converting xy coordinates to percentages of xy range"

print"    * working on position data..."
close (outfile)
outfile=basefile".pos"
for(z=1;z<=tpos;z++)    {
      x=((px[z]-xmin)/xrange)*100;y=((py[z]-ymin)/yrange)*100
      printf("%8.3f  %7.3f  %7.3f \n", pt[z],x,y) > outfile}
print"    * position data sent to \""outfile"\""

print"    * working on spike data..."
close (outfile)
outfile=basefile".spk"
for(z=1;z<=tspk;z++)    {
      x=((sx[z]-xmin)/xrange)*100;y=((sy[z]-ymin)/yrange)*100
      printf("%8.3f  %7.3f  %7.3f  %2s \n", st[z],x,y,sc[z]) > outfile}
print"    * position spike data sent to \""outfile"\""

close (outfile)
outfile=basefile".sta"
print"    * parameters sent to \""outfile"\"\n"
printf("%-4s (xmin)  %4s (ymin)  %4s (xmax)  %4s (ymax)  %6s (samples)
%6s (spikes)  %7.3f (time)\n",
       xmin,ymin,xmax,ymax,tpos,tspk,ttime) > outfile
z=0
```

```
for(c=0;c<=11;c++)
      if(ctot[c]!="") {
            z++;printf("%-2s %2s %4s %5.3f\n",c,z,ctot[c],ctot[c]/t)
>outfile
                  }
close (outfile)
status=status"BCD"
return
}


function PLACE2(basefile,xbintot,ybintot) {
#####################################################################
# calculate dwell-time per bin, spikes per bin
# calculate firing rates for each cluster in each bin
#####################################################################
print"\n    SORT SPIKE-EVENTS & DWELL-TIME INTO BINS"
print"    ---------------------------------------"
if(basefile=="") {print"    [ERROR]: set input file first!";return}
if(xbintot=="")  {print"    [ERROR]: set x-bins and y-bins
first!";return}
match(status,"B")
 if(RSTART<=0) {print"    [ERROR]: stats file (.sta) missing";return}
match(status,"C")
 if(RSTART<=0) {print"    [ERROR]: spike file (.spk) missing";return}
match(status,"D")
 if(RSTART<=0) {print"    [ERROR]: position file (.pos) missing";return}

FS=" "; infile=basefile".spk"; split("",spike); split("",samp)

z=0;infile=basefile".sta"
print"    * reading parameters from \""infile"\""
while((getline $0 < infile) > 0)    { z++
       if(z==1)
{xmin=$1;ymin=$3;xmax=$5;ymax=$7;tsamp=$9;tspike=$11;ttime=$13;continue}
       if($1!="")      {c=$1;id[c]=$2;cspike[c]=$3;crate[c]=$4}}
cmax=id[c]; close (infile)

infile=basefile".spk"
print"    * calculating spikes/bin/cluster from \""infile"\""
while((getline $0 < infile) > 0)           {
      if($2<=-0.001)
{xbin=ybin=0;spike[xbin,ybin,id[$4]]++;bspike[xbin,ybin]++;continue}
      xbin=int($2/(100.001/xbintot))+1;ybin=int($3/(100.001/ybintot))+1
      spike[xbin,ybin,id[$4]]++;bspike[xbin,ybin]++}
close (infile)

infile=basefile".pos"
print"    * calculating samples/bin from \""infile"\""
while((getline $0 < infile) > 0)           {
      if($2<=-0.001) {xbin=ybin=0;samp[xbin,ybin]++;continue}
      xbin=int($2/(100.001/xbintot))+1;ybin=int($3/(100.001/ybintot))+1
      samp[xbin,ybin]++}
close (infile)

print"    * calculating dwell time & firing rates/bin"
close (outfile)
```

```
outfile=basefile".bin"
for(xbin=0;xbin<=xbintot;xbin++)          {
for(ybin=0;ybin<=ybintot;ybin++)          {
if((xbin==0 && ybin==0) || (xbin!=0 && ybin!=0)){ # only process some
bins
      if(samp[xbin,ybin]=="") dwell[xbin,ybin]=0
      if(samp[xbin,ybin]!="")
dwell[xbin,ybin]=(samp[xbin,ybin]/tsamp)*ttime

printf("%3s %3s  %7.3f  ", xbin,ybin,dwell[xbin,ybin]) > outfile

for(c=1;c<=cmax;c++)     {
     x=0
     if(dwell[xbin,ybin]!=0) x=spike[xbin,ybin,c]/dwell[xbin,ybin]
     printf("  %6.3f",x) > outfile
                    }
print""> outfile
}}}
close (outfile)
print"     * data sent to \""outfile"\""
print"\n    Cluster    Id   Spikes  Meanrate\n"
for(c=0;c<=12;c++) {
      if(id[c]>="0") printf("     %3s    %4s    %4s
%8.3f\n",c,id[c],cspike[c],crate[c])}
status=status"E"
return
}


function PLOT1(basefile)       {
#########################################################################
if(basefile=="") {print"\[ERROR]: set input file first!";return}

FS=","
infile=basefile".txt"
outfile=basefile"_posall.tmp"

print"\n    PLOT ALL POINTS VISITED IN "infile
print"    ------------------------------------------------------------"
print "     * reading \""infile"\""
while((getline $0 < infile) > 0)     {
 if($1=="P")             {print $3,$4 > outfile}
                              }
close (infile)
close (outfile)

infile=outfile
outfile="./plot.tmp"
print "#! \/usr\/local\/bin\/gnuplot\n" > outfile
print "set bar 0.05\nset key\nset bmargin 3\nset lmargin 3\n" > outfile
print "set xlabel \"x-coordinate\" 0,-2" > outfile
print "set ylabel \"y-coordinate\" -2, 0" > outfile
print "set xtics axis mirror 50" > outfile
print "set ytics axis mirror 50" > outfile
print "set xrange [0:255]" > outfile
print "set yrange [0:255]" > outfile
print "set nogrid" > outfile
```

```
print "set key outside" > outfile
print "plot \\"    > outfile
print "\""infile"\" using 1:2 with dots"    > outfile

print "    * generating plot "basefile"_posplot.fig"
print "set term fig color" > outfile
print "set output \""basefile"_posplot.fig\"" > outfile
print "plot \\" > outfile
print "\""infile"\" using 1:2 with dots"    > outfile


print "\npause -1 \"* clear plot\"" > outfile
print "     quit" > outfile
close (outfile)
command="chmod u+x "outfile;system(command);system(outfile)
return
}



function PLOT2(basefile)       {
#########################################################################
# Plot position of specific spikes
#########################################################################
if(basefile=="") {print"\[ERROR]: set input file first!";return}

FS=" ";split("",tmp);y=0
infile=basefile".spk"
print"\n    PLOT SPIKE POSITIONS"
print"    -------------------------"
print "    * reading \""infile"\""
FS=" "
while((getline $0 < infile) > 0)     {
      if($2>=0.001)                  {
                                     {
                  outfile=basefile"_spk"$4".tmp"
                  tmp[$4]=1
                  printf("%9s %9s %3s\n",$2,$3,$4) > outfile
                                     }
                                     }
                                     }
close (infile); for(x in tmp) {outfile=basefile"_spk"x".tmp";close
(outfile)}

printf("    * executing spike plot of clusters ")
for (x=0;x<=14;x++) if(x in tmp) {y++;printf(x", ")}
print""
z=-1
outfile="./plot.tmp"
print "#! \/usr\/local\/bin\/gnuplot\n" > outfile
print "set bar 0.05" > outfile
print "set bmargin 3\nset lmargin 3\n" > outfile
print "set xlabel \"x-coordinate\" 0,-2" > outfile
print "set ylabel \"y-coordinate\" -2, 0" > outfile
print "set xtics axis mirror 10" > outfile
print "set ytics axis mirror 10" > outfile
```

```
print "set xrange [0:101]" > outfile
print "set yrange [0:101]" > outfile
print "set nogrid" > outfile
print "set nokey" > outfile
print "" > outfile
z=-1
print "    * generating plot "basefile"_spkplot.fig"
print "set term fig color portrait inches size "8.5*(y/2)" "11*(y/2) >
outfile
print "set output \""basefile"_spkplot.fig\"" > outfile
print "set multiplot" > outfile
print "set size "(0.5/y)","(0.5/y) > outfile
for (x=0;x<=15;x++) if(x in tmp) {{
        z++; infile=basefile"_spk"x".tmp"
        if(y<=1)   {
            print"set origin 0,0" > outfile
            print "set title \""infile"\"" > outfile
                  }
        if(y>=2)   {
            print"set origin "z*(1/(y+1.75))",.6" > outfile
            print "set title \""infile"\"" > outfile
                  }
        print "plot \""infile"\" using 1:2 with dots" > outfile
                          }}
print "set nomultiplot\n" > outfile
close (outfile)
command="chmod u+x "outfile;system(command);system(outfile)
return
}



function PLOT3(basefile,xbintot,ybintot) {
############################################################
# read means data, convert to proportion of max rate
# sort means for xy bins on scale of 1-10
# assign colors
# generate fig file
############################################################
FS=" "
xorig=100;yorig=100
lt=0;lw=1;size=250
color[1]="000000"
color[2]="000088"
color[3]="0000ff~"
color[4]="00979d"
color[5]="00c668"
color[6]="00ff00"
color[7]="caff00"
color[8]="fff382"
color[9]="ffffc8"
color[10]="ffffff"

print"\n    GENERATE XFIG PLOT OF CLUSTER FIRING RATES"
print"    ----------------------------------------"
if(basefile=="") {print"\[ERROR]: set input file first!";return}
if(xbintot=="")  {print"\[ERROR]: set x-bins and y-bins first!";return}
```

```
print"    * Plot of "basefile".bin in a "xbintot"x"ybintot" matrix."
close ("/dev/tty")

outfile=basefile"_rateplot.fig"
print"    * generating xfig file \""outfile"\""
print"#FIG 3.2" > outfile
print"Portrait" > outfile
print"Flush left" > outfile
print"Inches" > outfile
print"Letter" > outfile
print"100.00" > outfile
print"Single" > outfile
print"-2" > outfile
print"1200 2" > outfile
for(z=1;z<=10;z++) print"0 "31+z" #"color[z] > outfile

infile=basefile".sta"
print"    * reading parameters from \""infile"\""
z=0
while((getline $0 < infile) > 0)    {
      z++
        if(z==1)
{xmin=$1;ymin=$3;xmax=$5;ymax=$7;tsamp=$9;tspike=$11;ttime=$13;continue}
        if($1!="") {c=$1;id[c]=$2;cspike[c]=$3;crate[c]=$4}
                                          }
close (infile)

for(origcluster=0;origcluster<=11;origcluster++)       {
if(id[origcluster]<=0) continue

cluster=(id[origcluster]+3)
infile=basefile".bin"
cmax=0

print"    * reading cluster "origcluster" firing rates from
\""infile"\""
while((getline $0 < infile) > 0)                  {
      x=$1;y=$2;rate[x,y]=$cluster
      if(cmax<=$cluster) cmax=$cluster
                                       }
close (infile)

xmin=xorig+size;ymin=yorig+size
xmax=xorig+((xbintot+1)*size);ymax=yorig+((ybintot+1)*size)
print"6 "xmin" "ymin" "xmax" "ymax > outfile

xbin=ybin=0
modrate=int((rate[x,y]/(cmax+0.001))*10)+1
print"2 2 "lt" "lw" 4 "31+modrate" 0 9 20 0.0000 0 0 -1 0 0 5" > outfile
printf("      ") > outfile
printf(xorig+(xbin*size)" "yorig+(ybin*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(ybin*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(ybin*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(ybin*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(ybin*size)"\n") > outfile

for(xbin=1;xbin<=xbintot;xbin++) {
```

```
for(ybin=1;ybin<=ybintot;ybin++) {

y=ybintot+1-ybin
modrate=int((rate[xbin,ybin]/(cmax+0.001))*10)+1
print"2 2 "lt" "lw" 4 "31+modrate" 0 9 20 0.0000 0 0 -1 0 0 5" > outfile
printf("      ") > outfile
printf(xorig+(xbin*size)" "yorig+(y*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(y*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(y*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(y*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(y*size)"\n") > outfile
}
}
print"-6" > outfile
cluster=origcluster
printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s Cluster %s, %s %c001\n",
      xmin,ymax+(1*size),cluster,basefile,92) > outfile
printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s Maxrate = %s Hz%c001\n",
      xmin,ymax+(2*size),cmax,92) > outfile
printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s Meanrate= %s Hz%c001\n",
      xmin,ymax+(3*size),crate[cluster],92) > outfile


xorig+=(xbintot+2)*size
}

for(z=1;z<=10;z++)      {
      print"2 2 "lt" "lw" 4 "31+z" 0 9 20 0.0000 0 0 -1 0 0 5" > outfile
      printf("      ") > outfile
      printf(xmax+size  " "yorig+(z*size)" ") > outfile
      printf(xmax+2*size" "yorig+(z*size)" ") > outfile
      printf(xmax+2*size" "yorig+(z*size)+size" ") > outfile
      printf(xmax+size  " "yorig+(z*size)+size" ") > outfile
      printf(xmax+size  " "yorig+(z*size)"\n") > outfile
                  }
print"6 "xmax+size" "yorig+(size)" "xmax+2*size" "yorig+(10*size)+size >
outfile
for(z=1;z<=10;z++)      {
      printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s %2s-%2s%c of
max%c001\n",
            xmax+2.5*size,yorig+(z*size)+(0.75*size),(z-
1)*10,z*10,37,92) > outfile
                  }
close (outfile)



return
}


function FILE()    {
###############################################################################
####
print"\n    SET ORIGINAL FILENAME"
print"    ---------------------"
printf("    * Input filename: "); getline temp < "/dev/tty"
```

```
      if(temp=="") {print"     * invalid filename: ABORTED\n\n";return}
      close ("/dev/tty")

command="ls -l > file.tmp";system(command)
match(temp,"\\.");z=RSTART;if(z<=0)z=length(temp)+1;y=substr(temp,1,z-1)
infile="file.tmp"
status=""
while((getline $0 < "file.tmp") > 0)       {
      if($9==temp) {status=status"A"; print"    * Original datafile
found"}
      if($9==y".sta") {status=status"B"; print"    * Statistics file
found"}
      if($9==y".spk") {status=status"C"; print"    * Spike data
compiled"}
      if($9==y".pos") {status=status"D"; print"    * Position data
compiled"}
      if($9==y".bin") {status=status"E";z=y".bin"
            while((getline $0 < z) > 0) {xbintot=$1;ybintot=$2}
            close (z)
            print"    * Bin-sort completed: xbins="xbintot",
ybins="ybintot
                  }
      if($9==y".rot") {status=status"F"; print"    * Rate map rotation
performed"}
                              }
close ("file.tmp")
if(status=="") {print "    * ERROR - No files found matching
"temp;return}
match(status,"E");if(RSTART<=0)xbintot=ybintot=""
origfile=temp
basefile=y
return
}


function BINS()    {
##########################################################################
####
print"\n    SET NUMBER OF BINS TO DIVIDE ENVIRONMENT INTO"
print"    ------------------------------------------"
printf("    * Total x-bins: "); getline x < "/dev/tty"
      if(x=="") {print"     * invalid x-bins: ABORTED\n\n";return}
printf("    * Total y-bins: "); getline y < "/dev/tty"
      if(y=="") {print"    * invalid y-bins: ABORTED\n\n";return}
printf("    * Proceed (y/n)? "); getline GO < "/dev/tty"
       if(GO!="y") {printf("    * ABORTED\n\n"); return}
close ("/dev/tty")
z=0;if(x!=xbintot || y!=ybintot) z=1
xbintot=x;ybintot=y
if(z==1) PLACE2(basefile,xbintot,ybintot)
return
}


function ROTATE(basefile)      {
##########################################################################
####
```

```
# Make rotated versions of place field maps
# Input = .bin files (output from PLACE program)...
#      $1 = xbin
#      $2 = ybin
#      $3 = dwelltime in that bin
#      $4 = firing rate for cluster in that bin
#
# Output on each line...  xbin ybin R  0deg 90deg 180deg 270deg
#######################################################################
print"    MAKE ROTATED VERSIONS OF RATE MAP"
print"    --------------------------------"
if(basefile=="") {print"    [ERROR]: set input file first!";return}
match(status,"E")
if(RSTART<=0)    {print"    [ERROR]: create rate (.bin) file
first!";return}

split("",rate1);split("",rate2);split("",rate3);split("",rate4)
infile=basefile".bin"
outfile=basefile".rot"
print"    * reading min/max bins from "infile
while((getline $0 < infile) > 0)     {xmax=$1;ymax=$2}
close (infile)
print"    * creating outfile "outfile
while((getline $0 < infile) > 0)     {
      xbin=$1;ybin=$2;z=$4
      if(xbin==0) {print $0 > outfile; continue}
      rate1[xbin,ybin]=z
      rate2[ybin,xmax-(xbin-1)]=z
      rate3[xmax-(xbin-1),ymax-(ybin-1)]=z
      rate4[ymax-(ybin-1),xbin]=z
                                }
close (infile)
for(x=1;x<=xmax;x++)     {
for(y=1;y<=ymax;y++)     {
      printf("%3s %3s   %7.3f  %7.3f  %7.3f  %7.3f\n",
      x,y,rate1[x,y],rate2[x,y],rate3[x,y],rate4[x,y]) > outfile
                  }
                  }
close (outfile)
status=status"F"
return
}


function SPLIT(basefile)       {
#######################################################################
####
print"    SPLIT ORIGINAL DATAFILE ("basefile".txt) IN HALF"
print"    ------------------------------------------------"
if(basefile=="") {print"\[ERROR]: set input file first!";return}

FS=","
x=y=z=0
temp="h123456789"
infile=basefile".txt"
while((getline $0 < infile) > 0) {x++}
close (infile)
```

```
outfile=basefile"_a.txt"
print"    * sending 1st half to "outfile
z=0
while((getline $0 < infile) > 0) {
      y++
      if(y>=x/2) {
            outfile=basefile"_b.txt"
            if(z==0) {
                  close (outfile)
                  print"    * sending 2nd half to "outfile
                   }
            z=1
               }
      print $0 > outfile
                        }
close (infile); close (outfile)
return
}



function CLUSTERS(basefile)    {
###############################################################################
####
print"\n    * MODIFY CLUSTERS IN "origfile
print"    * ------------------------------------"
if(basefile=="") {print"\[ERROR]: set input file first!";return}

printf("    * Replace cluster#: "); getline x < "/dev/tty"
      if(x=="") {print"    * invalid cluster: ABORTED\n\n";return}
printf("    *              with: "); getline y < "/dev/tty"
      if(y=="") {print"    * Remove cluster #"x}
printf("    * THIS IS NON-REVERSIBLE! PROCEDE? ");getline z < "/dev/tty"
 if(z!="y") {print"    * ABORTED\n\n";return}
close ("/dev/tty")
z=0
FS=","
infile=origfile
outfile="file.tmp"
while((getline $0 < infile) > 0)     {
      if($1!="S" && $1!="E") {print $0 > outfile;continue}
      if($3!=x) {print $0 > outfile;continue}
      w++
      if(y=="") continue
      $3=y
      for(z=1;z<=NF-1;z++) printf($z",") > outfile
      print $z  > outfile
                              }
close (infile); close (outfile)
command="mv "outfile" "infile;system(command)
print "    * "w" replacements made"
return
}

function OPTIONS()       {
###############################################################################
####
```

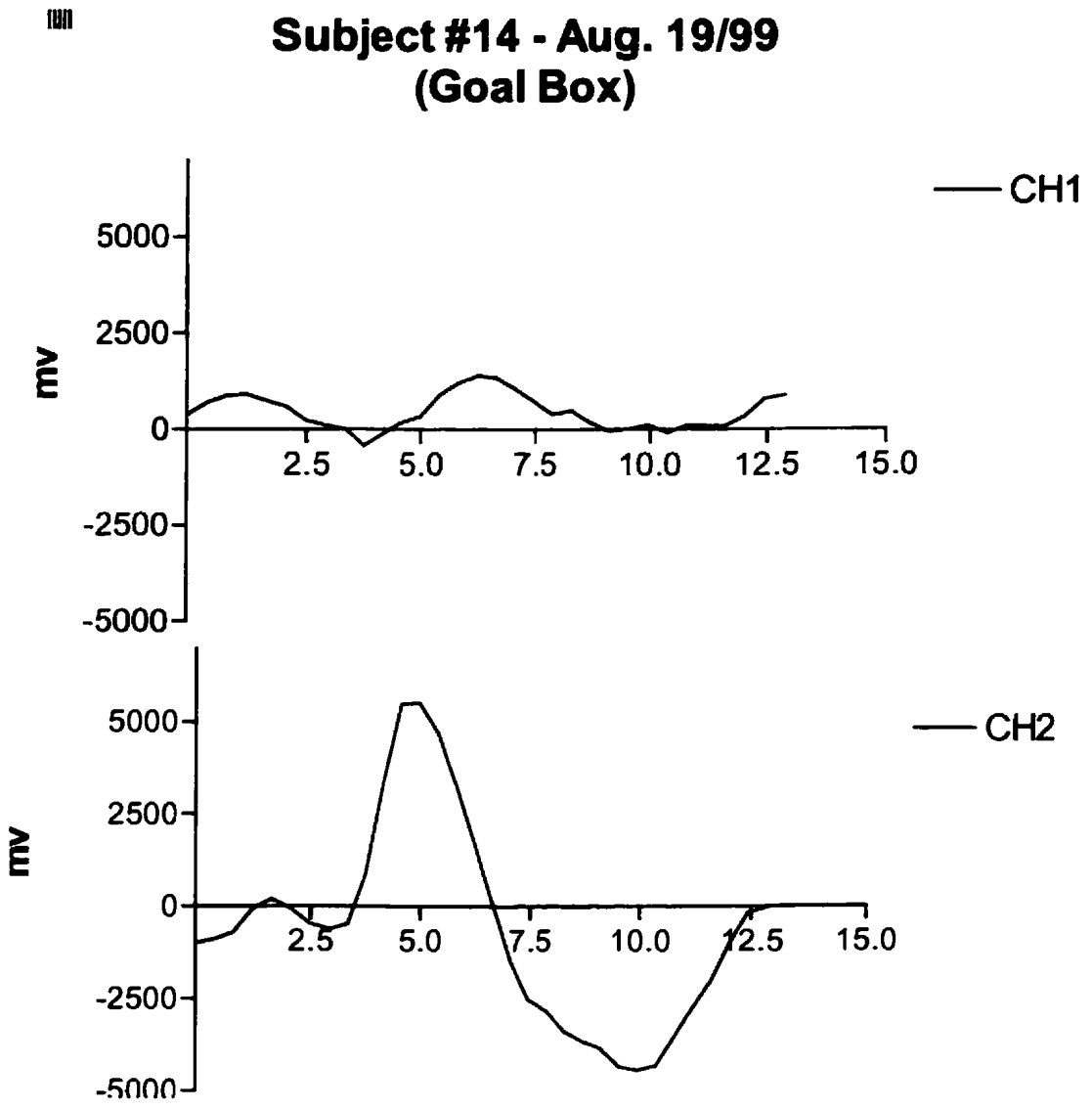
```
print ""
print"    1: read data file"
print"    2: calculate bin firing rates"
print""
print"    p1: plot all position samples"
print"    p2: plot spike positions by cluster"
print"    p3: generate rate map"
print""
print"    o: options"
print"    f: set input filename"
print"    c: modify clusters in original file"
print"    b: set x-bins & y-bins"
print"    ls: directory"
print"    r: create rotated versions of rate map"
print"    s: split original data file in two"
print"    q: quit"
}


function QUIT()    {
##########################################################################
####
print"\nQUIT PROGRAM\n";exit
}

function LS()            {
##########################################################################
####
print"";command=$0;system(command)
}


'
rm -f *_pos*.tmp
rm -f *_spk*.tmp
rm -f file.tmp
```

Appendix B

Electrophysiology

Cell Waveforms

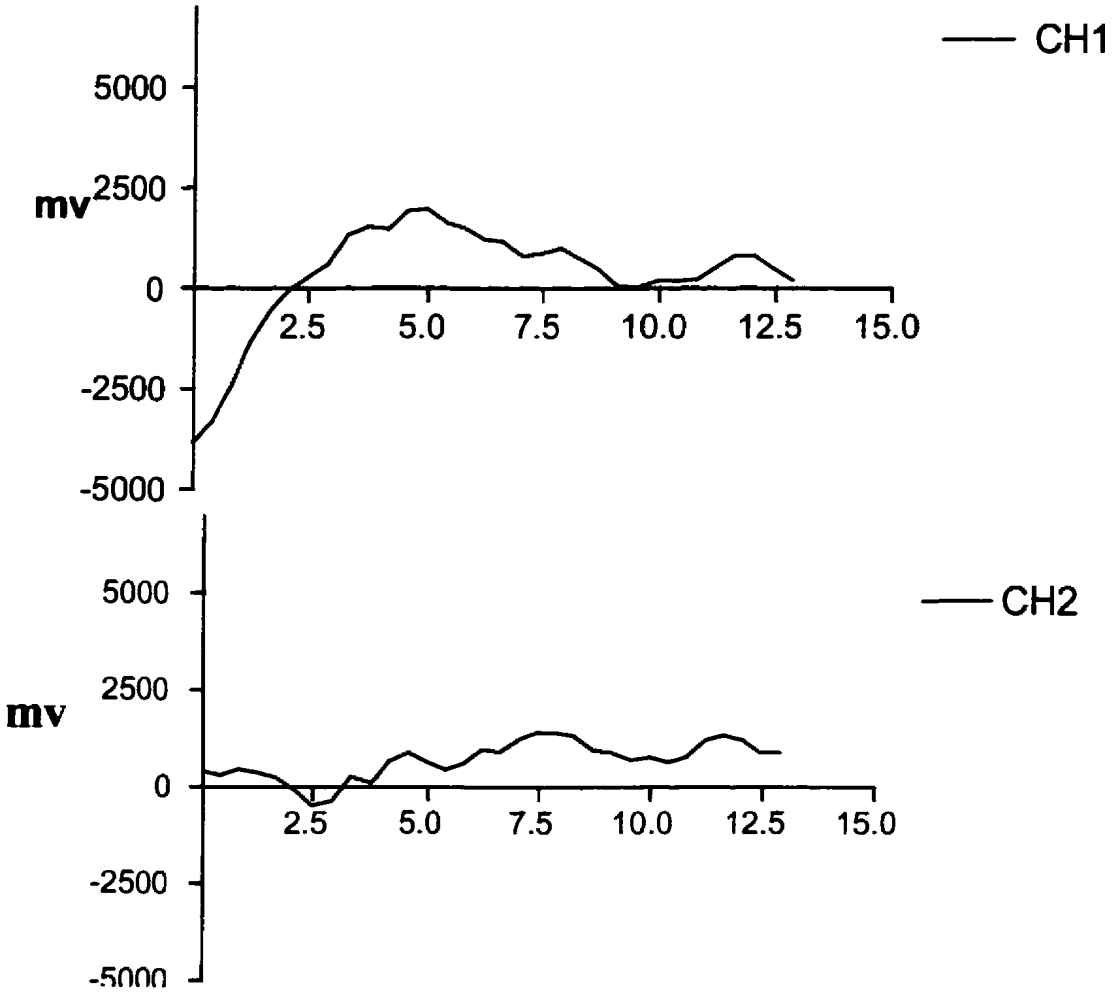
Note: Goal box wave forms
not used in analysis

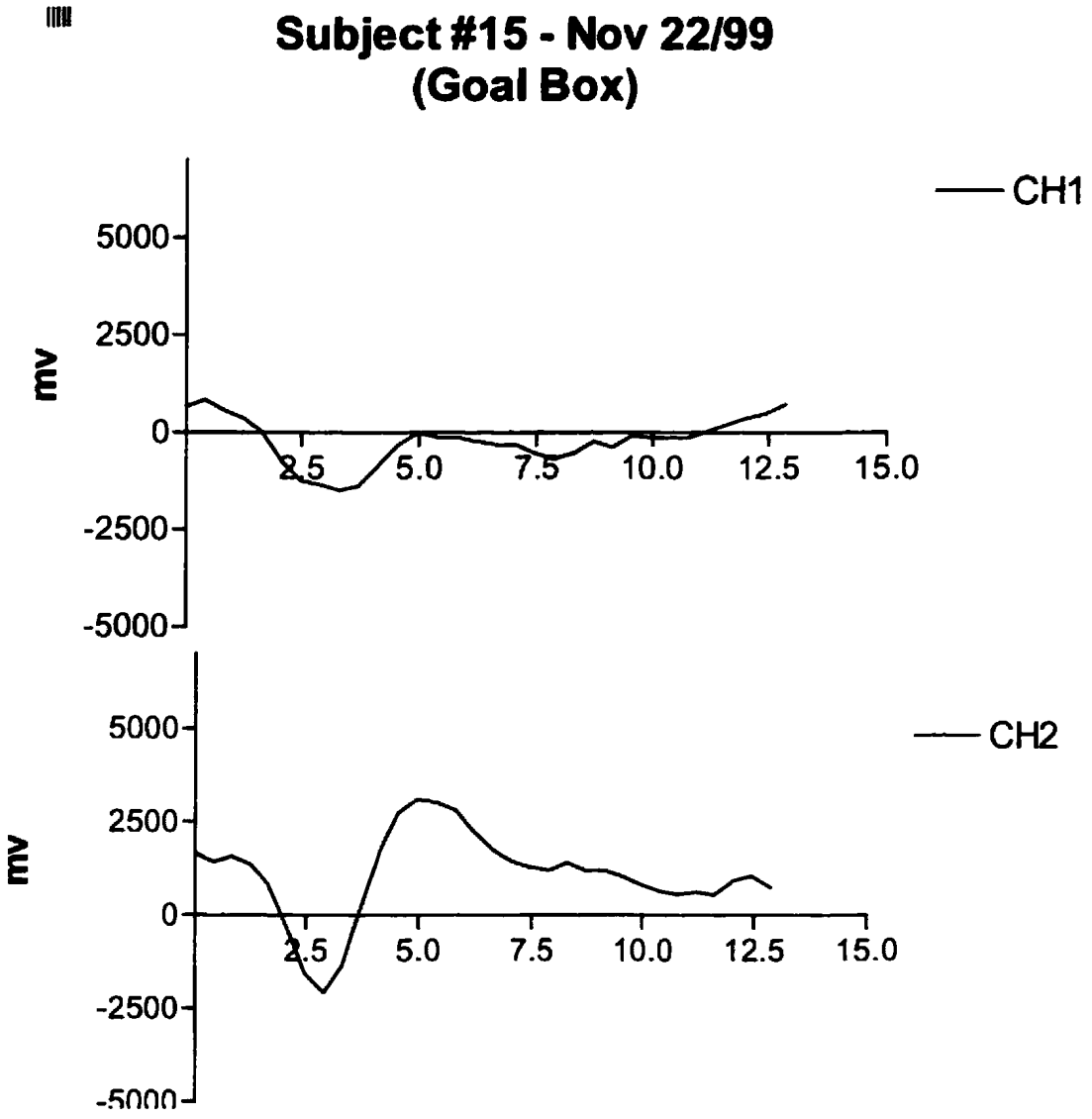

Subject #14 - Aug. 19/99
(Goal Box)
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0

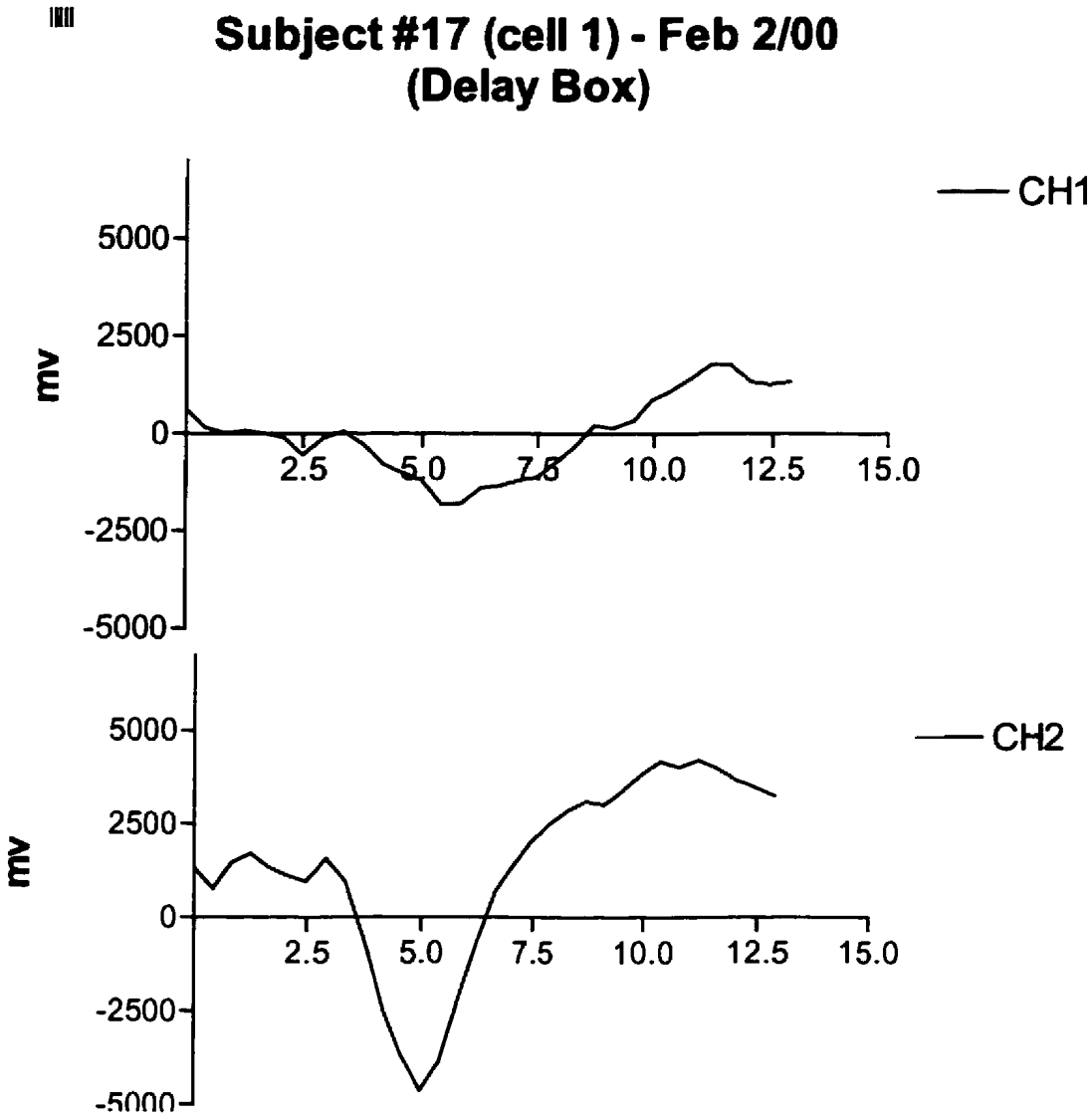
IIIII

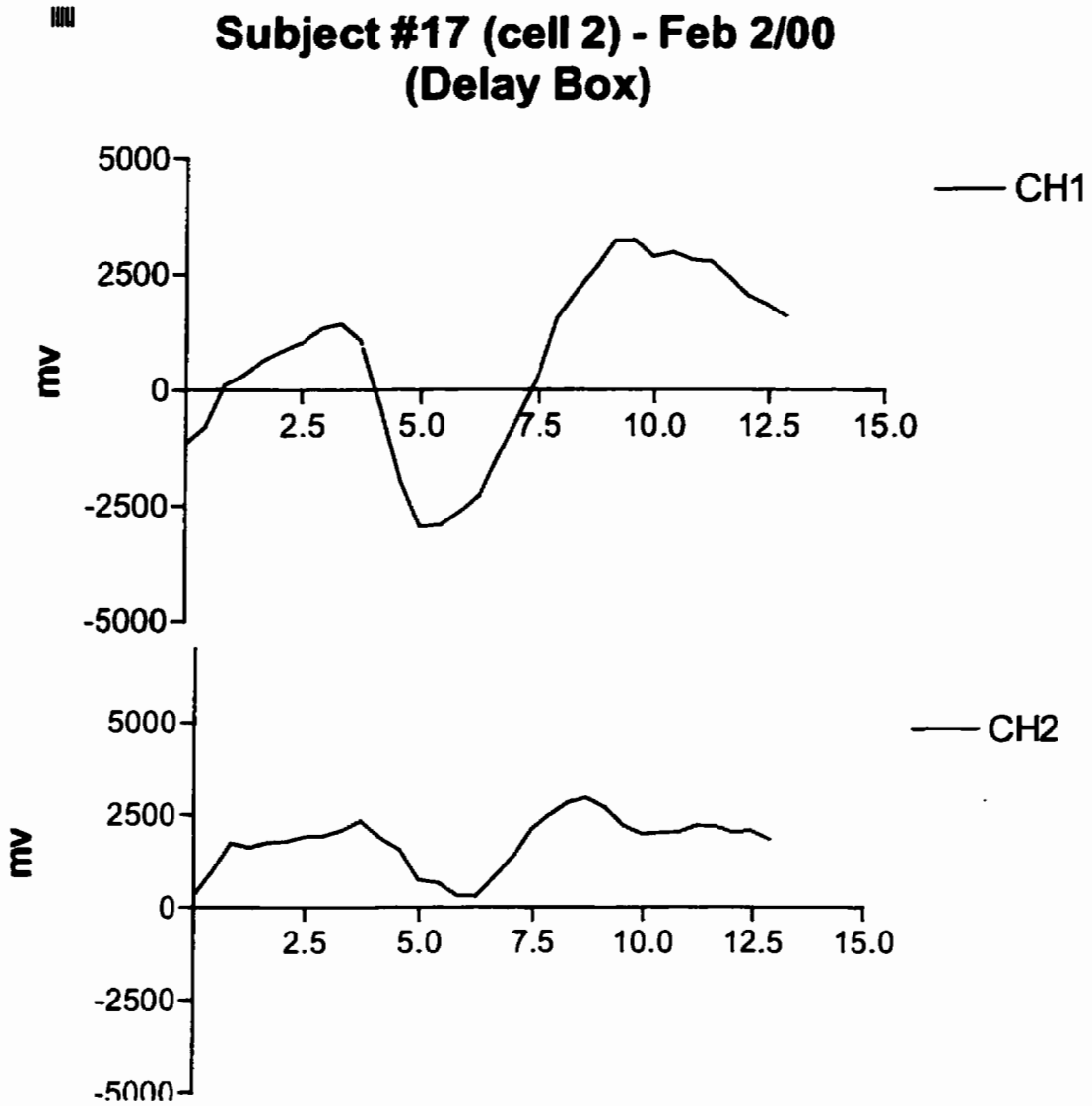
Subject #15 - Nov 25/99

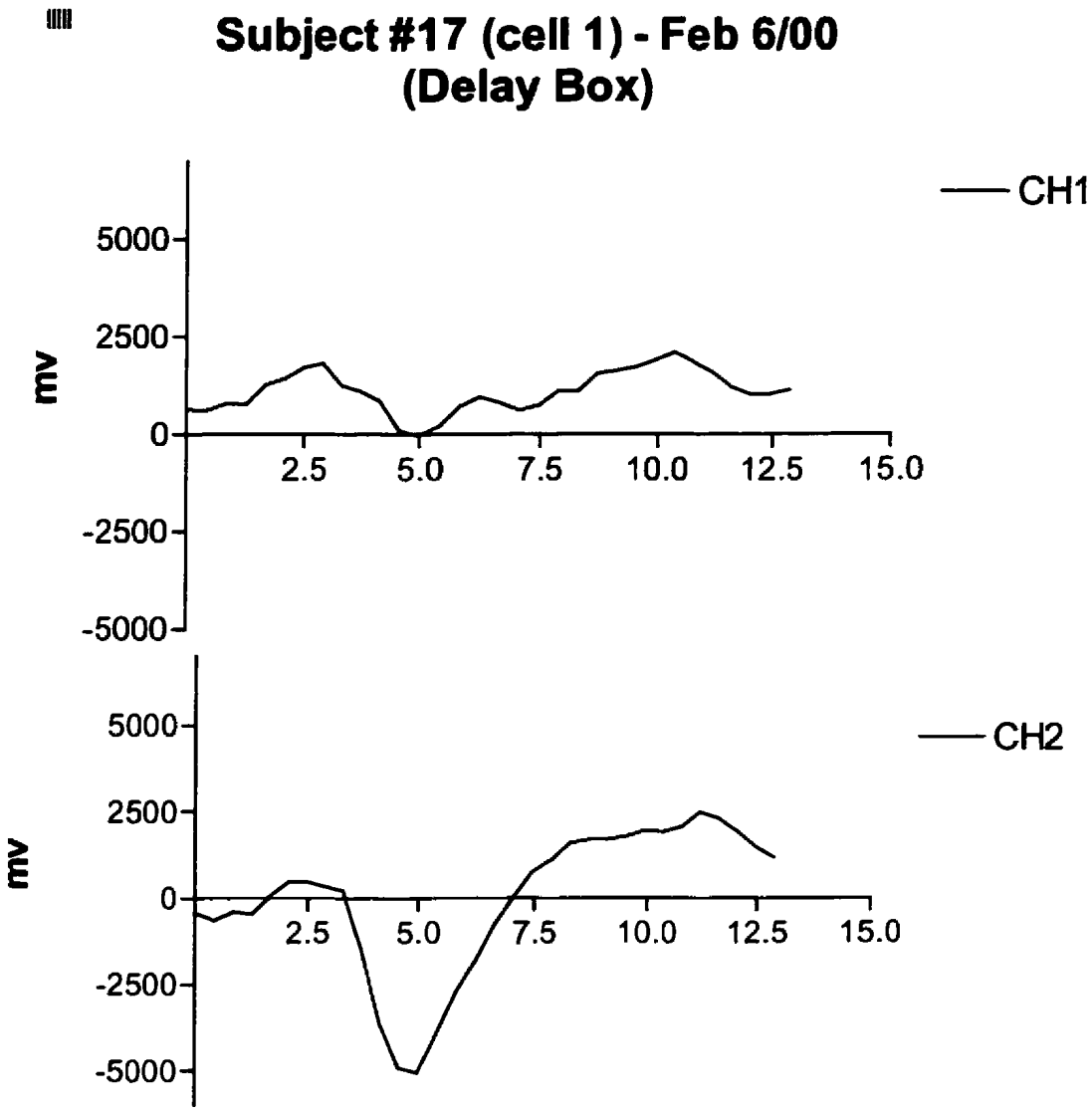
(Delay Box) note: only caught "upswing" of cell

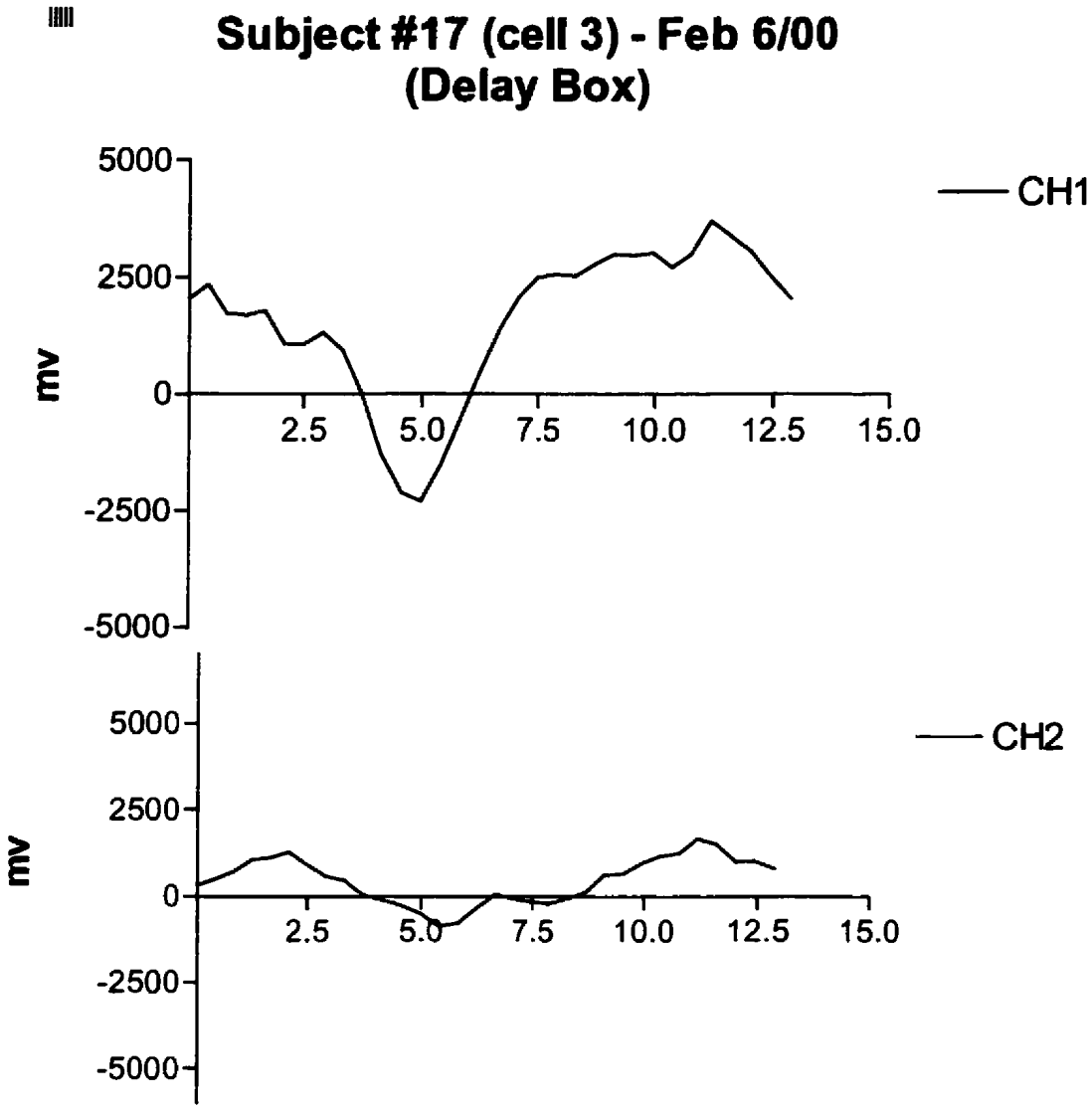


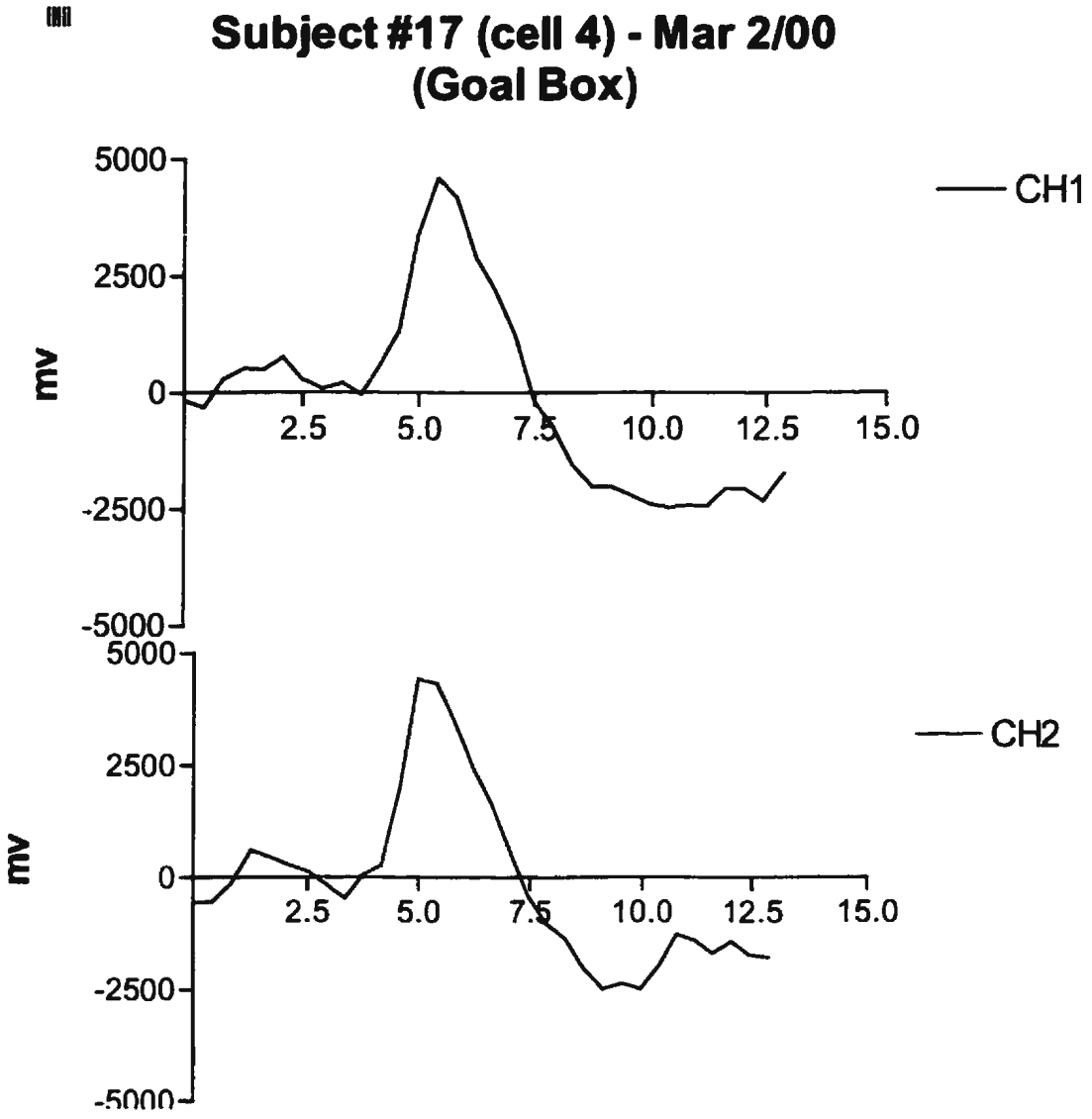

Subject #15 - Nov 22/99
(Goal Box)
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0

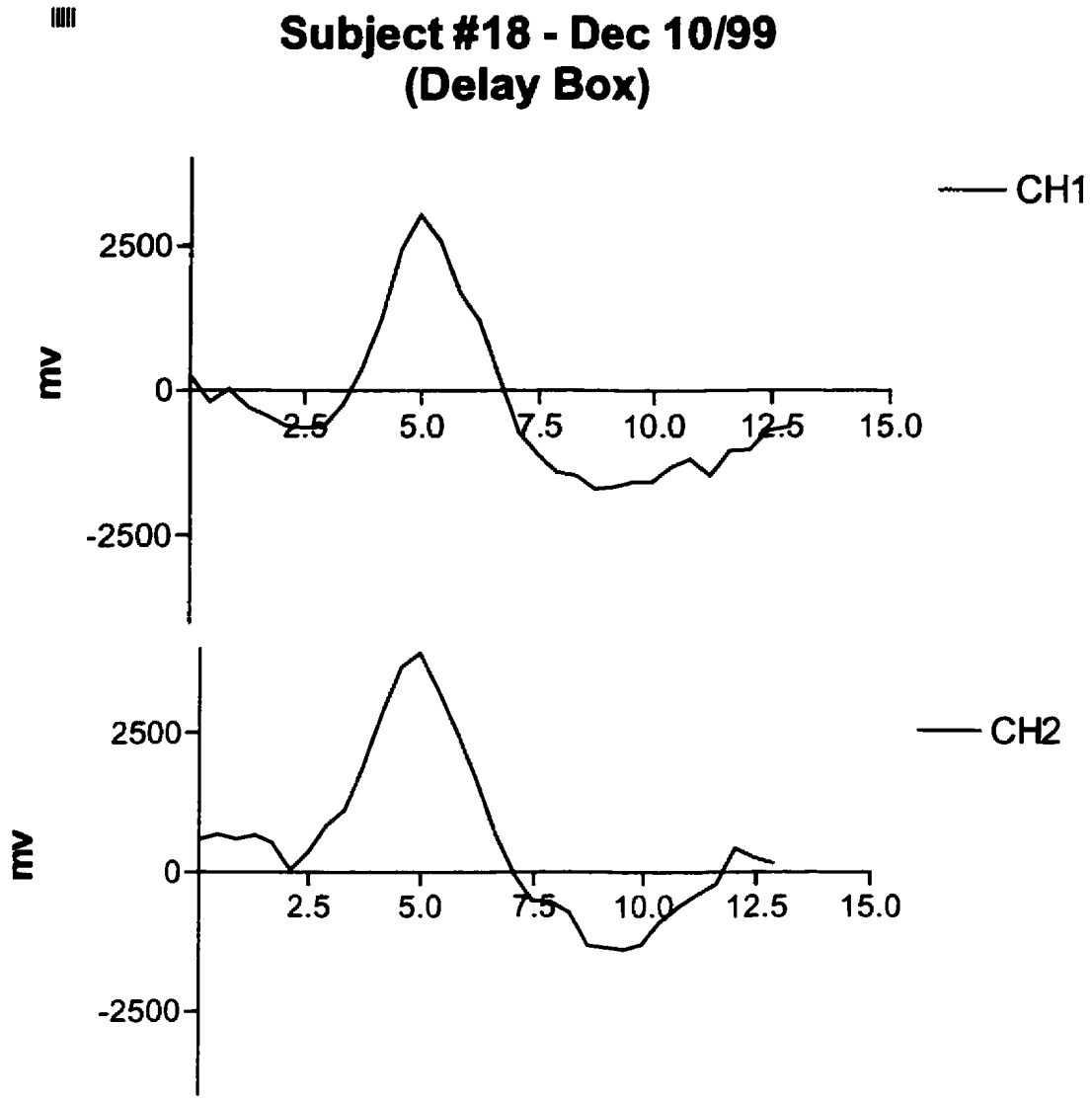

Subject #17 (cell 1) - Feb 2/00
(Delay Box)
CH1
CH2
mv
mv
5000
2500
0
-2500
-5000
2.5
5.0
7.5
10.0
12.5
15.0

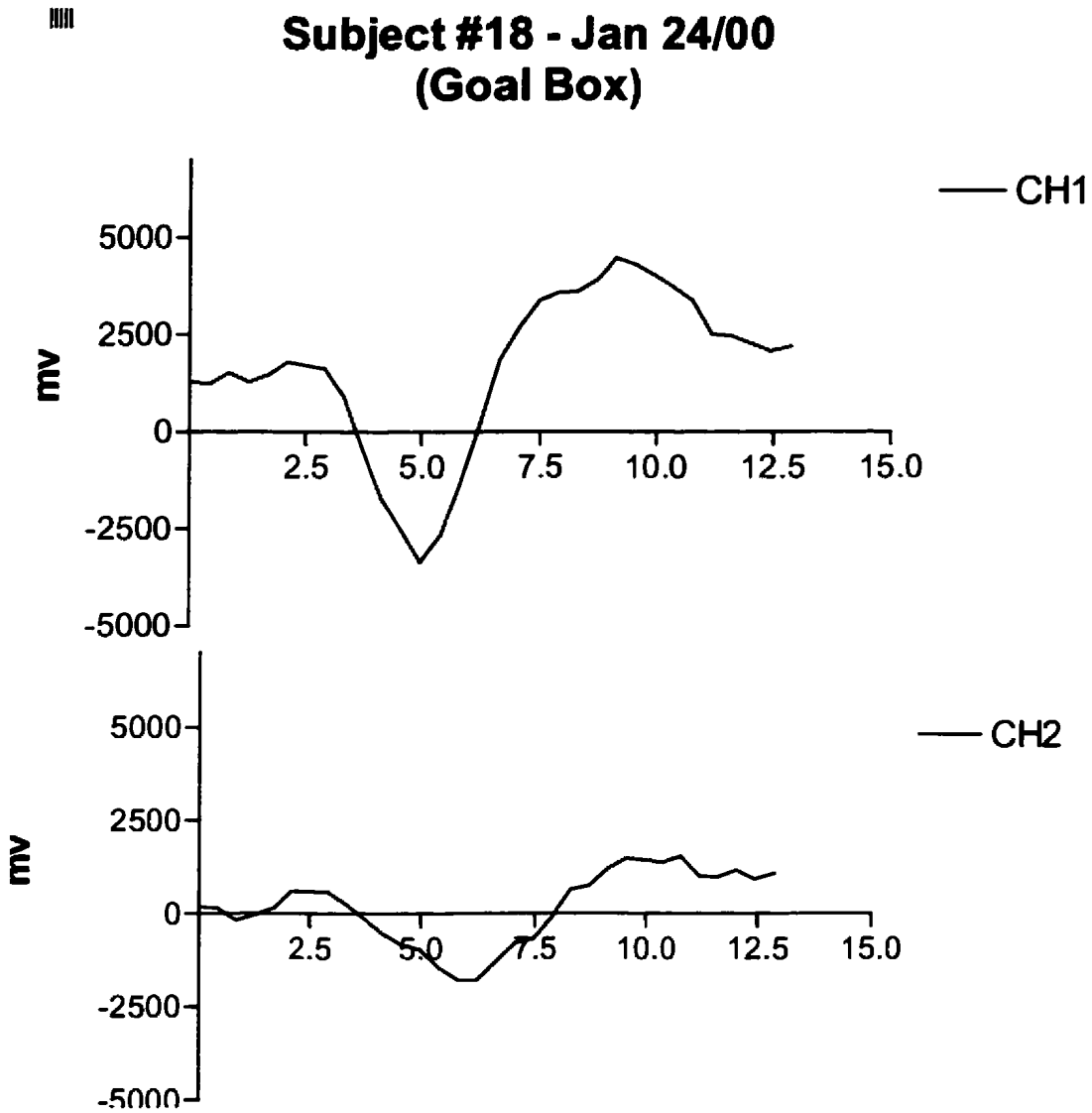

Subject #17 (cell 2) - Feb 2/00
(Delay Box)
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2


Subject #17 (cell 1) - Feb 6/00
(Delay Box)
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0


Subject #17 (cell 3) - Feb 6/00
(Delay Box)
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2

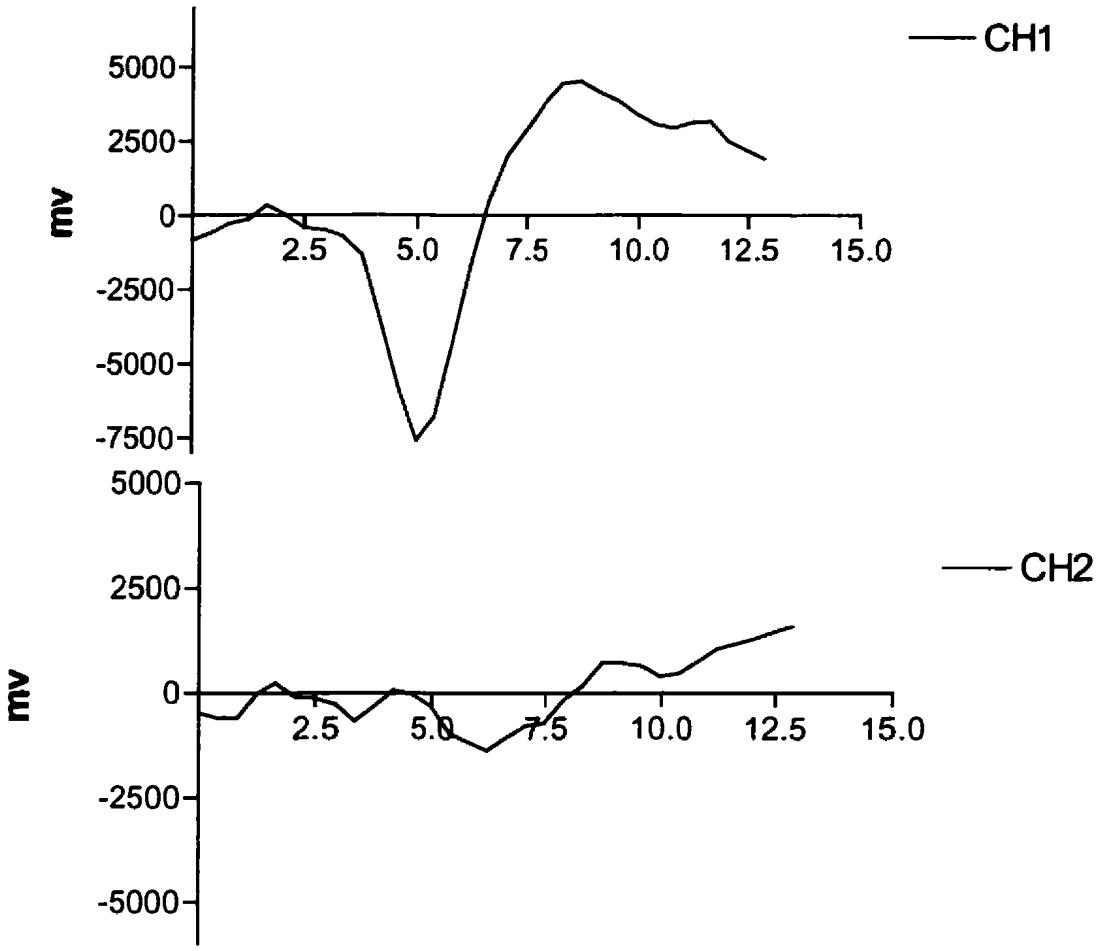

Subject #17 (cell 4) - Mar 2/00
(Goal Box)
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH1
CH2


Subject #18 - Dec 10/99
(Delay Box)
CH1
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0

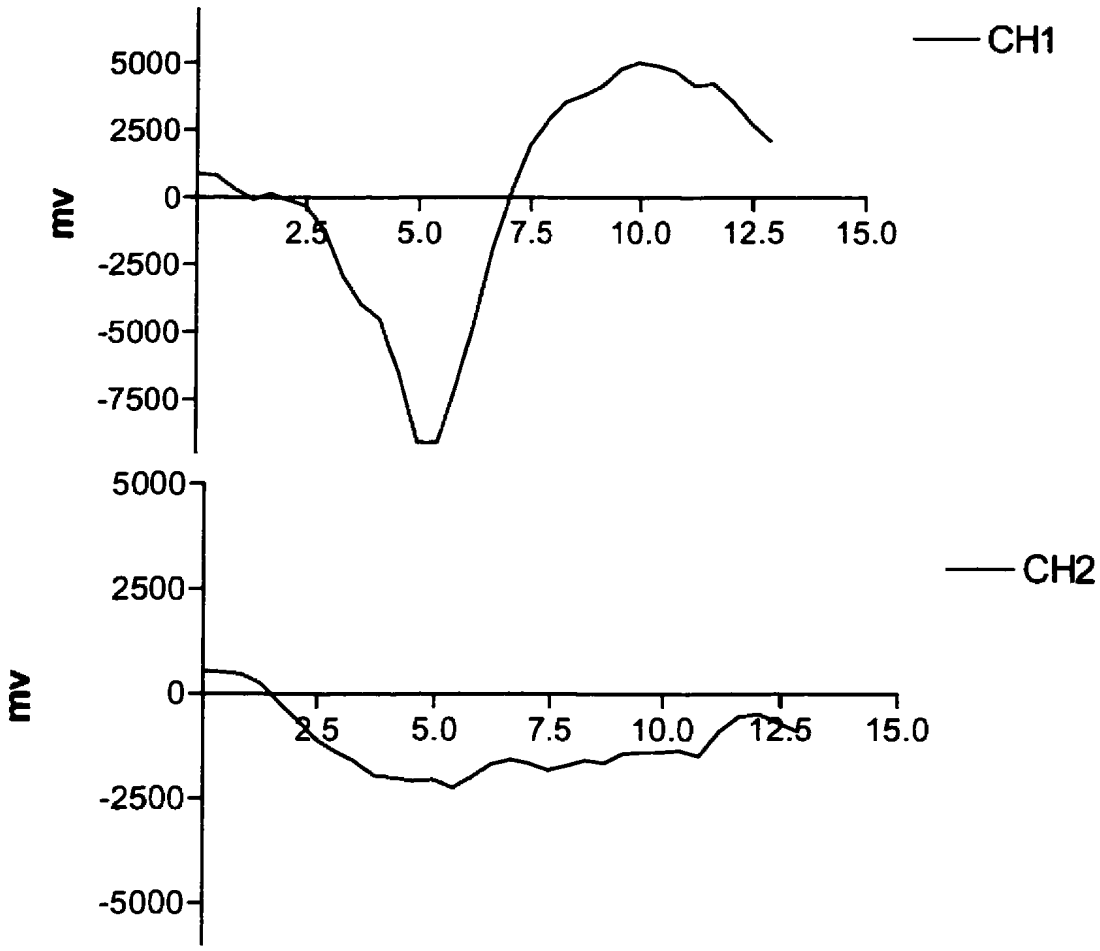

Subject #18 - Jan 24/00
(Goal Box)
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0

IIIII

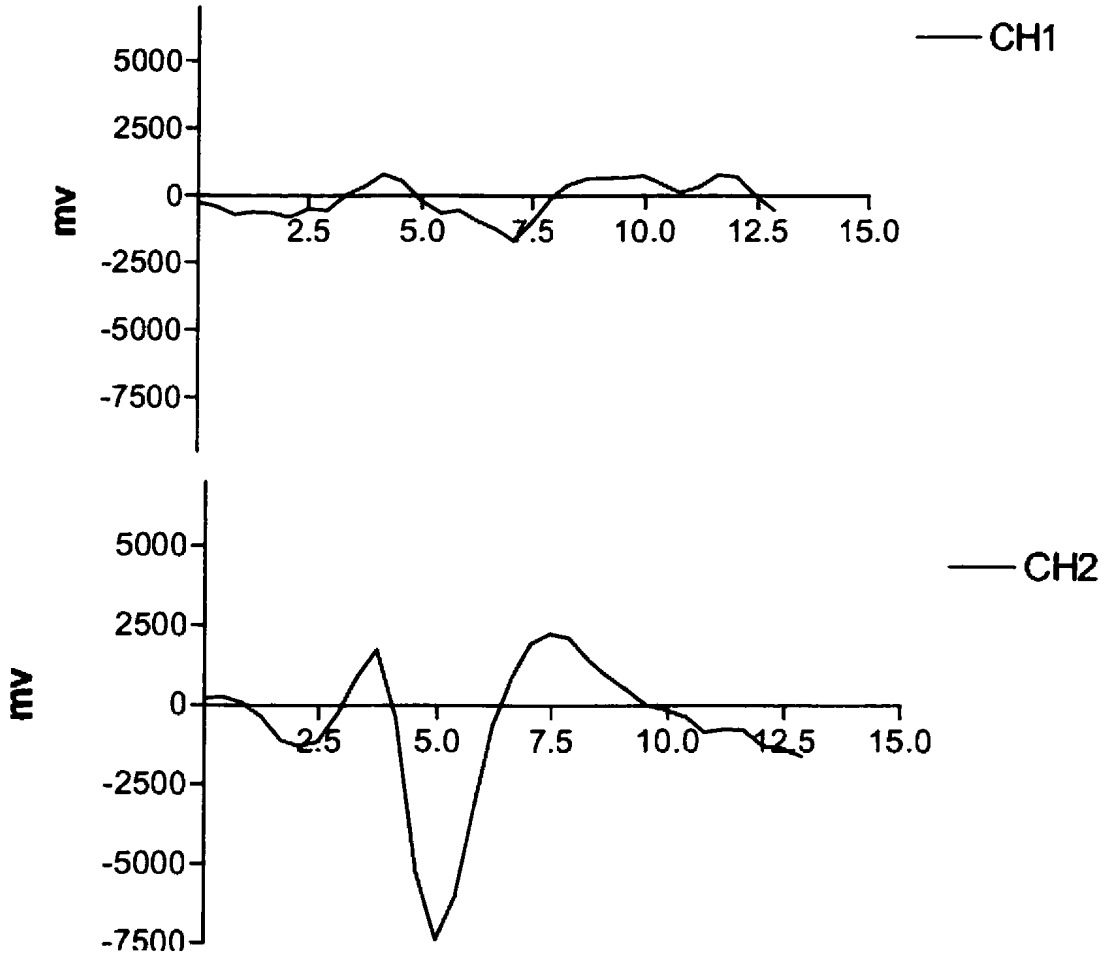
Subject #23 - Mar 23/00
(Delay Box)



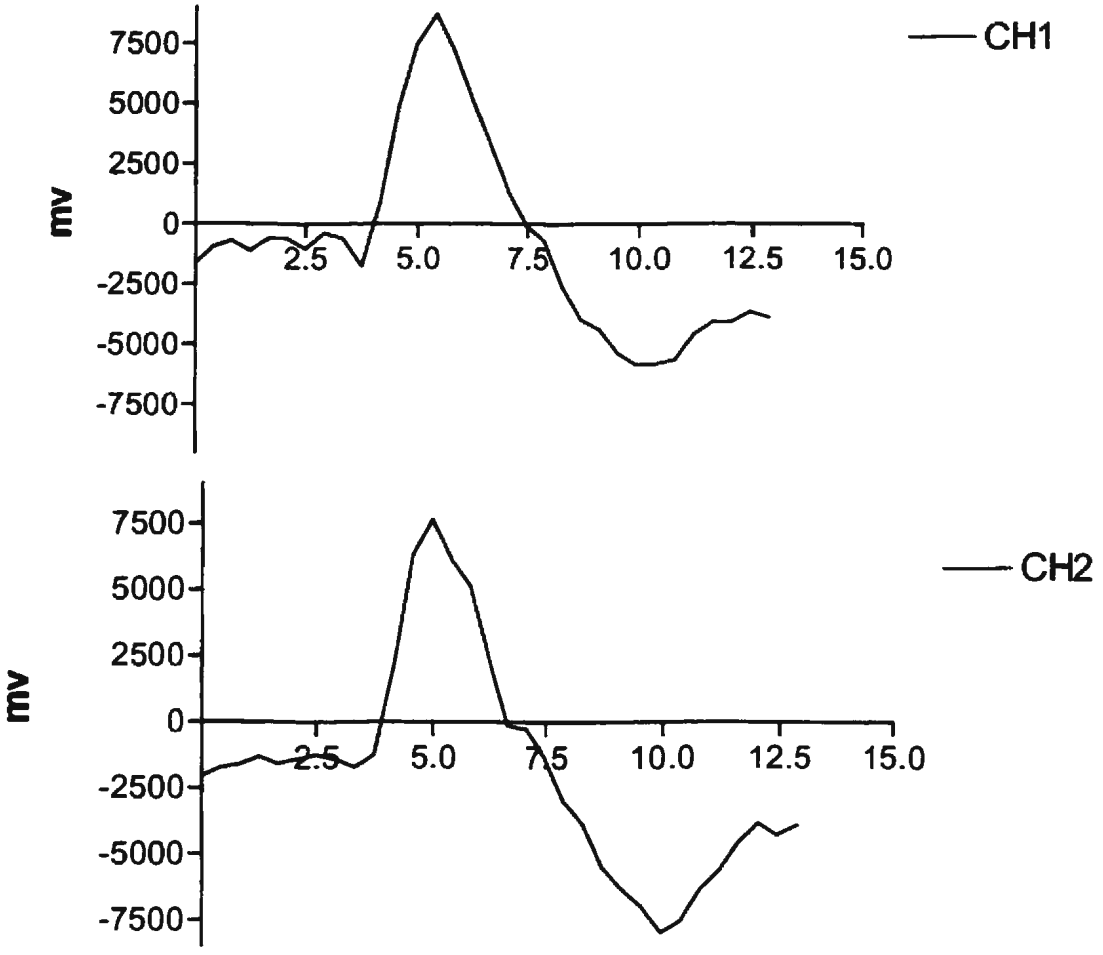
Subject #26 - Aug 29/00
(Delay Box)

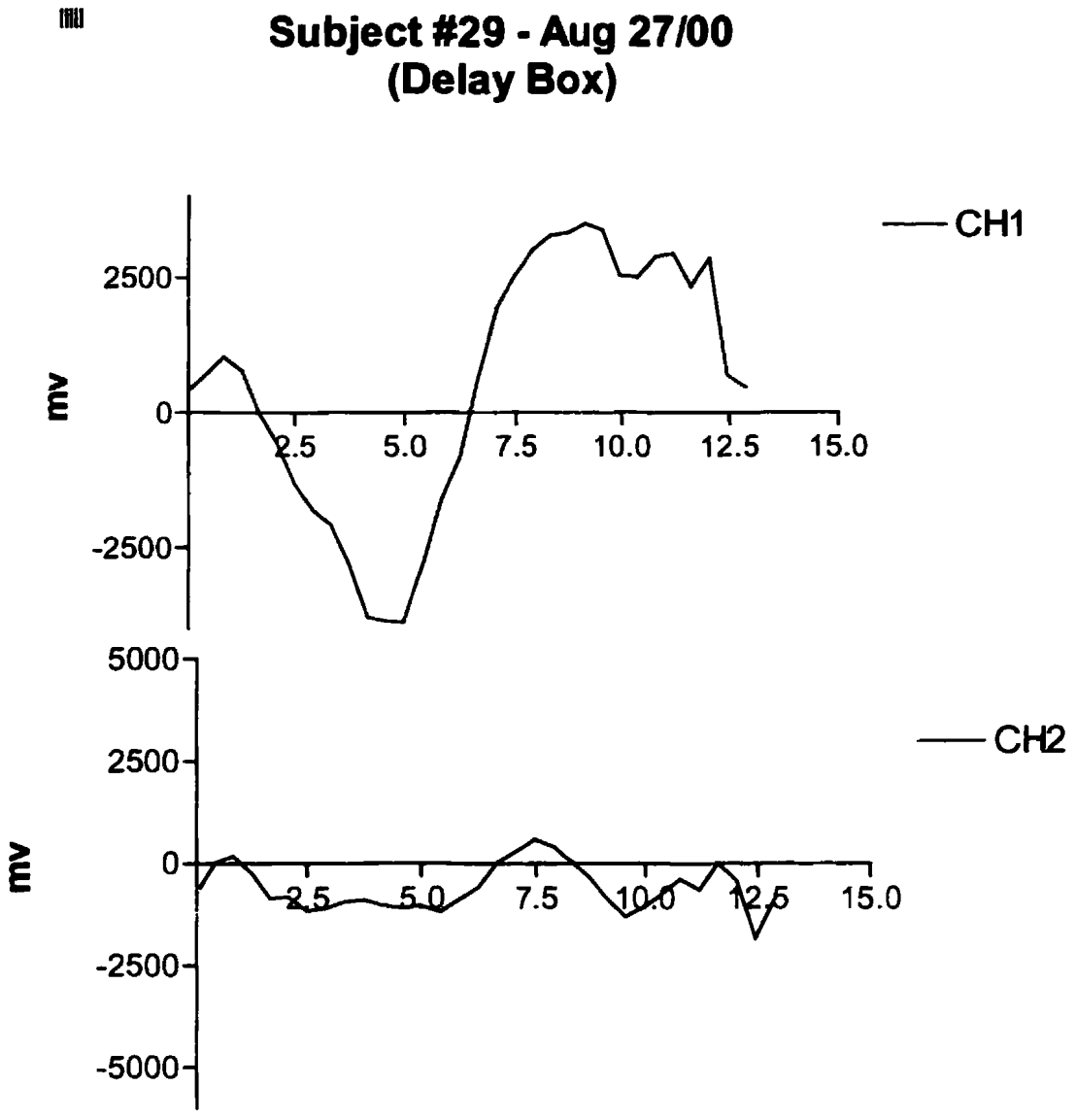


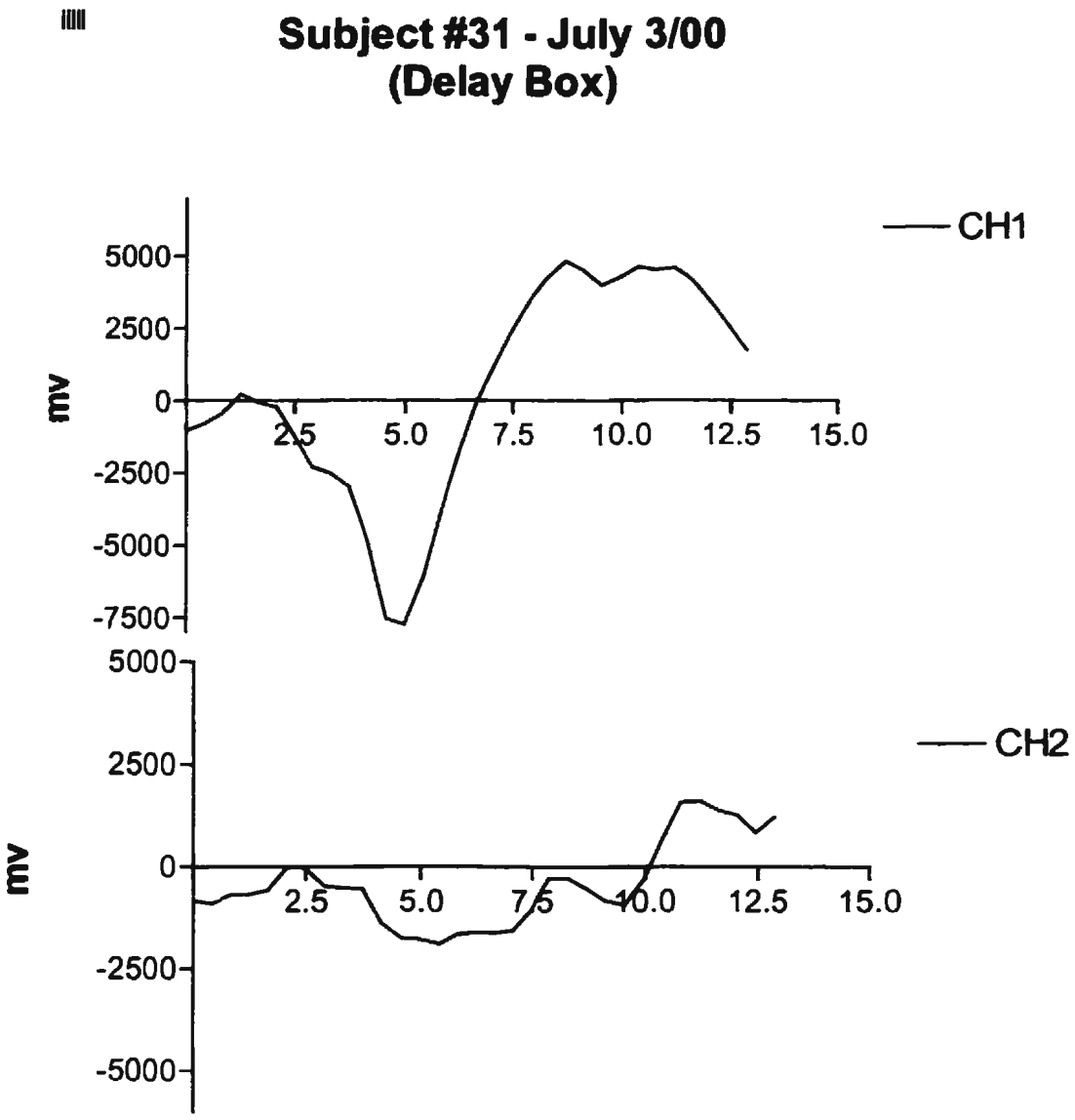
Subject #26 (Cell 2) - Sept 2/00
(Delay Box)



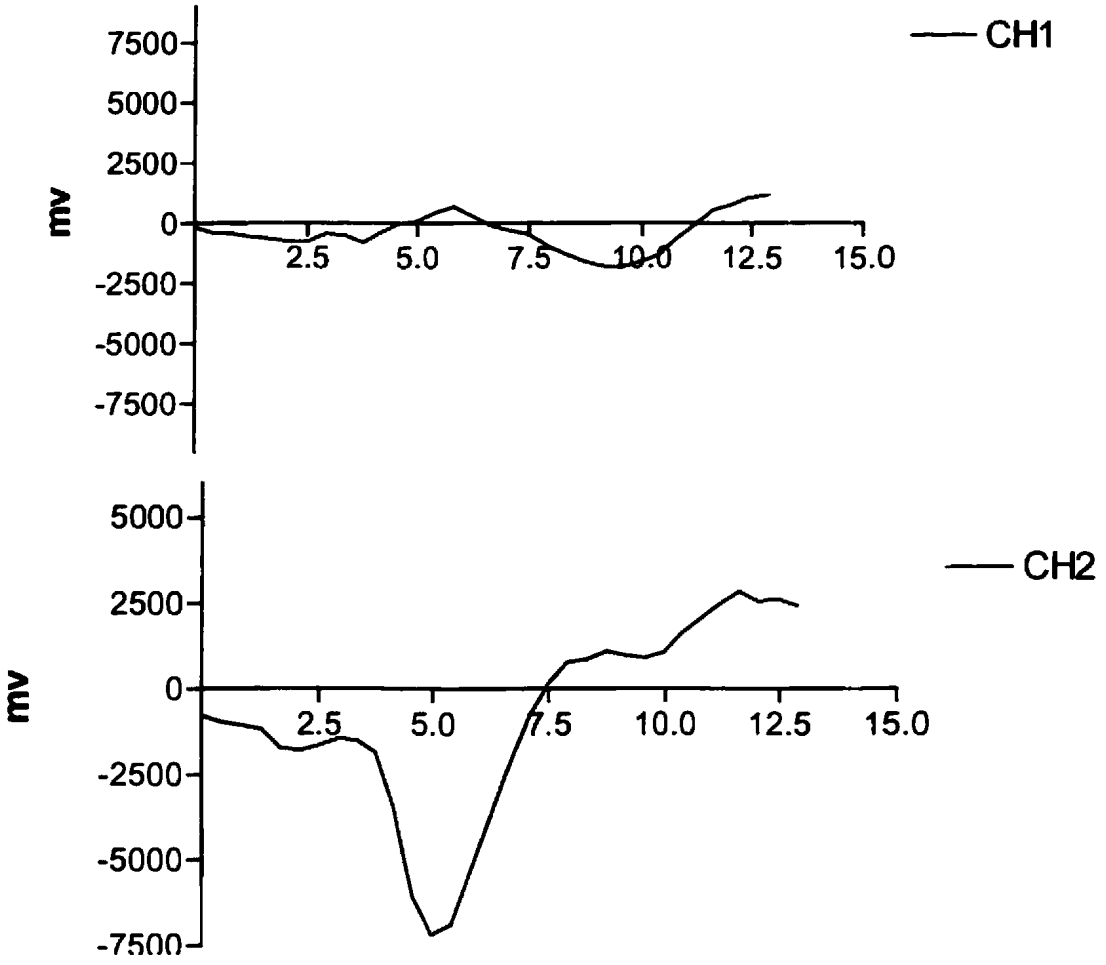
Subject #26 (Cell 3) - Sept 6/00 (Goal Box)

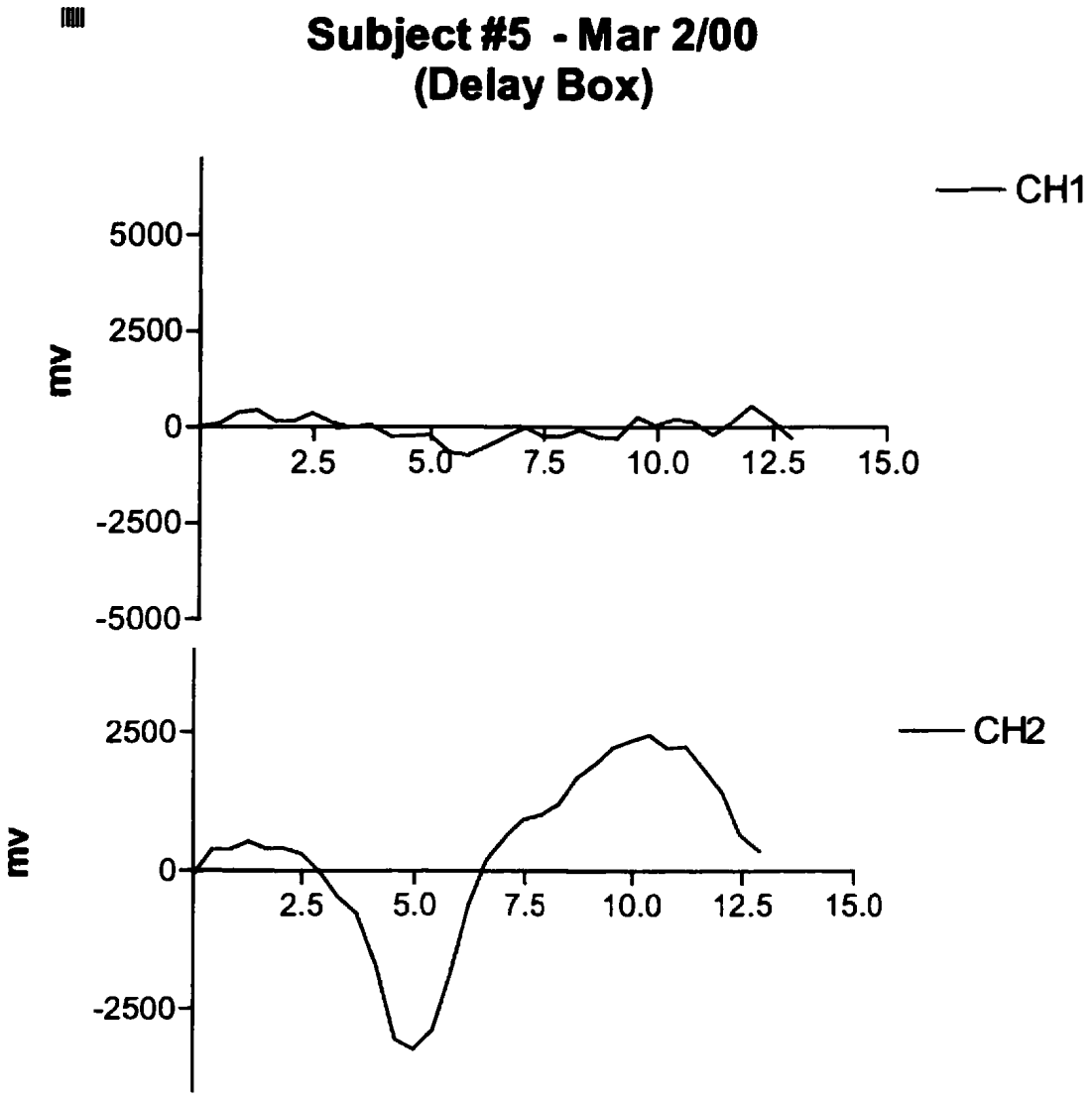


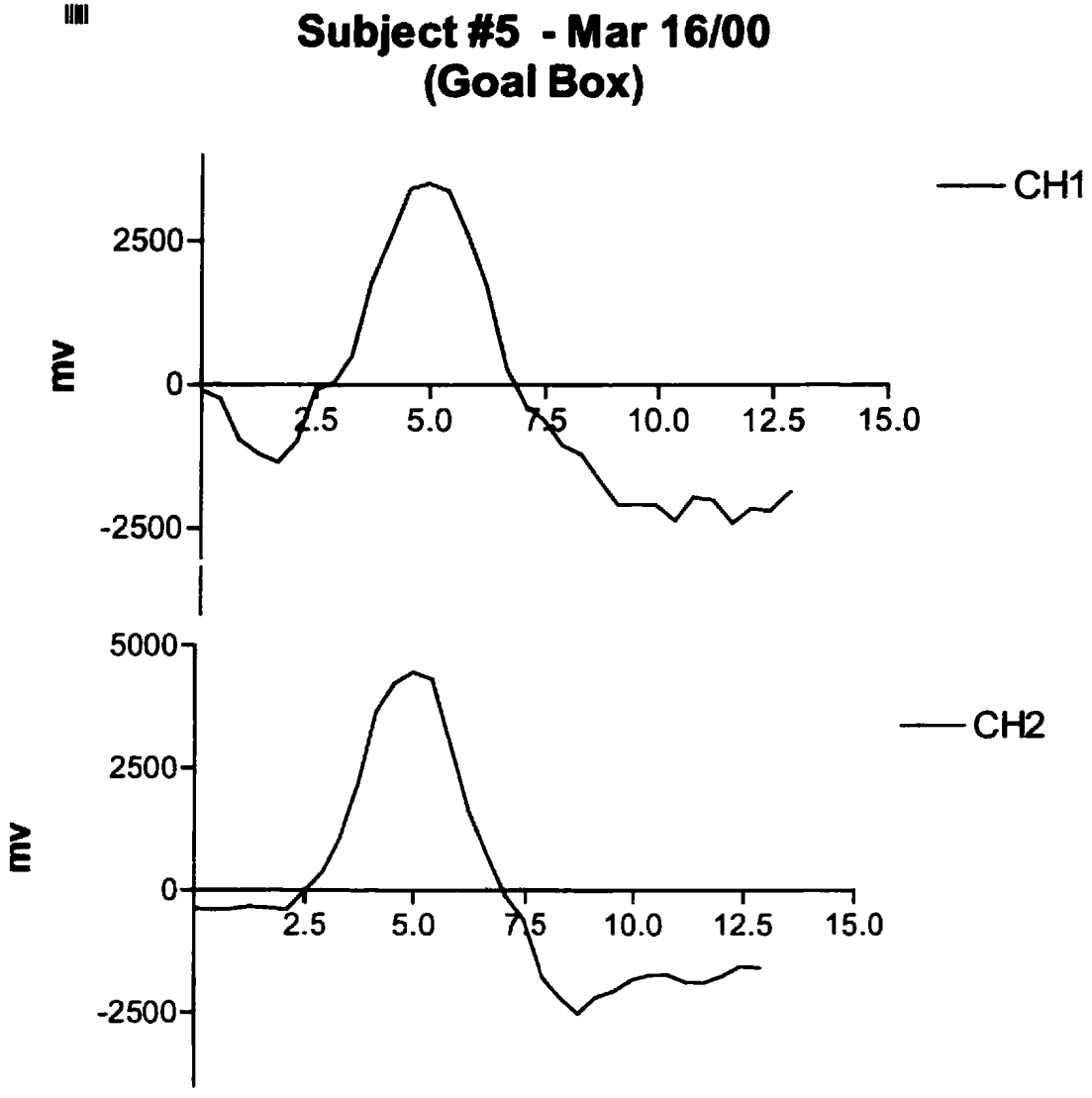

Subject #29 - Aug 27/00
(Delay Box)
CH1
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0

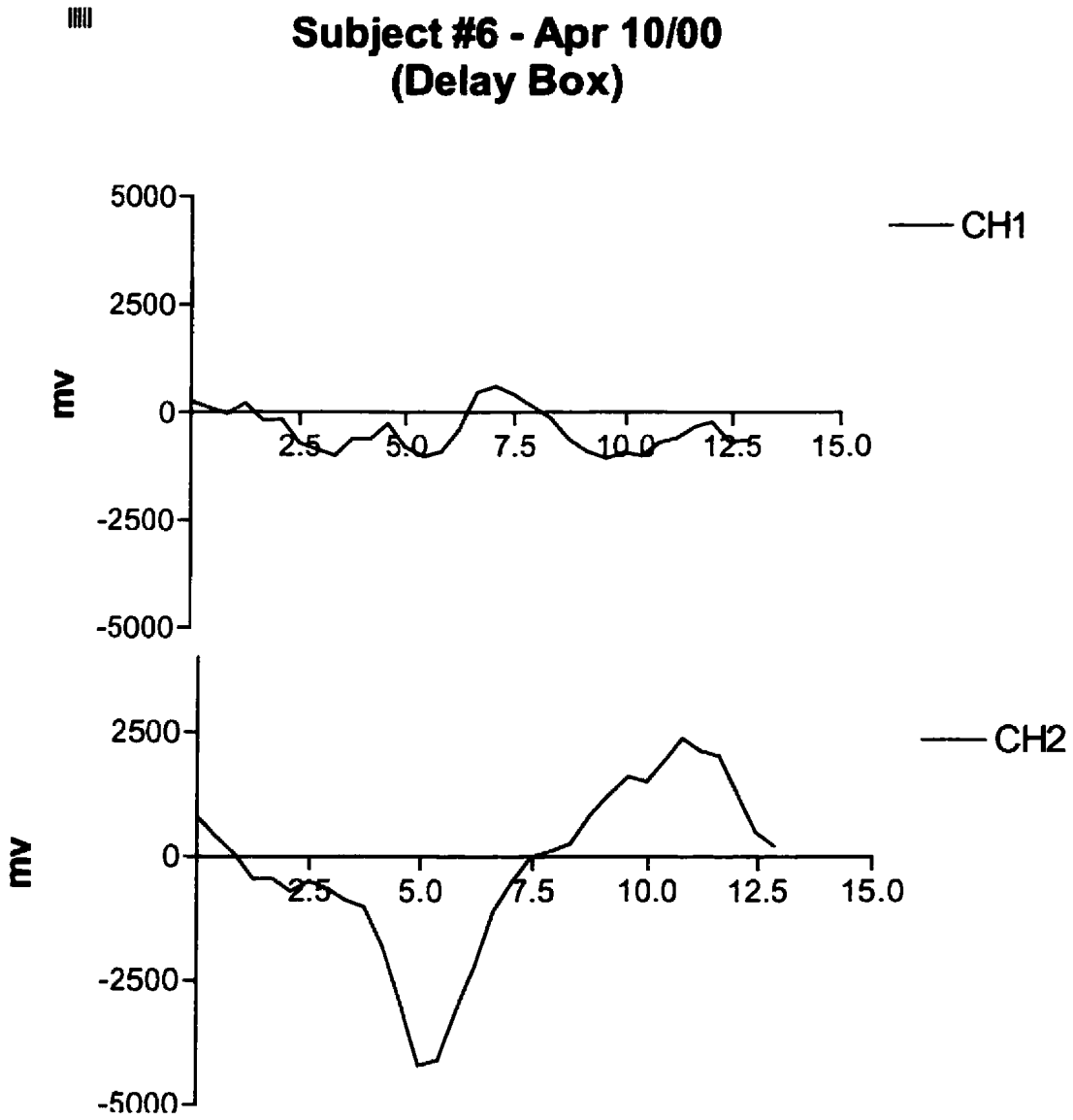

Subject #31 - July 3/00
(Delay Box)
CH1
5000
2500
0
-2500
-5000
-7500
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0

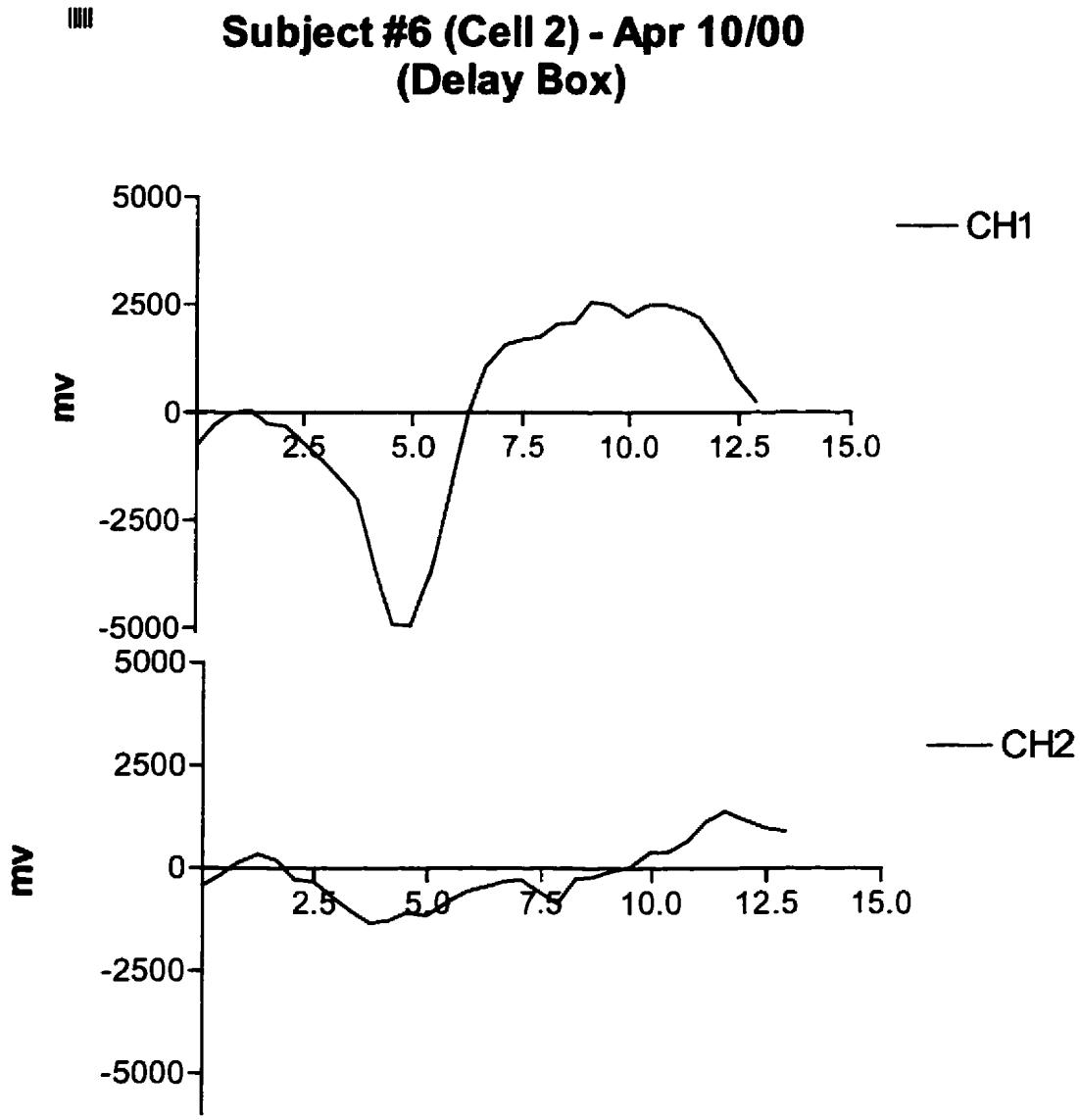
Subject #33 - Sept 5/00
(Goal Box)




Subject #5 - Mar 2/00
(Delay Box)
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0


Subject #5 - Mar 16/00
(Goal Box)
CH1
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0
5000
2500
0
-2500
mv
CH2
2.5
5.0
7.5
10.0
12.5
15.0


Subject #6 - Apr 10/00
(Delay Box)
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH1
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2


Subject #6 (Cell 2) - Apr 10/00
(Delay Box)
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH1
CH2

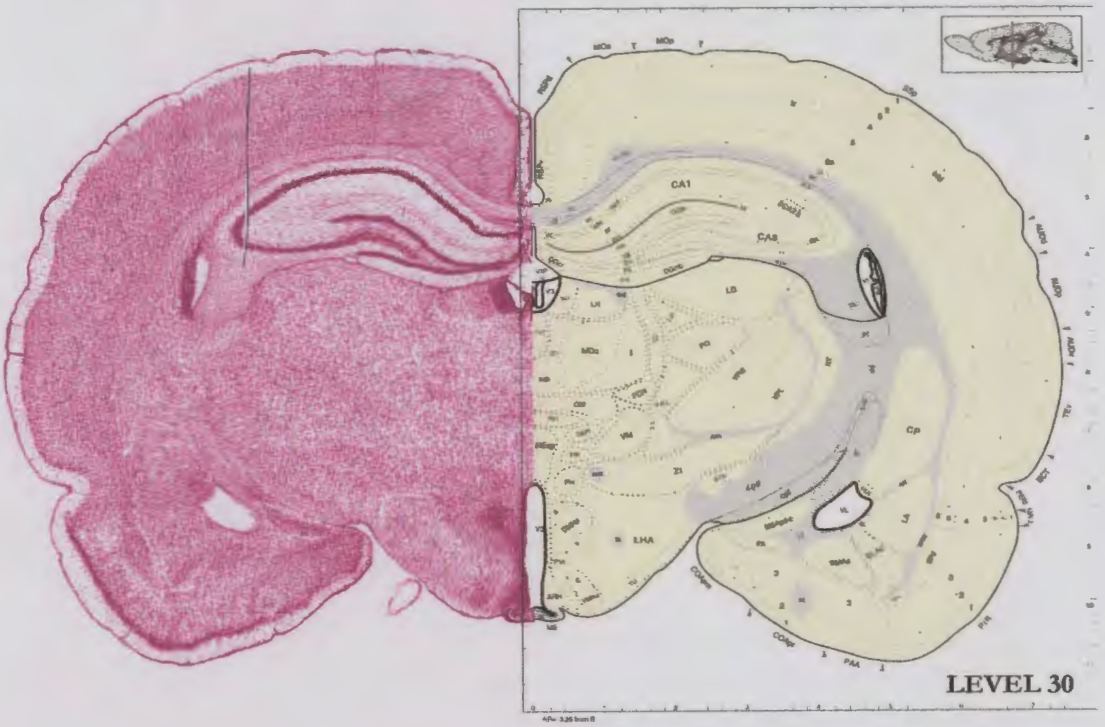
Appendix C

Histology

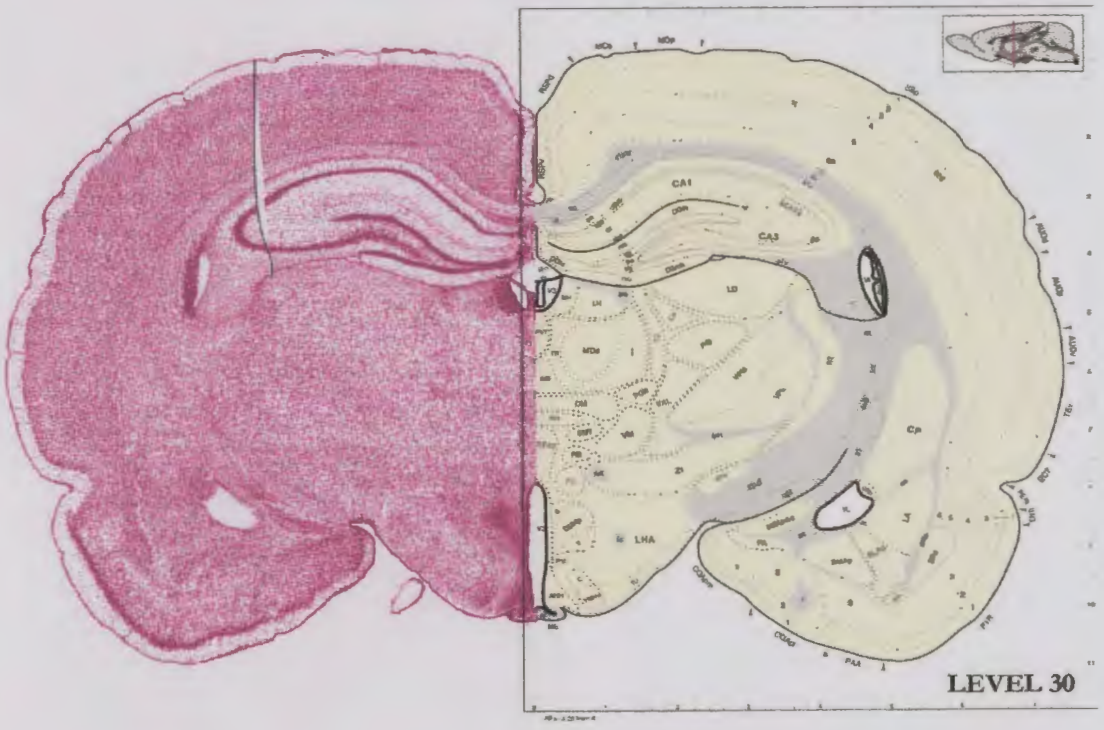
Subject 5

Subject 6

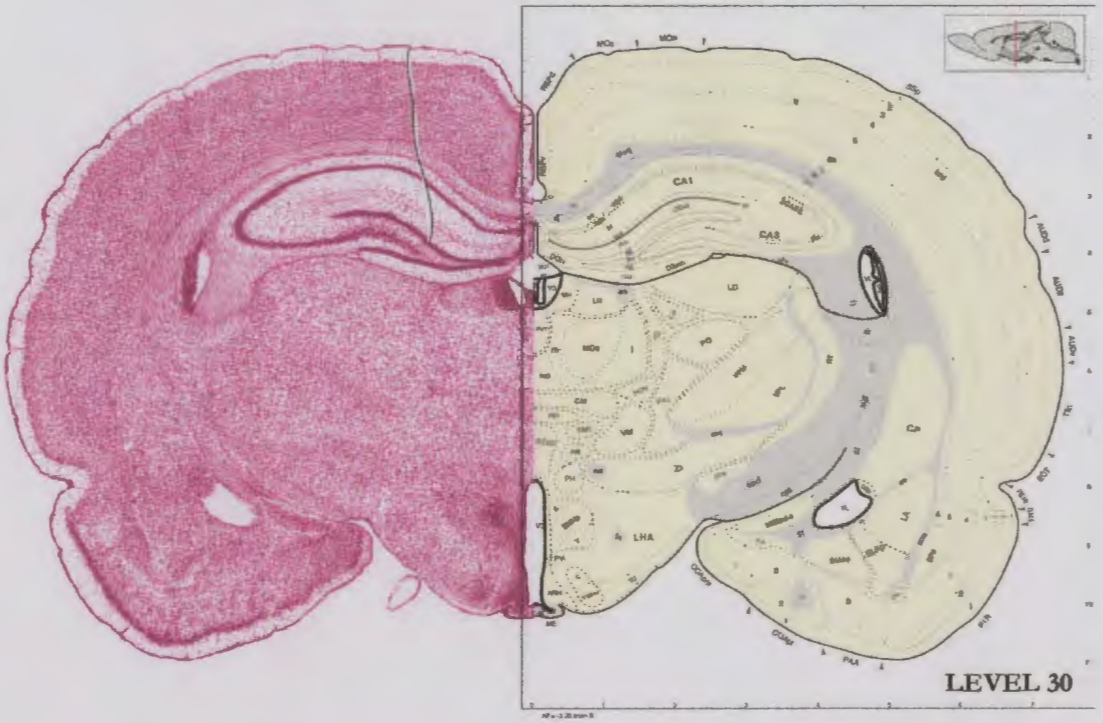
Subject 14



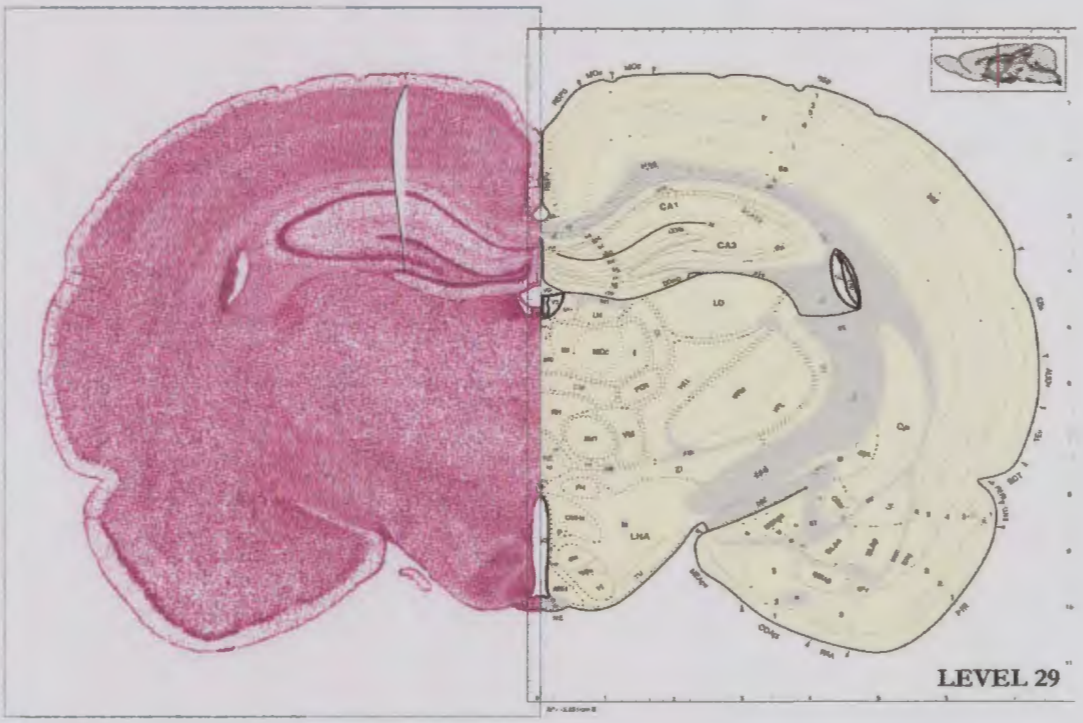
Subject 15



Subject 17



Subject 18

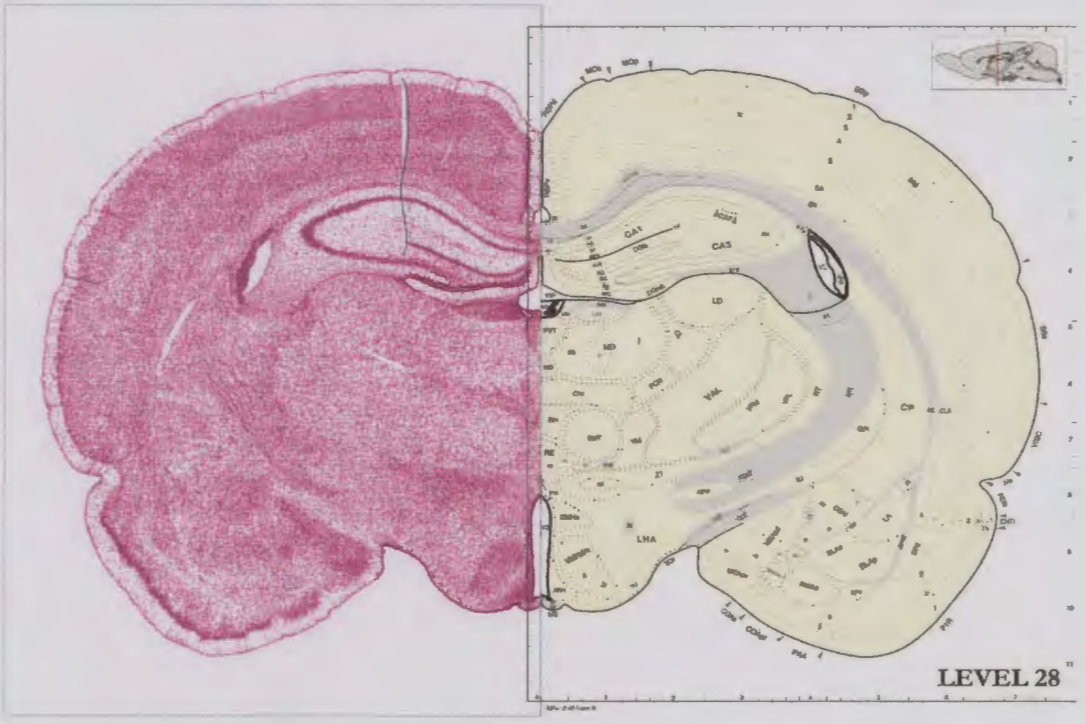


Subject 23

Subject 26

Subject 29

Subject 31



Subject 33

NOTE TO USERS

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UMI

Appendix A

Firing Rate Map Program

(John Huxter, 1998)

```
#!/bin/sh

gawk '

BEGIN {
printf("\n\n\nPLACE CELL ANALYSIS PROGRAM: by J.Huxter, 1998")
while(choice!="q")      {
print"\n*****************************************************************
"
print"MAIN MENU: file = \""origfile"\", x-bins="xbintot", y-
bins="ybintot

printf("Choice ([o]ptions) ? "); getline $0 < "/dev/tty"
if($1=="1") PLACE1(origfile,basefile)
if($1=="2") PLACE2(basefile,xbintot,ybintot)
if($1=="p1") PLOT1(basefile)
if($1=="p2") PLOT2(basefile)
if($1=="p3") PLOT3(basefile,xbintot,ybintot)
if($1=="f") FILE()
if($1=="c") CLUSTERS(basefile)
if($1=="b") BINS()
if($1=="r") ROTATE(basefile)
if($1=="s") SPLIT(basefile)
if($1=="o") OPTIONS()
if($1=="ls") LS()
if($1=="q") QUIT()
                }
exit
}


function PLACE1(origfile,basefile) {
############################################################
# eliminate commas, control-characters from discovery file
# convert numeric cluster ids to numbers (A -> 11, B -> 12)
# convert time-stamp into seconds
# calculate max/min xy coordinates
#     infile = file to read data from with following format
#           "P",timestamp,x,y
#           "E"or"S",timestamp,cluster,electrode channel
#
#     x = x coordinate from infile
#     y = y coordinate from infile
#     c = cluster number from infile
#     t = timestamp (converted to seconds from start) from infile
#     tmin = time at start of record (in ms)
#     xmin/xmax = min and max x-coordinates sampled
#     ymin/ymax = min and max y-coordinates sampled
#     tmin = time stamp at start of record
#     tpos = total number of position samples
#     ttime = time at end of record (in seconds, from start)
#     tspk = total number of spike events
#     sx[]/sy[]/st[] = x-coord/y-coord/time for each spike
#     px[]/py[]/pt[] = x-coord/y-coord/time for each position sample
#     ctot[] = total number of spikes from each cluster
#
```

```
# output =  *.sta (statistics)
#           *.spk (position & spike data)
############################################################
print"\n    CONVERT INPUT FILE & GENERATE STATS"
print"    ----------------------------------"
if(origfile=="") {print"\[ERROR]: set input file first!";return}
infile=origfile
FS=","
z=tpos=tspk=ttime=tmin=0
xmin=ymin=999999;xmax=ymax=0
print "    * reading \""infile"\""
print "    * converting timestamp, adding xy data to spikes"
while((getline $0 < infile) > 0)    {
      z++
      if(z==1) tmin=$2
      t=($2-tmin)/10000
 if($1=="P")      {
      tpos++
      x=$3;y=$4
      if(xmin>=x && x!=0) xmin=x; if(xmax<=x) xmax=x
      if(ymin>=y && x!=0) ymin=y; if(ymax<=y) ymax=y
      px[tpos]=x;py[tpos]=y;pt[tpos]=t
            }
 if($1=="E" || $1=="S")    {
      tspk++
      c=$3; if(c=="A")c="10"; if(c=="B")c="11"
      sx[tspk]=x;sy[tspk]=y;st[tspk]=t;sc[tspk]=c;ctot[c]++
            }
                              }
close (infile)
ttime=t
xrange=xmax-xmin;yrange=ymax-ymin
print"    * converting xy coordinates to percentages of xy range"

print"    * working on position data..."
close (outfile)
outfile=basefile".pos"
for(z=1;z<=tpos;z++)    {
      x=((px[z]-xmin)/xrange)*100;y=((py[z]-ymin)/yrange)*100
      printf("%8.3f  %7.3f  %7.3f \n", pt[z],x,y) > outfile}
print"    * position data sent to \""outfile"\""

print"    * working on spike data..."
close (outfile)
outfile=basefile".spk"
for(z=1;z<=tspk;z++)    {
      x=((sx[z]-xmin)/xrange)*100;y=((sy[z]-ymin)/yrange)*100
      printf("%8.3f  %7.3f  %7.3f  %2s \n", st[z],x,y,sc[z]) > outfile}
print"    * position spike data sent to \""outfile"\""

close (outfile)
outfile=basefile".sta"
print"    * parameters sent to \""outfile"\"\n"
printf("%-4s (xmin)  %4s (ymin)  %4s (xmax)  %4s (ymax)  %6s (samples)
%6s (spikes)  %7.3f (time)\n",
       xmin,ymin,xmax,ymax,tpos,tspk,ttime) > outfile
z=0
```

```
for(c=0;c<=11;c++)
      if(ctot[c]!="") {
            z++;printf("%-2s %2s %4s %5.3f\n",c,z,ctot[c],ctot[c]/t)
>outfile
                  }
close (outfile)
status=status"BCD"
return
}


function PLACE2(basefile,xbintot,ybintot) {
#####################################################################
# calculate dwell-time per bin, spikes per bin
# calculate firing rates for each cluster in each bin
#####################################################################
print"\n    SORT SPIKE-EVENTS & DWELL-TIME INTO BINS"
print"    ---------------------------------------"
if(basefile=="") {print"    [ERROR]: set input file first!";return}
if(xbintot=="")  {print"    [ERROR]: set x-bins and y-bins
first!";return}
match(status,"B")
 if(RSTART<=0) {print"    [ERROR]: stats file (.sta) missing";return}
match(status,"C")
 if(RSTART<=0) {print"    [ERROR]: spike file (.spk) missing";return}
match(status,"D")
 if(RSTART<=0) {print"    [ERROR]: position file (.pos) missing";return}

FS=" "; infile=basefile".spk"; split("",spike); split("",samp)

z=0;infile=basefile".sta"
print"    * reading parameters from \""infile"\""
while((getline $0 < infile) > 0)   { z++
        if(z==1)
{xmin=$1;ymin=$3;xmax=$5;ymax=$7;tsamp=$9;tspike=$11;ttime=$13;continue}
        if($1!="")      {c=$1;id[c]=$2;cspike[c]=$3;crate[c]=$4}}
cmax=id[c]; close (infile)

infile=basefile".spk"
print"    * calculating spikes/bin/cluster from \""infile"\""
while((getline $0 < infile) > 0)          {
      if($2<=-0.001)
{xbin=ybin=0;spike[xbin,ybin,id[$4]]++;bspike[xbin,ybin]++;continue}
      xbin=int($2/(100.001/xbintot))+1;ybin=int($3/(100.001/ybintot))+1
      spike[xbin,ybin,id[$4]]++;bspike[xbin,ybin]++}
close (infile)

infile=basefile".pos"
print"    * calculating samples/bin from \""infile"\""
while((getline $0 < infile) > 0)          {
      if($2<=-0.001) {xbin=ybin=0;samp[xbin,ybin]++;continue}
      xbin=int($2/(100.001/xbintot))+1;ybin=int($3/(100.001/ybintot))+1
      samp[xbin,ybin]++}
close (infile)

print"    * calculating dwell time & firing rates/bin"
close (outfile)
```

```
outfile=basefile".bin"
for(xbin=0;xbin<=xbintot;xbin++)          {
for(ybin=0;ybin<=ybintot;ybin++)          {
if((xbin==0 && ybin==0) || (xbin!=0 && ybin!=0)){ # only process some
bins
      if(samp[xbin,ybin]=="") dwell[xbin,ybin]=0
      if(samp[xbin,ybin]!="")
dwell[xbin,ybin]=(samp[xbin,ybin]/tsamp)*ttime

printf("%3s %3s  %7.3f  ", xbin,ybin,dwell[xbin,ybin]) > outfile

for(c=1;c<=cmax;c++)    {
     x=0
     if(dwell[xbin,ybin]!=0) x=spike[xbin,ybin,c]/dwell[xbin,ybin]
     printf("  %6.3f",x) > outfile
                  }
print""> outfile
}}}
close (outfile)
print"    * data sent to \""outfile"\""
print"\n    Cluster    Id   Spikes  Meanrate\n"
for(c=0;c<=12;c++) {
      if(id[c]>="0") printf("     %3s    %4s   %4s
%8.3f\n",c,id[c],cspike[c],crate[c])}
status=status"E"
return
}


function PLOT1(basefile)      {
#########################################################################
if(basefile=="") {print"\[ERROR]: set input file first!";return}

FS=","
infile=basefile".txt"
outfile=basefile"_posall.tmp"

print"\n    PLOT ALL POINTS VISITED IN "infile
print"    ------------------------------------------------------------"
print "    * reading \""infile"\""
while((getline $0 < infile) > 0)    {
 if($1=="P")            {print $3,$4 > outfile}
                            }
close (infile)
close (outfile)

infile=outfile
outfile="./plot.tmp"
print "#! \/usr\/local\/bin\/gnuplot\n" > outfile
print "set bar 0.05\nset key\nset bmargin 3\nset lmargin 3\n" > outfile
print "set xlabel \"x-coordinate\" 0,-2" > outfile
print "set ylabel \"y-coordinate\" -2, 0" > outfile
print "set xtics axis mirror 50" > outfile
print "set ytics axis mirror 50" > outfile
print "set xrange [0:255]" > outfile
print "set yrange [0:255]" > outfile
print "set nogrid" > outfile
```

```
print "set key outside" > outfile
print "plot \\"   > outfile
print "\""infile"\" using 1:2 with dots"  > outfile

print "    * generating plot "basefile"_posplot.fig"
print "set term fig color" > outfile
print "set output \""basefile"_posplot.fig\"" > outfile
print "plot \\" > outfile
print "\""infile"\" using 1:2 with dots"  > outfile


print "\npause -1 \"* clear plot\"" > outfile
print "     quit" > outfile
close (outfile)
command="chmod u+x "outfile;system(command);system(outfile)
return
}



function PLOT2(basefile)      {
#########################################################################
# Plot position of specific spikes
#########################################################################
if(basefile=="") {print"\[ERROR]: set input file first!";return}

FS=" ";split("",tmp);y=0
infile=basefile".spk"
print"\n    PLOT SPIKE POSITIONS"
print"    ------------------------"
print "    * reading \""infile"\""
FS=" "
while((getline $0 < infile) > 0)    {
      if($2>=0.001)                 {
                                    {
                  outfile=basefile"_spk"$4".tmp"
                  tmp[$4]=1
                  printf("%9s %9s %3s\n",$2,$3,$4) > outfile
                                    }
                                    }
                                    }
close (infile); for(x in tmp) {outfile=basefile"_spk"x".tmp";close
(outfile)}

printf("    * executing spike plot of clusters ")
for (x=0;x<=14;x++) if(x in tmp) {y++;printf(x", ")}
print""
z=-1
outfile="./plot.tmp"
print "#! \/usr\/local\/bin\/gnuplot\n" > outfile
print "set bar 0.05" > outfile
print "set bmargin 3\nset lmargin 3\n" > outfile
print "set xlabel \"x-coordinate\" 0,-2" > outfile
print "set ylabel \"y-coordinate\" -2, 0" > outfile
print "set xtics axis mirror 10" > outfile
print "set ytics axis mirror 10" > outfile
```

```
print "set xrange [0:101]" > outfile
print "set yrange [0:101]" > outfile
print "set nogrid" > outfile
print "set nokey" > outfile
print "" > outfile
z=-1
print "    * generating plot "basefile"_spkplot.fig"
print "set term fig color portrait inches size "8.5*(y/2)" "11*(y/2) >
outfile
print "set output \""basefile"_spkplot.fig\"" > outfile
print "set multiplot" > outfile
print "set size "(0.5/y)","(0.5/y) > outfile
for (x=0;x<=15;x++) if(x in tmp) {{
        z++; infile=basefile"_spk"x".tmp"
        if(y<=1)  {
            print"set origin 0,0" > outfile
            print "set title \""infile"\"" > outfile
                  }
        if(y>=2)  {
            print"set origin "z*(1/(y+1.75))",.6" > outfile
            print "set title \""infile"\"" > outfile
                  }
        print "plot \""infile"\" using 1:2 with dots" > outfile
                        }}
print "set nomultiplot\n" > outfile
close (outfile)
command="chmod u+x "outfile;system(command);system(outfile)
return
}



function PLOT3(basefile,xbintot,ybintot) {
#########################################################
# read means data, convert to proportion of max rate
# sort means for xy bins on scale of 1-10
# assign colors
# generate fig file
#########################################################
FS=" "
xorig=100;yorig=100
lt=0;lw=1;size=250
color[1]="000000"
color[2]="000088"
color[3]="0000ff~"
color[4]="00979d"
color[5]="00c668"
color[6]="00ff00"
color[7]="caff00"
color[8]="fff382"
color[9]="ffffc8"
color[10]="ffffff"

print"\n    GENERATE XFIG PLOT OF CLUSTER FIRING RATES"
print"    ----------------------------------------"
if(basefile=="") {print"\[ERROR]: set input file first!";return}
if(xbintot=="")  {print"\[ERROR]: set x-bins and y-bins first!";return}
```

```
print"    * Plot of "basefile".bin in a "xbintot"x"ybintot" matrix."
close ("/dev/tty")

outfile=basefile"_rateplot.fig"
print"    * generating xfig file \""outfile"\""
print"#FIG 3.2" > outfile
print"Portrait" > outfile
print"Flush left" > outfile
print"Inches" > outfile
print"Letter" > outfile
print"100.00" > outfile
print"Single" > outfile
print"-2" > outfile
print"1200 2" > outfile
for(z=1;z<=10;z++) print"0 "31+z" #"color[z] > outfile

infile=basefile".sta"
print"    * reading parameters from \""infile"\""
z=0
while((getline $0 < infile) > 0)    {
      z++
        if(z==1)
{xmin=$1;ymin=$3;xmax=$5;ymax=$7;tsamp=$9;tspike=$11;ttime=$13;continue}
        if($1!="") {c=$1;id[c]=$2;cspike[c]=$3;crate[c]=$4}
                                          }
close (infile)

for(origcluster=0;origcluster<=11;origcluster++)      {
if(id[origcluster]<=0) continue

cluster=(id[origcluster]+3)
infile=basefile".bin"
cmax=0

print"    * reading cluster "origcluster" firing rates from
\""infile"\""
while((getline $0 < infile) > 0)                {
      x=$1;y=$2;rate[x,y]=$cluster
      if(cmax<=$cluster) cmax=$cluster
                                      }
close (infile)

xmin=xorig+size;ymin=yorig+size
xmax=xorig+((xbintot+1)*size);ymax=yorig+((ybintot+1)*size)
print"6 "xmin" "ymin" "xmax" "ymax > outfile

xbin=ybin=0
modrate=int((rate[x,y]/(cmax+0.001))*10)+1
print"2 2 "lt" "lw" 4 "31+modrate" 0 9 20 0.0000 0 0 -1 0 0 5" > outfile
printf("      ") > outfile
printf(xorig+(xbin*size)" "yorig+(ybin*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(ybin*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(ybin*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(ybin*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(ybin*size)"\n") > outfile

for(xbin=1;xbin<=xbintot;xbin++) {
```

```
for(ybin=1;ybin<=ybintot;ybin++) {

y=ybintot+1-ybin
modrate=int((rate[xbin,ybin]/(cmax+0.001))*10)+1
print"2 2 "lt" "lw" 4 "31+modrate" 0 9 20 0.0000 0 0 -1 0 0 5" > outfile
printf("      ") > outfile
printf(xorig+(xbin*size)" "yorig+(y*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(y*size)" ") > outfile
printf(xorig+(xbin*size)+size" "yorig+(y*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(y*size)+size" ") > outfile
printf(xorig+(xbin*size)" "yorig+(y*size)"\n") > outfile
}
}
print"-6" > outfile
cluster=origcluster
printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s Cluster %s, %s %c00i\n",
      xmin,ymax+(1*size),cluster,basefile,92) > outfile
printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s Maxrate = %s Hz%c001\n",
      xmin,ymax+(2*size),cmax,92) > outfile
printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s Meanrate= %s Hz%c001\n",
      xmin,ymax+(3*size),crate[cluster],92) > outfile


xorig+=(xbintot+2)*size
}

for(z=1;z<=10;z++)      {
      print"2 2 "lt" "lw" 4 "31+z" 0 9 20 0.0000 0 0 -1 0 0 5" > outfile
      printf("      ") > outfile
      printf(xmax+size  " "yorig+(z*size)" ") > outfile
      printf(xmax+2*size" "yorig+(z*size)" ") > outfile
      printf(xmax+2*size" "yorig+(z*size)+size" ") > outfile
      printf(xmax+size  " "yorig+(z*size)+size" ") > outfile
      printf(xmax+size  " "yorig+(z*size)"\n") > outfile
                  }
print"6 "xmax+size" "yorig+(size)" "xmax+2*size" "yorig+(10*size)+size >
outfile
for(z=1;z<=10;z++)      {
      printf("4 0 0 0 0 0 14 0.0000 4 0 0 %s %s %2s-%2s%c of
max%c001\n",
            xmax+2.5*size,yorig+(z*size)+(0.75*size),(z-
1)*10,z*10,37,92) > outfile
                  }
close (outfile)



return
}


function FILE()   {
##############################################################################
####
print"\n    SET ORIGINAL FILENAME"
print"    ---------------------"
printf("    * Input filename: "); getline temp < "/dev/tty"
```

```
      if(temp=="") {print"     * invalid filename: ABORTED\n\n";return}
      close ("/dev/tty")

command="ls -l > file.tmp";system(command)
match(temp,"\\.");z=RSTART;if(z<=0)z=length(temp)+1;y=substr(temp,1,z-1)
infile="file.tmp"
status=""
while((getline $0 < "file.tmp") > 0)      {
      if($9==temp) {status=status"A"; print"    * Original datafile
found"}
      if($9==y".sta") {status=status"B"; print"    * Statistics file
found"}
      if($9==y".spk") {status=status"C"; print"    * Spike data
compiled"}
      if($9==y".pos") {status=status"D"; print"    * Position data
compiled"}
      if($9==y".bin") {status=status"E";z=y".bin"
            while((getline $0 < z) > 0) {xbintot=$1;ybintot=$2}
            close (z)
            print"    * Bin-sort completed: xbins="xbintot",
ybins="ybintot
                  }
      if($9==y".rot") {status=status"F"; print"    * Rate map rotation
performed"}
                              }
close ("file.tmp")
if(status=="") {print "    * ERROR - No files found matching
"temp;return}
match(status,"E");if(RSTART<=0)xbintot=ybintot=""
origfile=temp
basefile=y
return
}


function BINS()   {
##########################################################################
####
print"\n    SET NUMBER OF BINS TO DIVIDE ENVIRONMENT INTO"
print"    ------------------------------------------"
printf("    * Total x-bins: "); getline x < "/dev/tty"
      if(x=="") {print"     * invalid x-bins: ABORTED\n\n";return}
printf("    * Total y-bins: "); getline y < "/dev/tty"
      if(y=="") {print"    * invalid y-bins: ABORTED\n\n";return}
printf("    * Proceed (y/n)? "); getline GO < "/dev/tty"
        if(GO!="y") {printf("    * ABORTED\n\n"); return}
close ("/dev/tty")
z=0;if(x!=xbintot || y!=ybintot) z=1
xbintot=x;ybintot=y
if(z==1) PLACE2(basefile,xbintot,ybintot)
return
}


function ROTATE(basefile)     {
##########################################################################
####
```

```
# Make rotated versions of place field maps
# Input = .bin files (output from PLACE program)...
#      $1 = xbin
#      $2 = ybin
#      $3 = dwelltime in that bin
#      $4 = firing rate for cluster in that bin
#
# Output on each line...  xbin ybin R  0deg 90deg 180deg 270deg
#########################################################################
print"    MAKE ROTATED VERSIONS OF RATE MAP"
print"    --------------------------------"
if(basefile=="") {print"    [ERROR]: set input file first!";return}
match(status,"E")
if(RSTART<=0)    {print"    [ERROR]: create rate (.bin) file
first!";return}

split("",rate1);split("",rate2);split("",rate3);split("",rate4)
infile=basefile".bin"
outfile=basefile".rot"
print"    * reading min/max bins from "infile
while((getline $0 < infile) > 0)    {xmax=$1;ymax=$2}
close (infile)
print"    * creating outfile "outfile
while((getline $0 < infile) > 0)    {
      xbin=$1;ybin=$2;z=$4
      if(xbin==0) {print $0 > outfile; continue}
      rate1[xbin,ybin]=z
      rate2[ybin,xmax-(xbin-1)]=z
      rate3[xmax-(xbin-1),ymax-(ybin-1)]=z
      rate4[ymax-(ybin-1),xbin]=z
                              }
close (infile)
for(x=1;x<=xmax;x++)    {
for(y=1;y<=ymax;y++)    {
      printf("%3s %3s   %7.3f  %7.3f  %7.3f  %7.3f\n",
      x,y,rate1[x,y],rate2[x,y],rate3[x,y],rate4[x,y]) > outfile
                  }
                  }
close (outfile)
status=status"F"
return
}


function SPLIT(basefile)      {
#########################################################################
####
print"    SPLIT ORIGINAL DATAFILE ("basefile".txt) IN HALF"
print"    ------------------------------------------------"
if(basefile=="") {print"\[ERROR]: set input file first!";return}

FS=","
x=y=z=0
temp="h123456789"
infile=basefile".txt"
while((getline $0 < infile) > 0) {x++}
close (infile)
```

```
outfile=basefile"_a.txt"
print"     * sending 1st half to "outfile
z=0
while((getline $0 < infile) > 0) {
      y++
      if(y>=x/2) {
            outfile=basefile"_b.txt"
            if(z==0) {
                  close (outfile)
                  print"     * sending 2nd half to "outfile
                   }
            z=1
               }
      print $0 > outfile
                        }
close (infile); close (outfile)
return
}



function CLUSTERS(basefile)   {
###############################################################################
####
print"\n     * MODIFY CLUSTERS IN "origfile
print"     * ----------------------------------"
if(basefile=="") {print"\[ERROR]: set input file first!";return}

printf("     * Replace cluster#: "); getline x < "/dev/tty"
      if(x=="") {print"     * invalid cluster: ABORTED\n\n";return}
printf("     *              with: "); getline y < "/dev/tty"
      if(y=="") {print"     * Remove cluster #"x}
printf("     * THIS IS NON-REVERSIBLE! PROCEDE? ");getline z < "/dev/tty"
 if(z!="y") {print"     * ABORTED\n\n";return}
close ("/dev/tty")
z=0
FS=","
infile=origfile
outfile="file.tmp"
while((getline $0 < infile) > 0)    {
      if($1!="S" && $1!="E") {print $0 > outfile;continue}
      if($3!=x) {print $0 > outfile;continue}
      w++
      if(y=="") continue
      $3=y
      for(z=1;z<=NF-1;z++) printf($z",") > outfile
      print $z  > outfile
                              }
close (infile); close (outfile)
command="mv "outfile" "infile;system(command)
print "     * "w" replacements made"
return
}

function OPTIONS()      {
###############################################################################
####
```

```
print ""
print"    1: read data file"
print"    2: calculate bin firing rates"
print""
print"    p1: plot all position samples"
print"    p2: plot spike positions by cluster"
print"    p3: generate rate map"
print""
print"    o: options"
print"    f: set input filename"
print"    c: modify clusters in original file"
print"    b: set x-bins & y-bins"
print"    ls: directory"
print"    r: create rotated versions of rate map"
print"    s: split original data file in two"
print"    q: quit"
}


function QUIT()   {
##########################################################################
####
print"\nQUIT PROGRAM\n";exit
}

function LS()           {
##########################################################################
####
print"";command=$0;system(command)
}


'
rm -f *_pos*.tmp
rm -f *_spk*.tmp
rm -f file.tmp
```

Appendix B

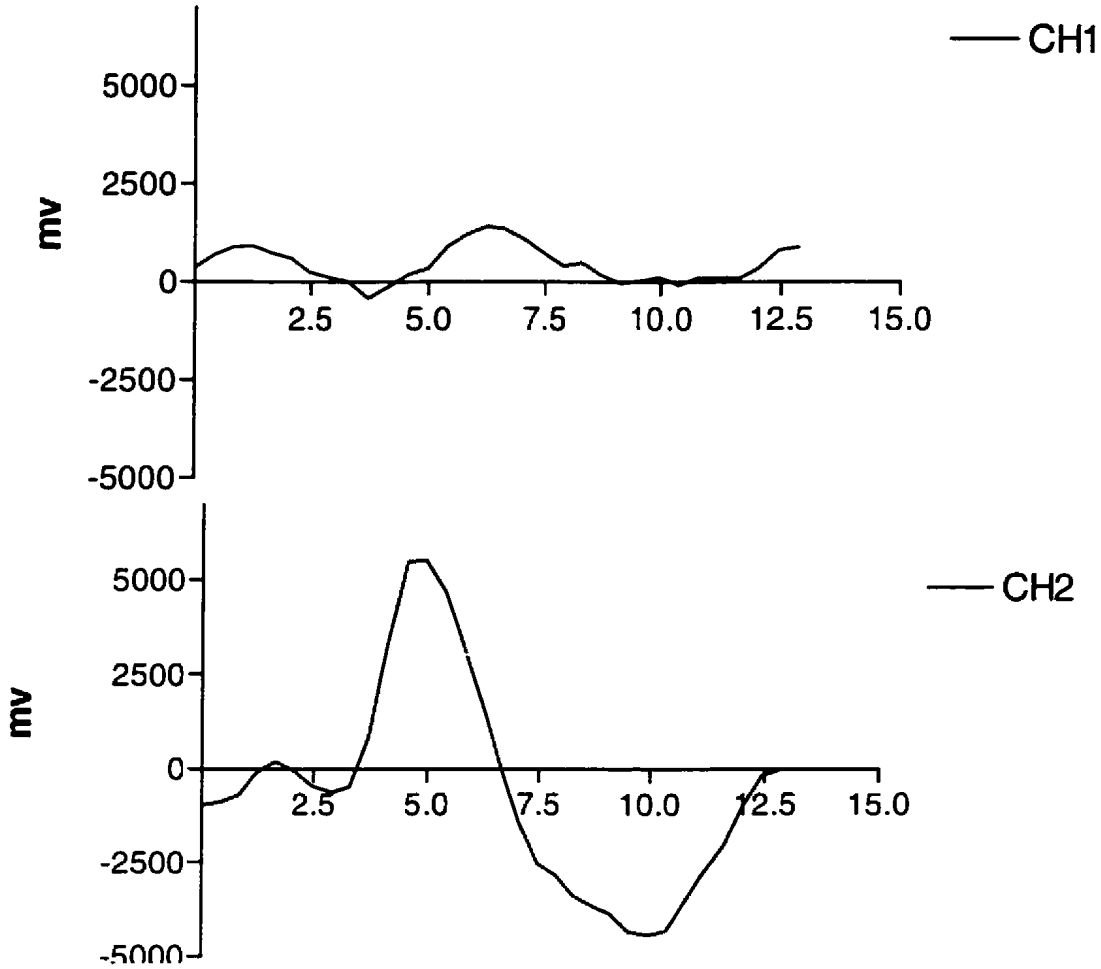
Electrophysiology

Cell Waveforms

Note: Goal box wave forms
not used in analysis

IIIII

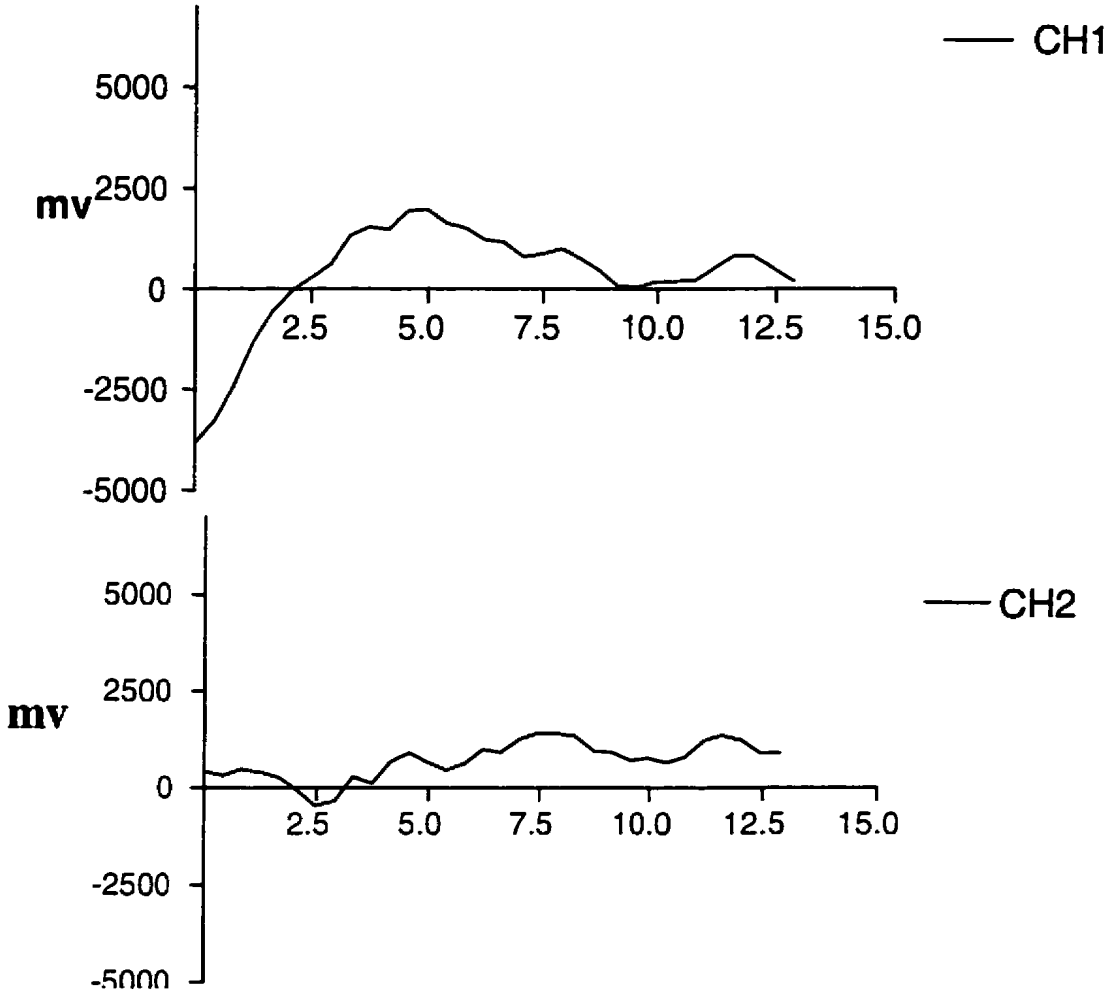
Subject #14 - Aug. 19/99
(Goal Box)

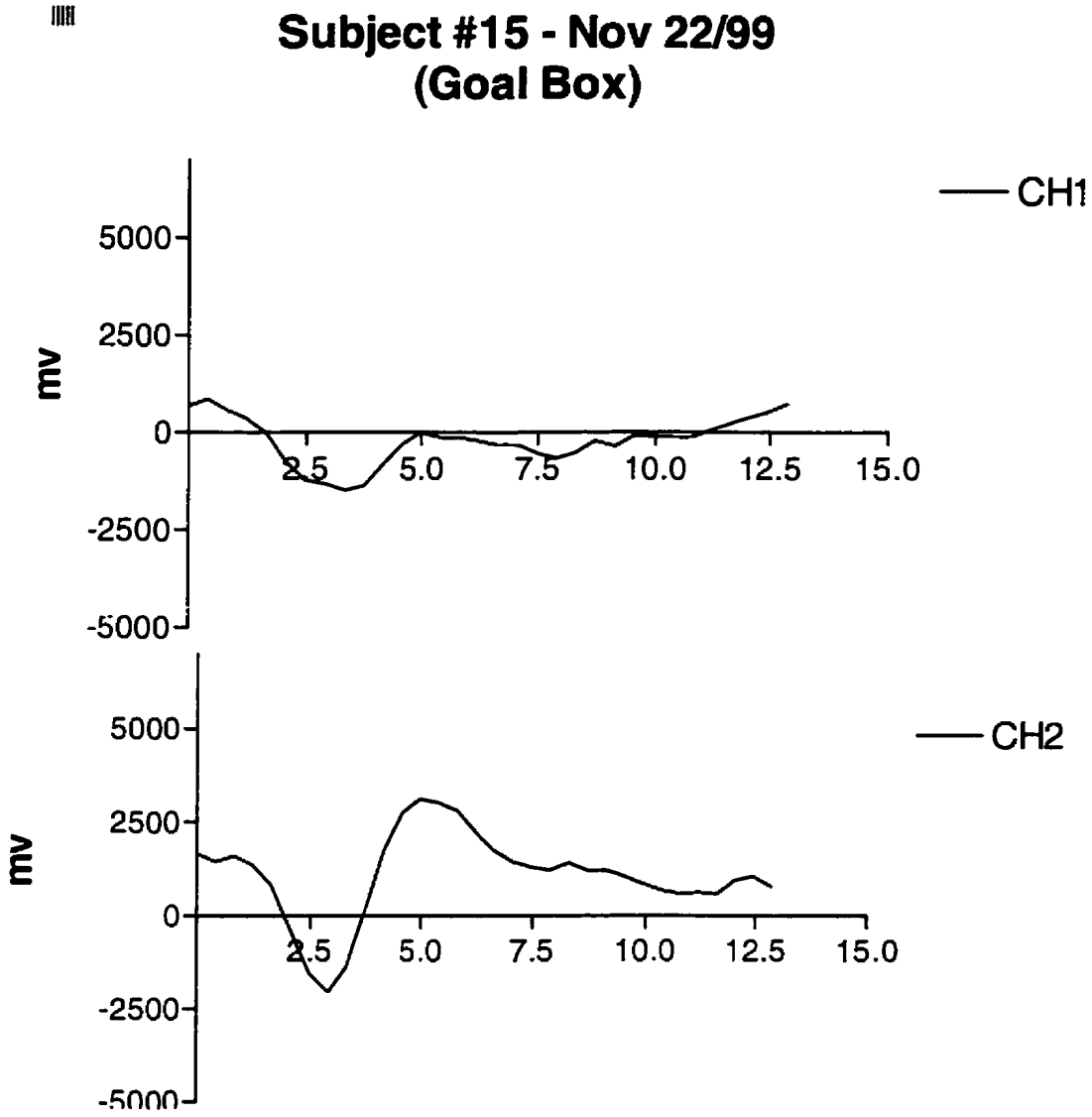


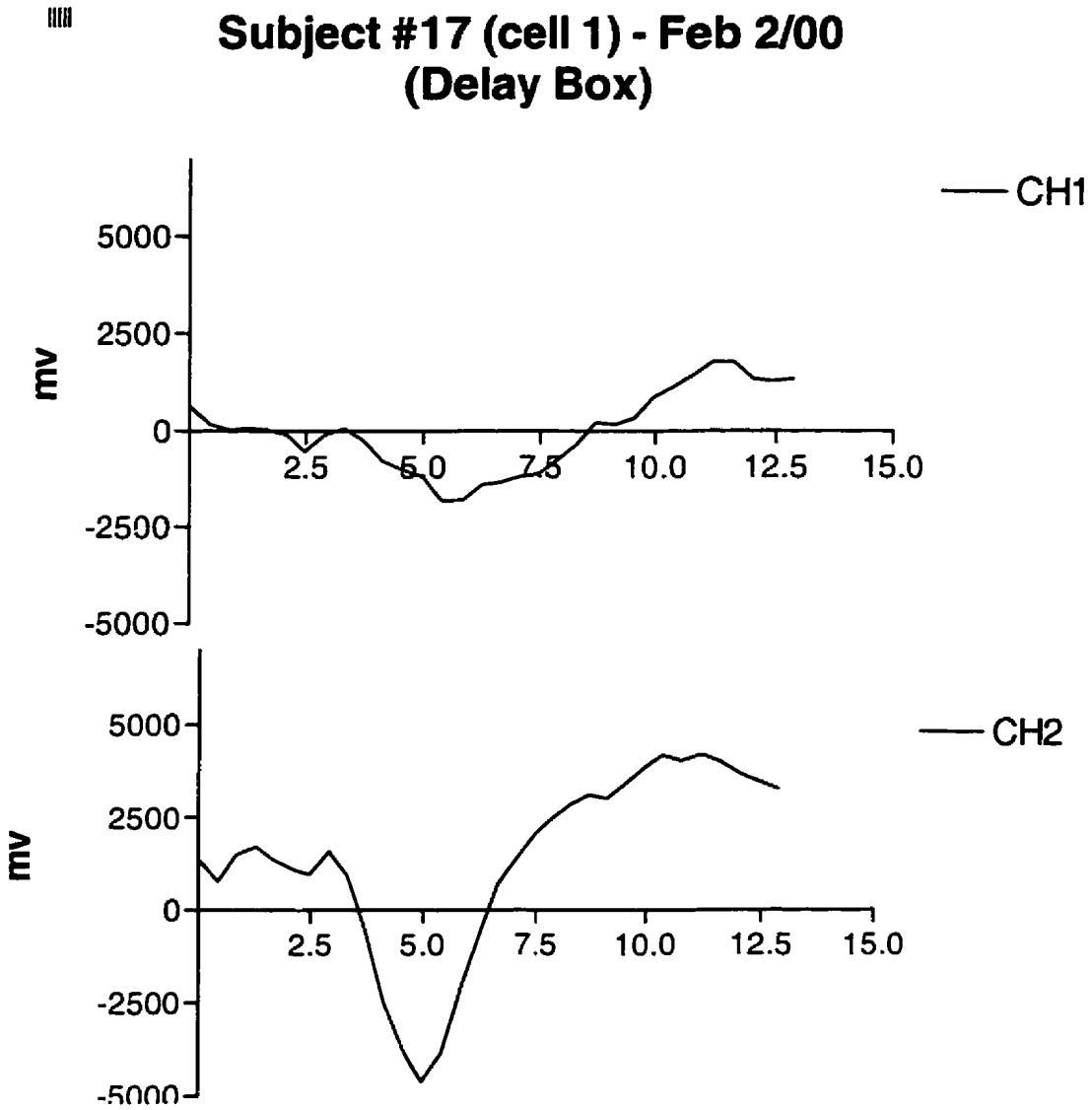
IIIII

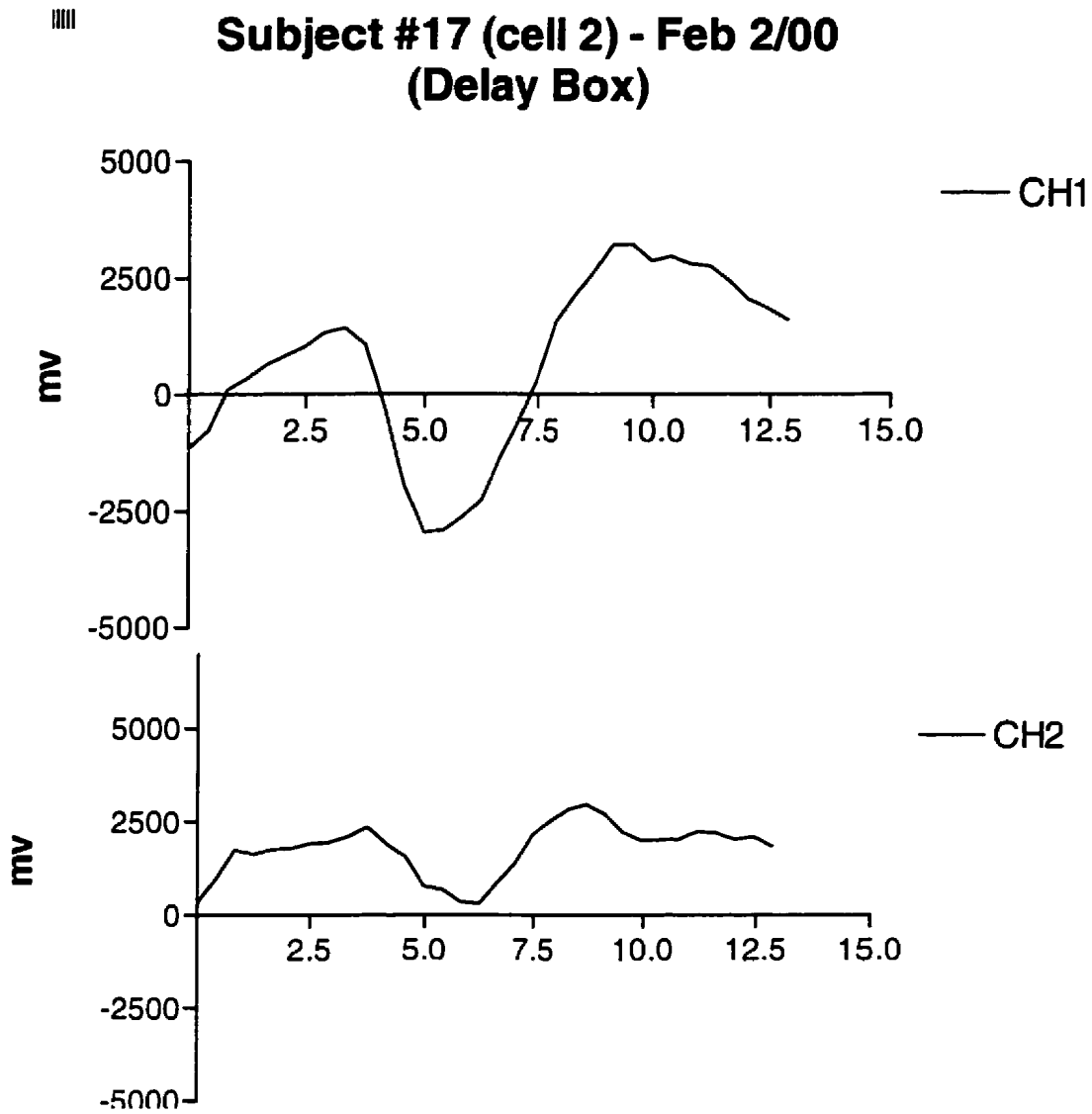
Subject #15 - Nov 25/99

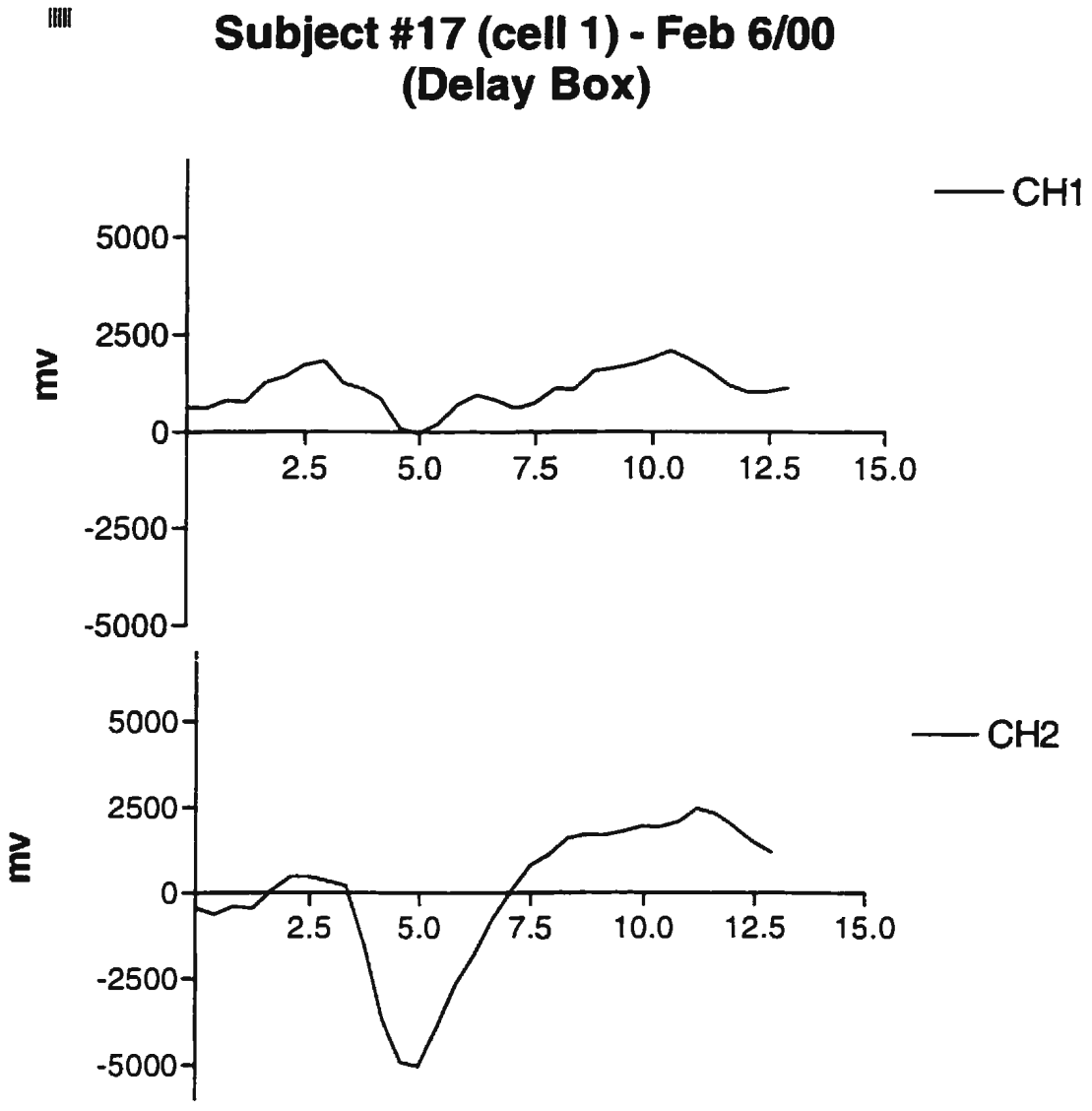
(Delay Box) note: only caught "upswing" of cell

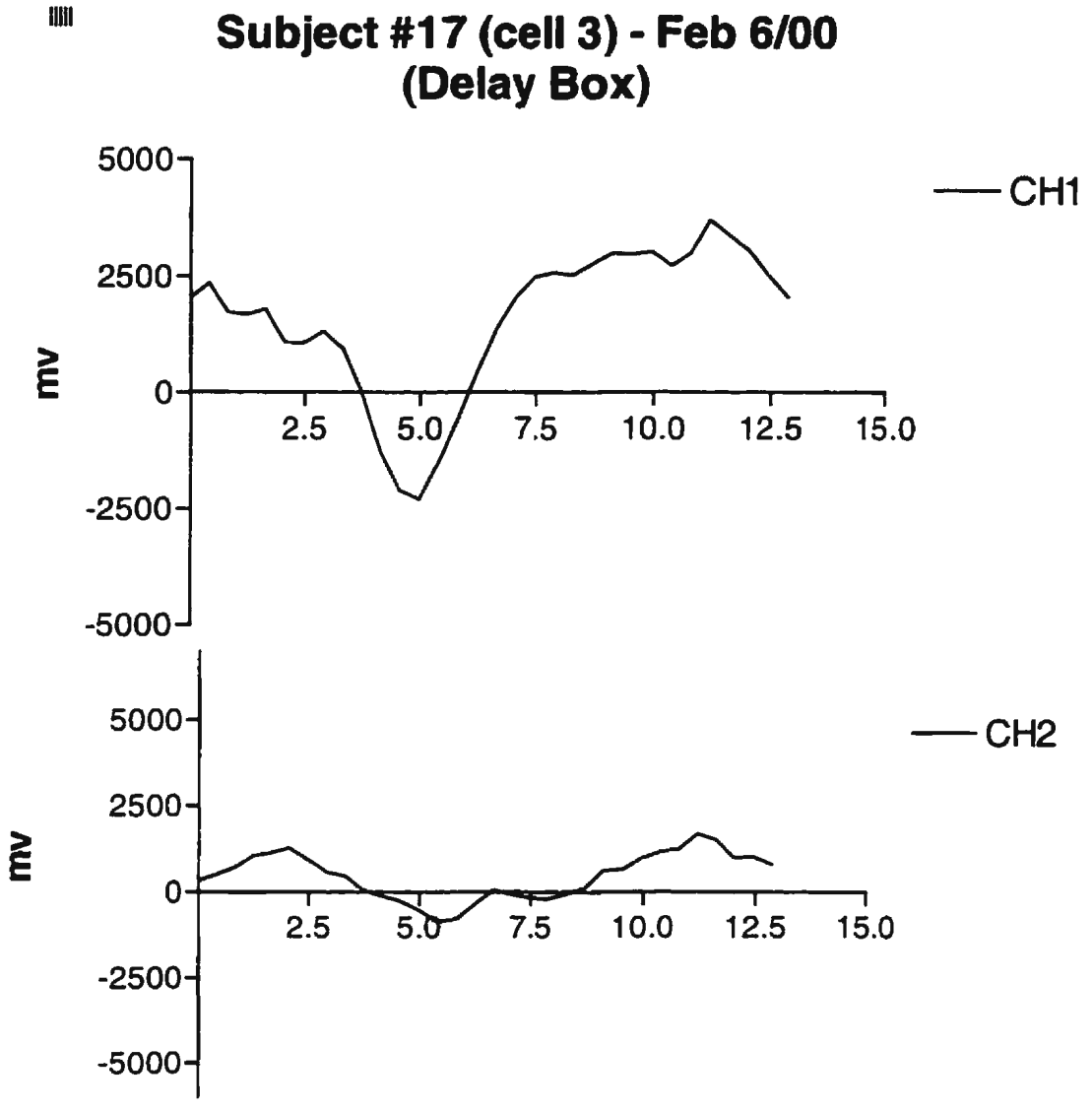


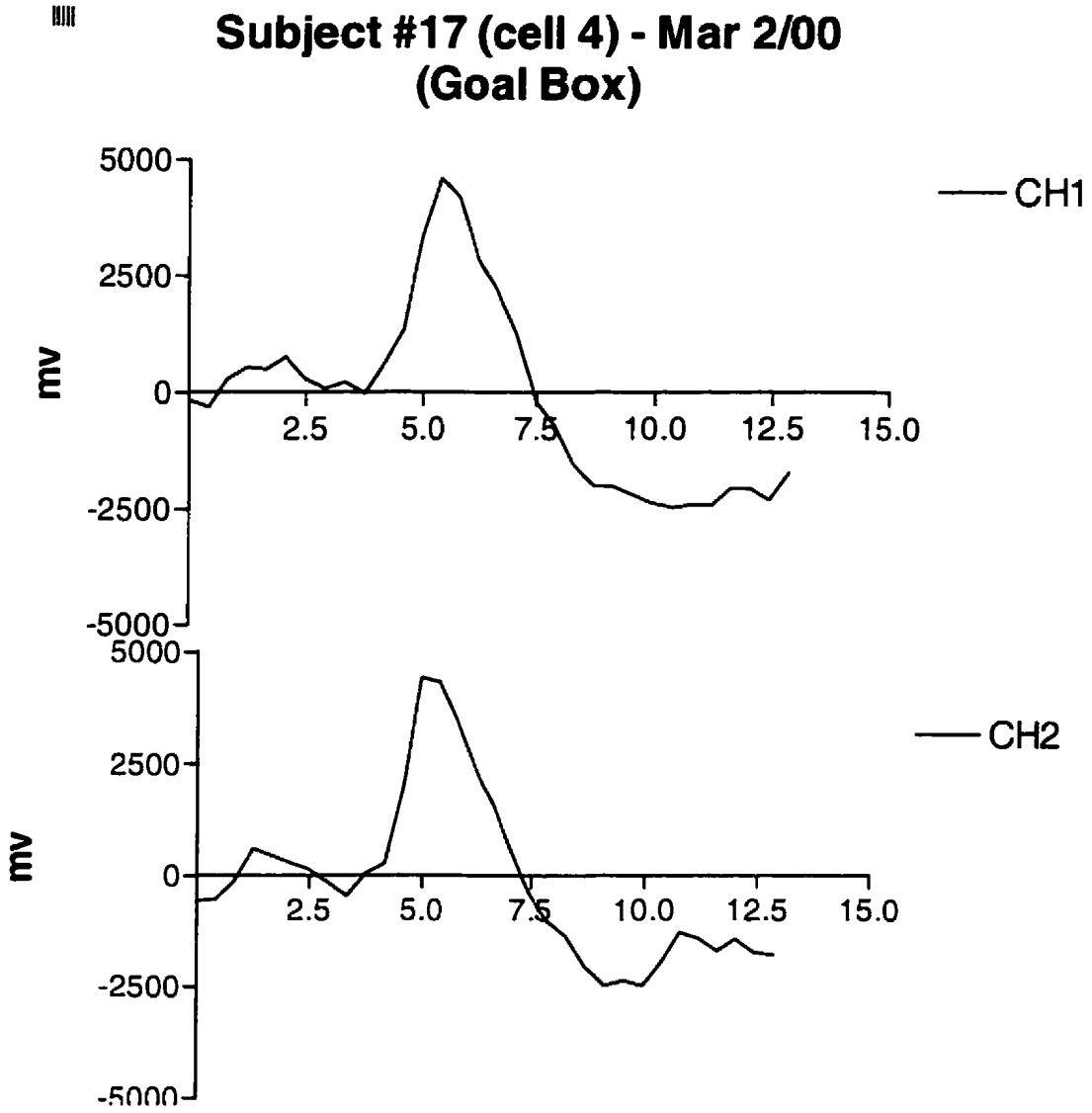

Subject #15 - Nov 22/99
(Goal Box)
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0

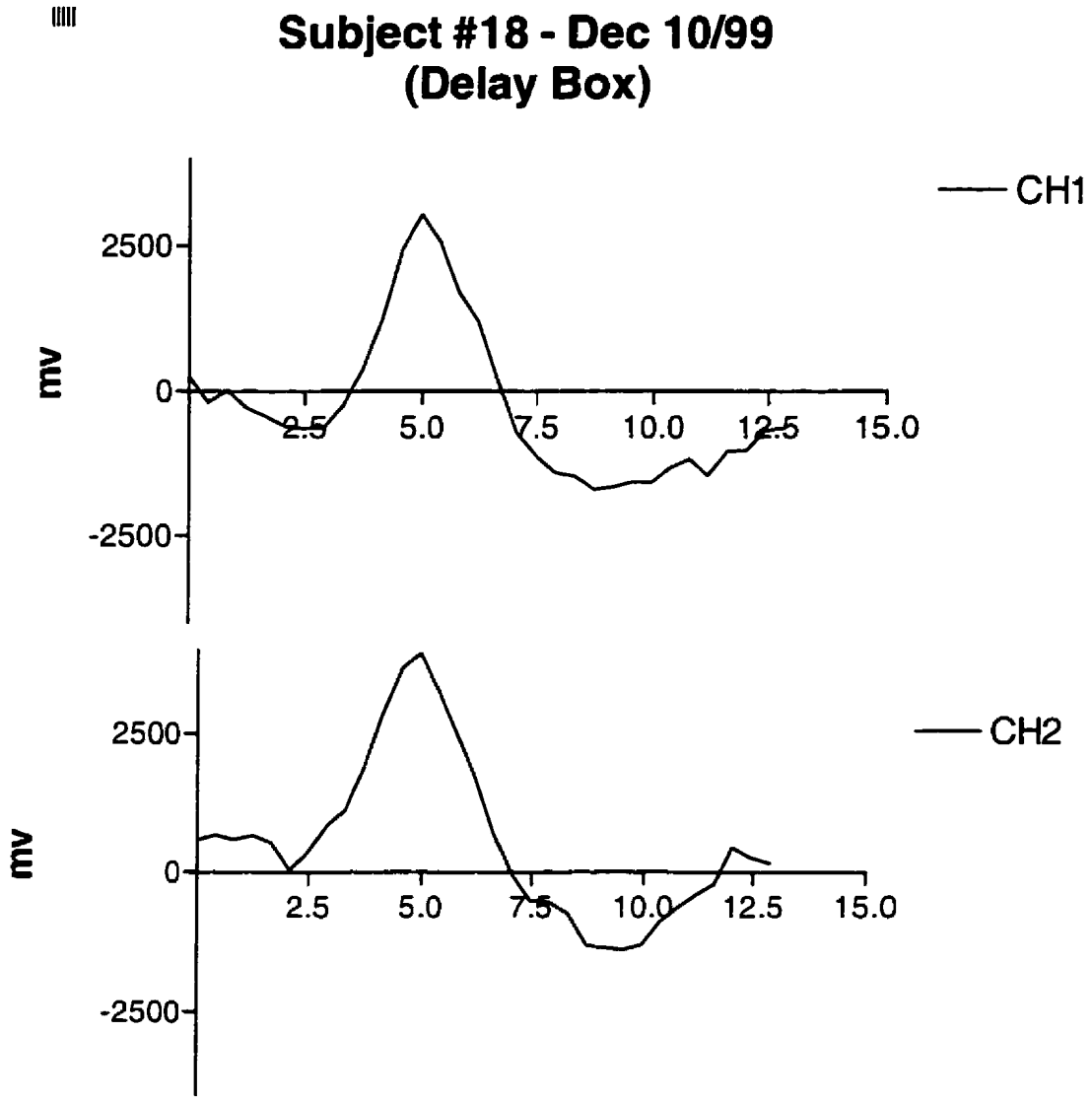

Subject #17 (cell 1) - Feb 2/00
(Delay Box)
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0

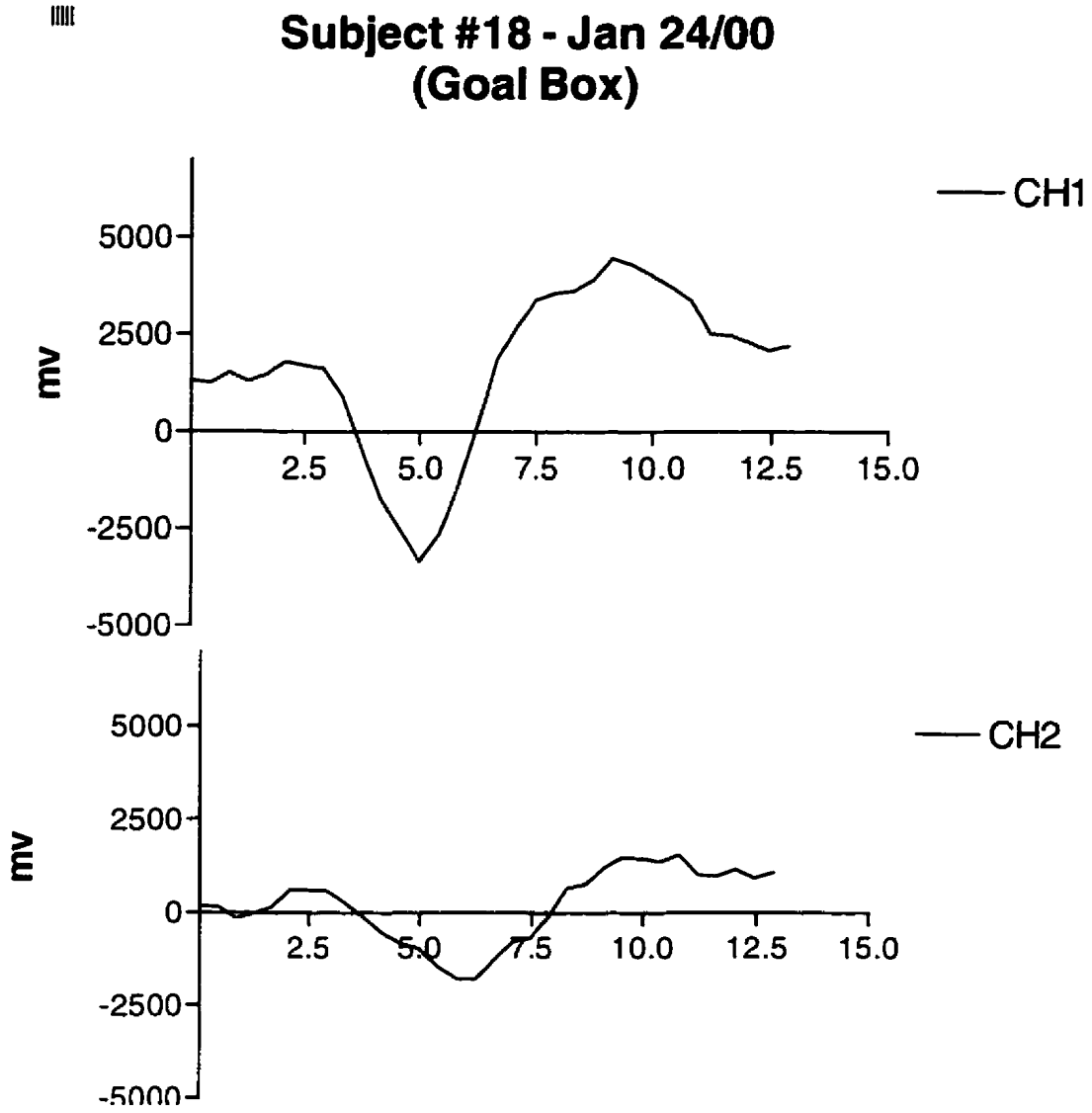

Subject #17 (cell 2) - Feb 2/00
(Delay Box)
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2

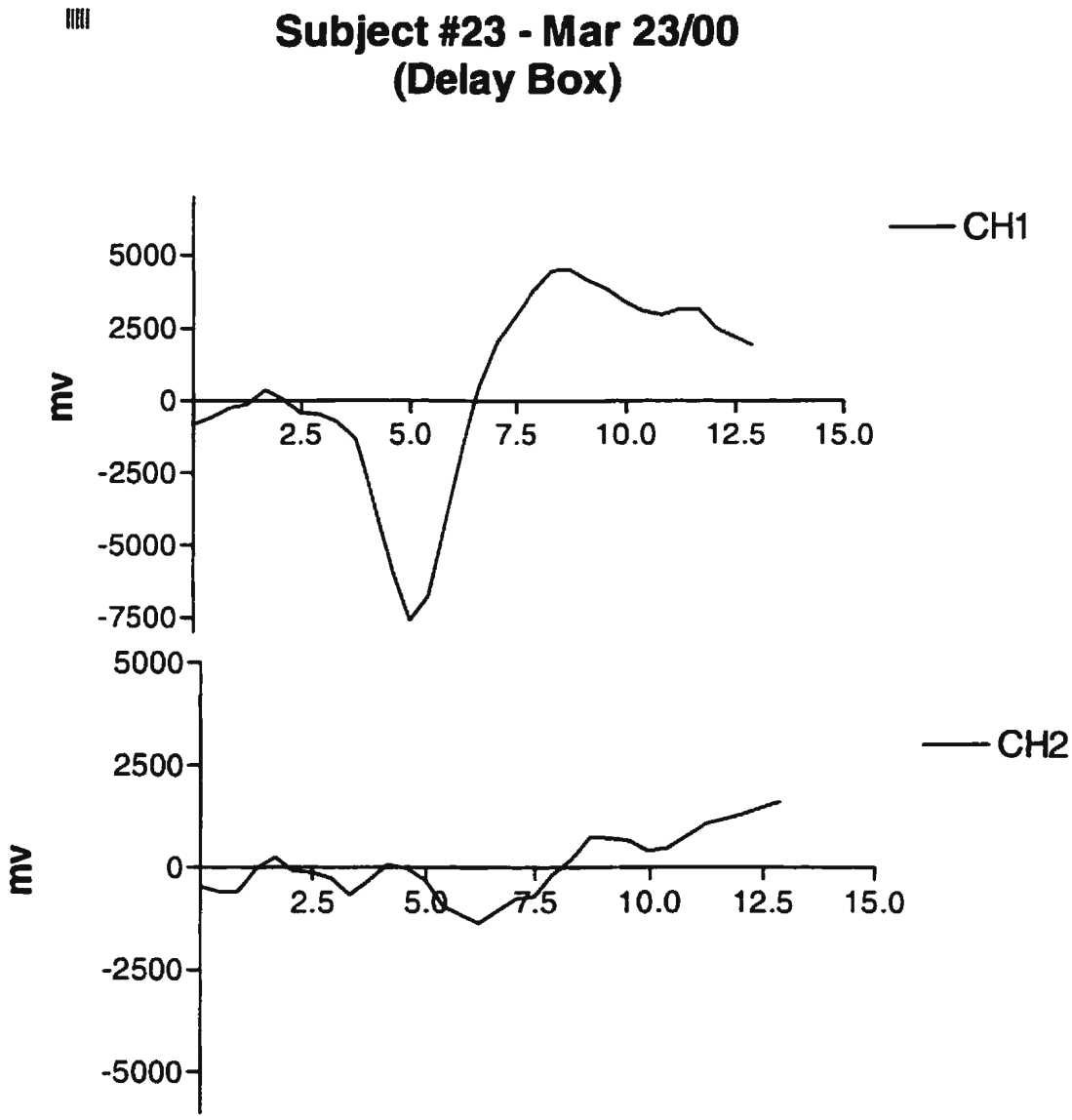

Subject #17 (cell 1) - Feb 6/00
(Delay Box)
CH1
CH2
mv
mv
5000
2500
0
-2500
-5000
2.5
5.0
7.5
10.0
12.5
15.0


Subject #17 (cell 3) - Feb 6/00
(Delay Box)
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2

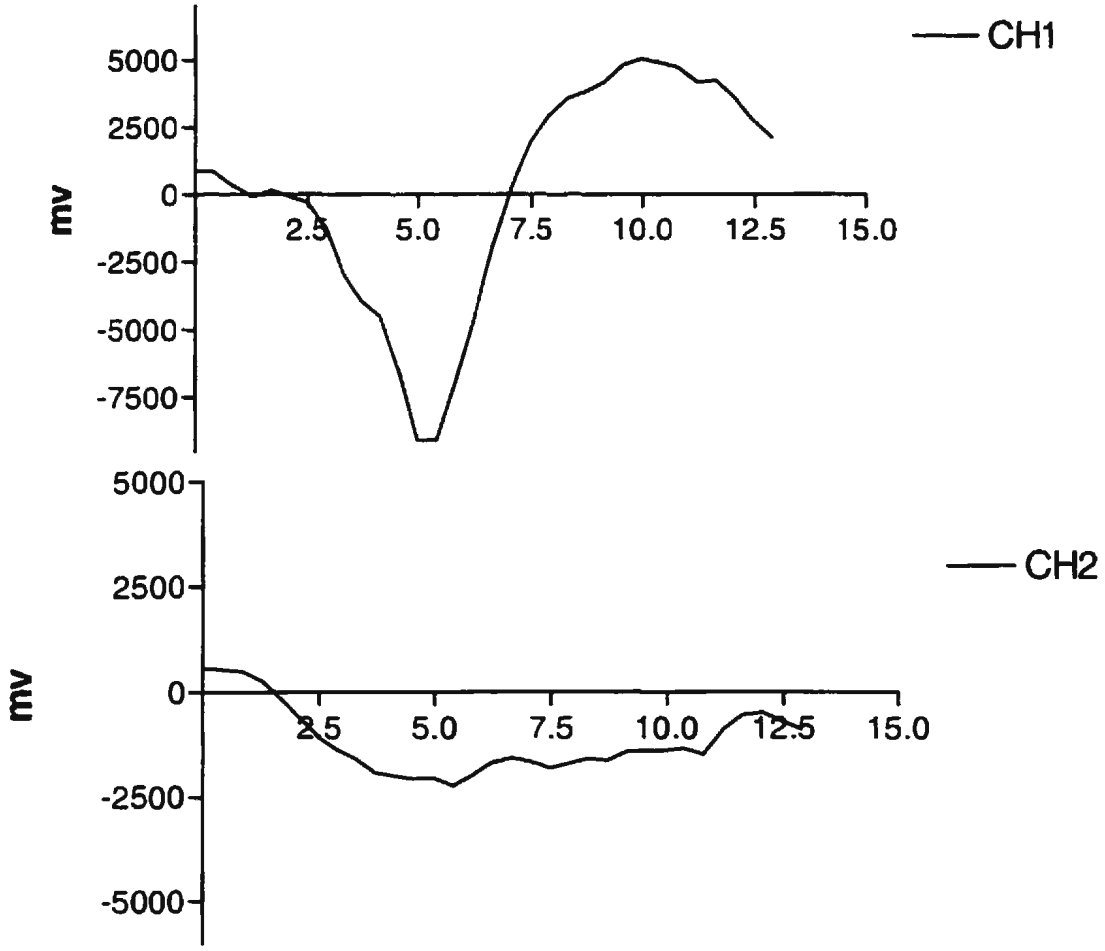

Subject #17 (cell 4) - Mar 2/00
(Goal Box)
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH1
CH2


Subject #18 - Dec 10/99
(Delay Box)
CH1
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0

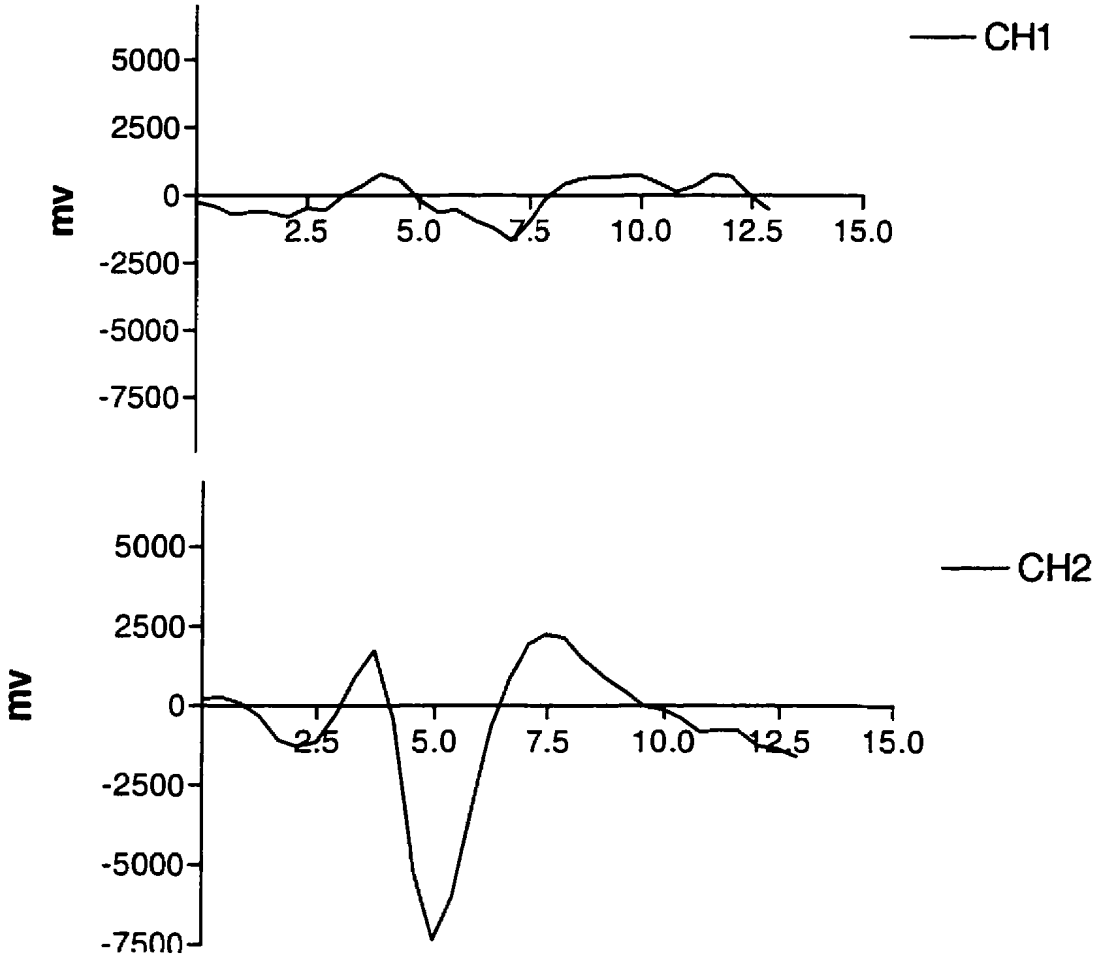

Subject #18 - Jan 24/00
(Goal Box)
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0


Subject #23 - Mar 23/00
(Delay Box)
CH1
5000
2500
0
-2500
-5000
-7500
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0

Subject #26 - Aug 29/00
(Delay Box)

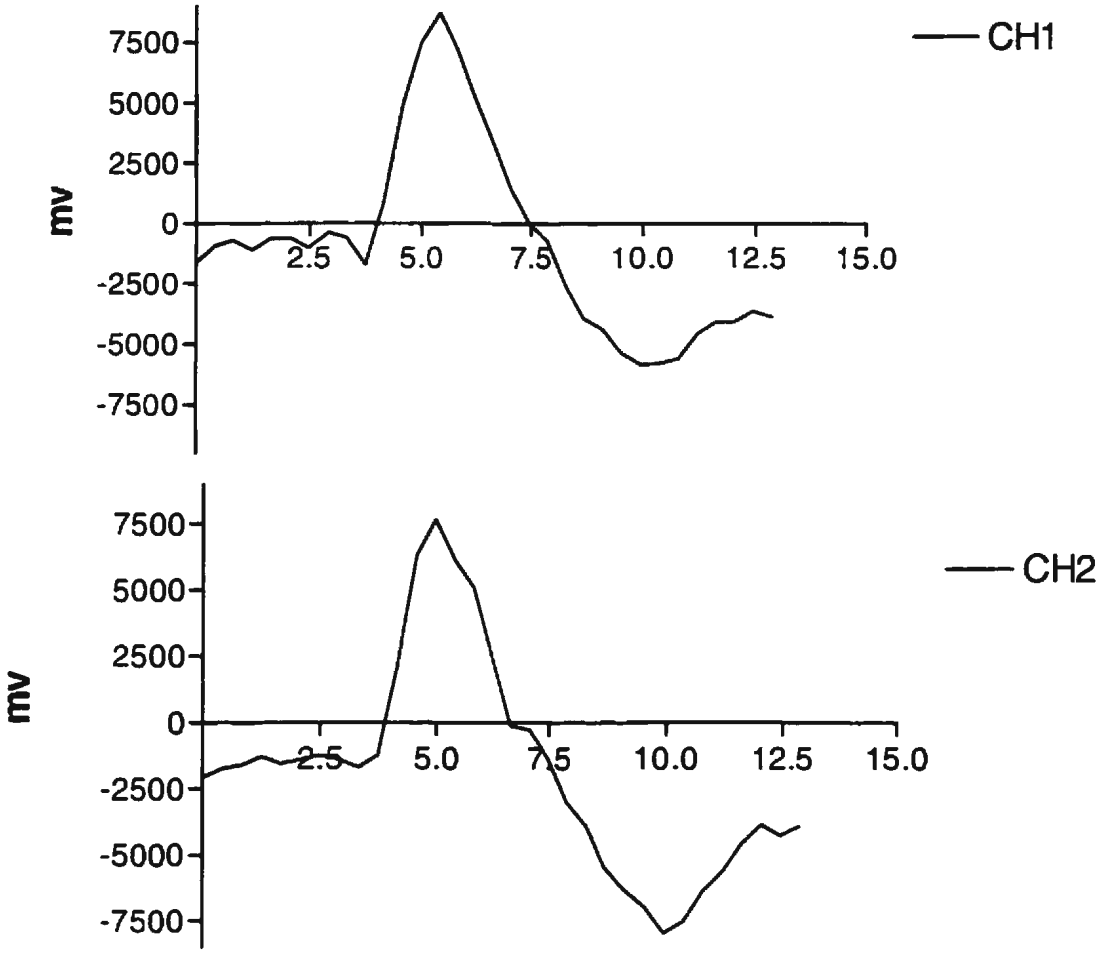


Subject #26 (Cell 2) - Sept 2/00
(Delay Box)



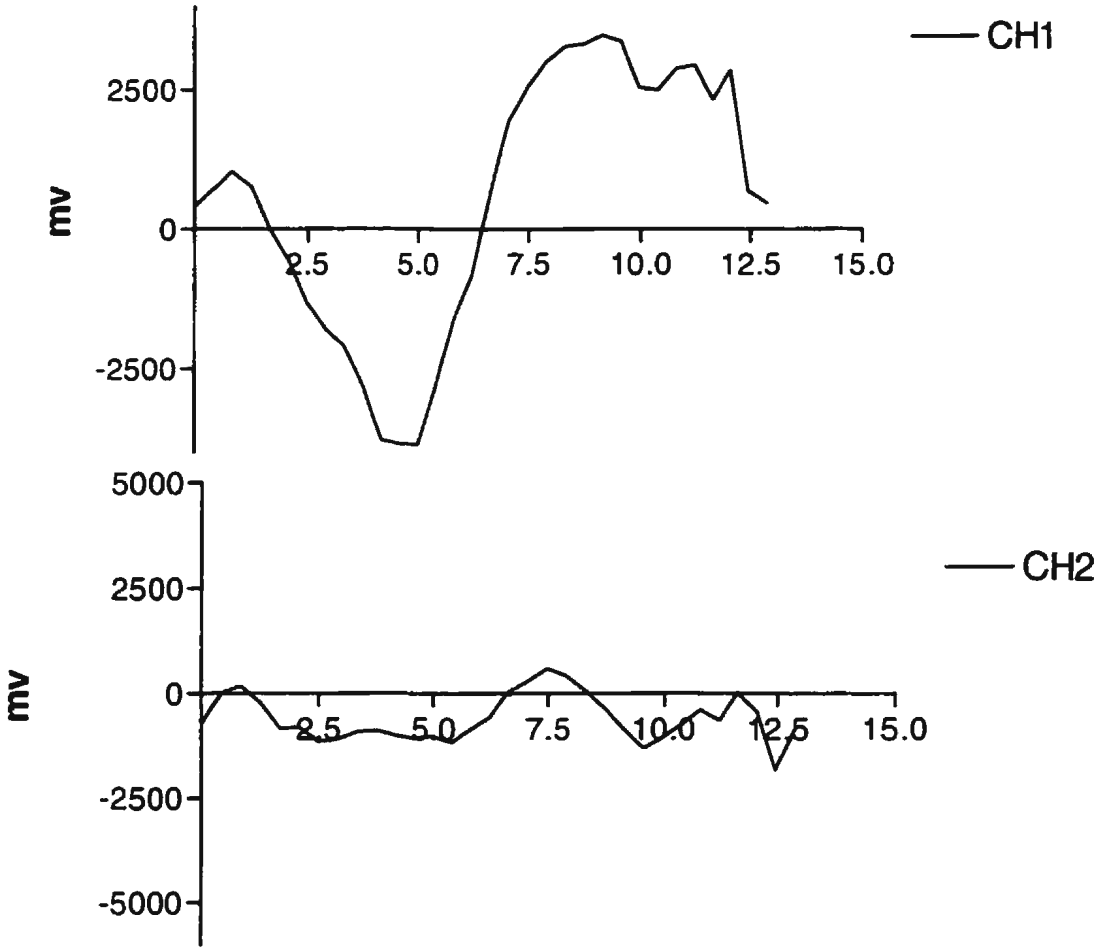
IIIII

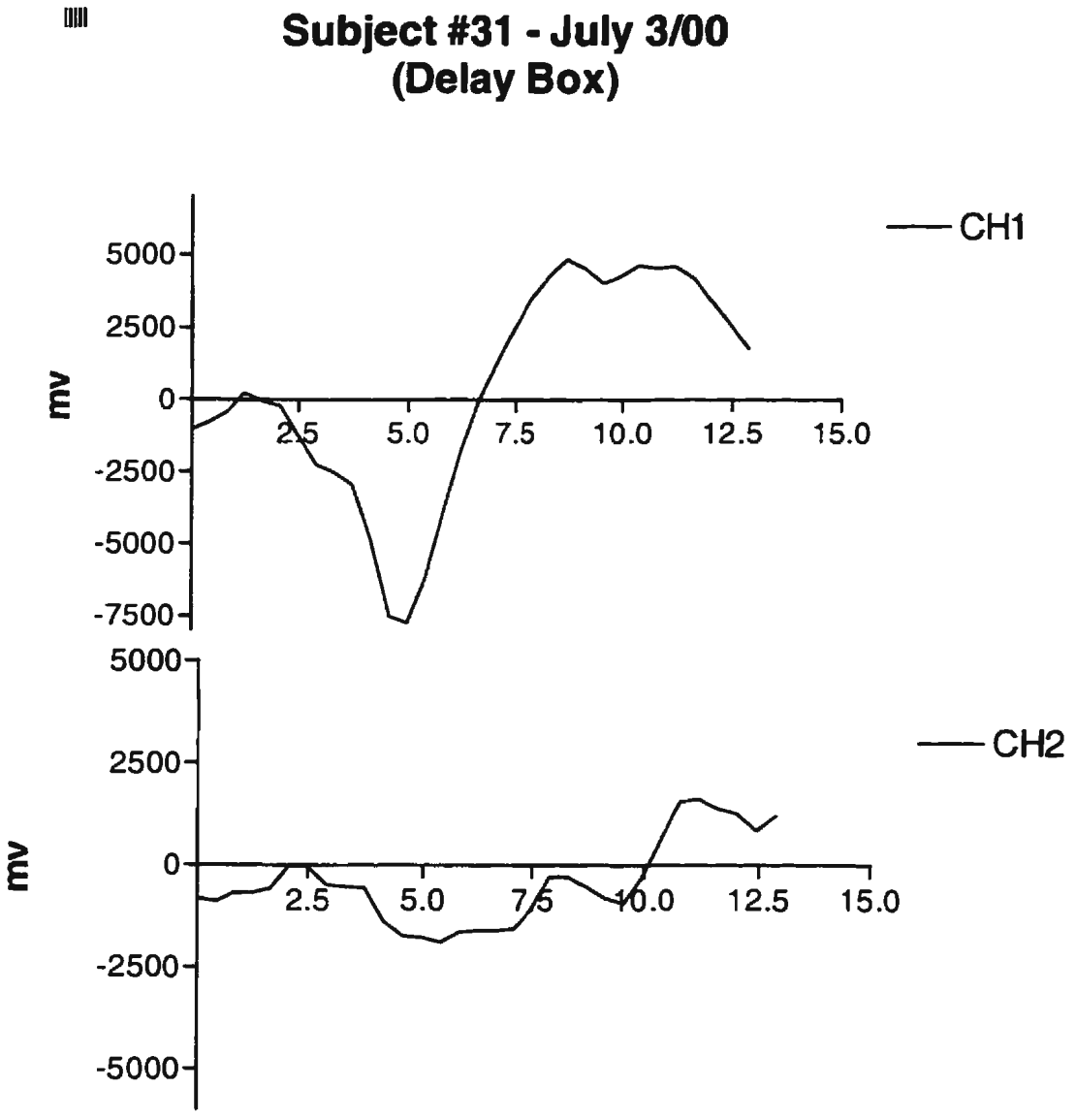
Subject #26 (Cell 3) - Sept 6/00 (Goal Box)



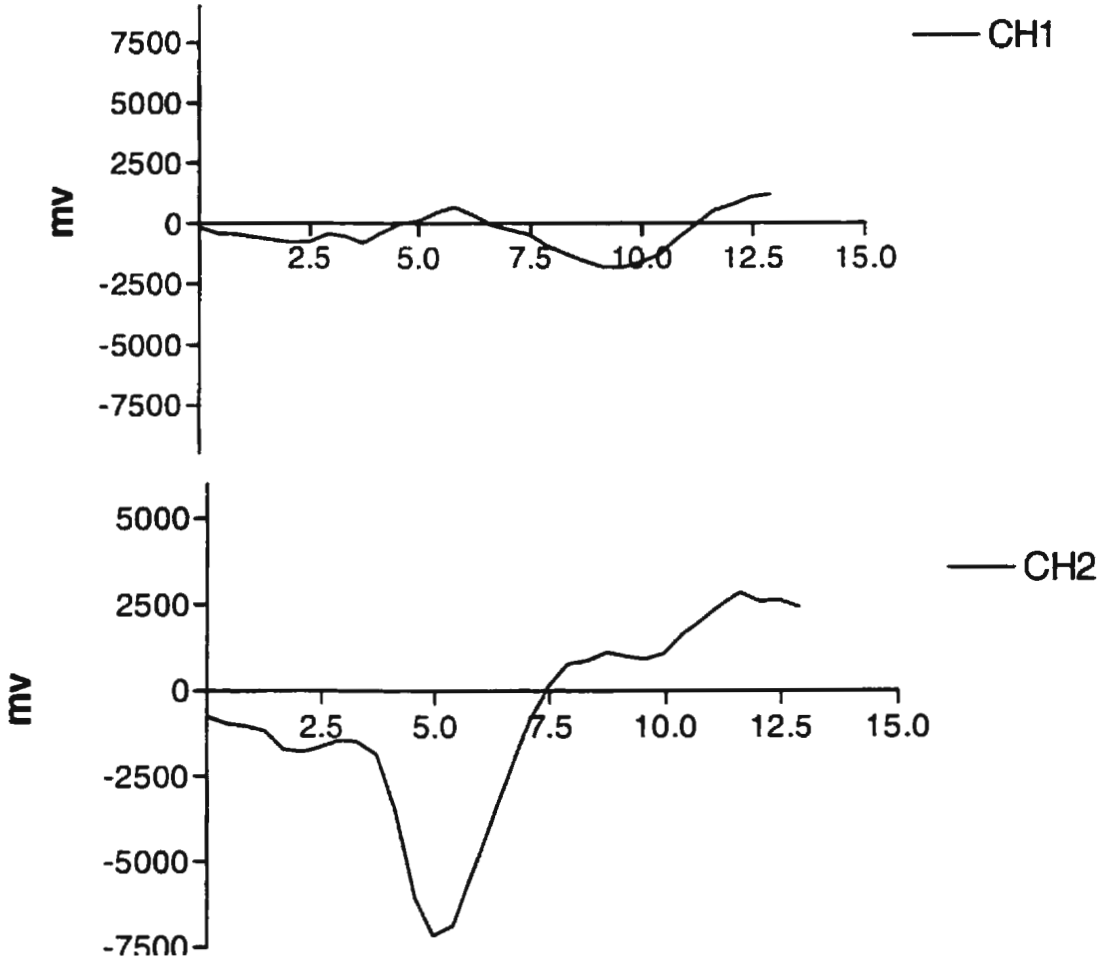
IIIII

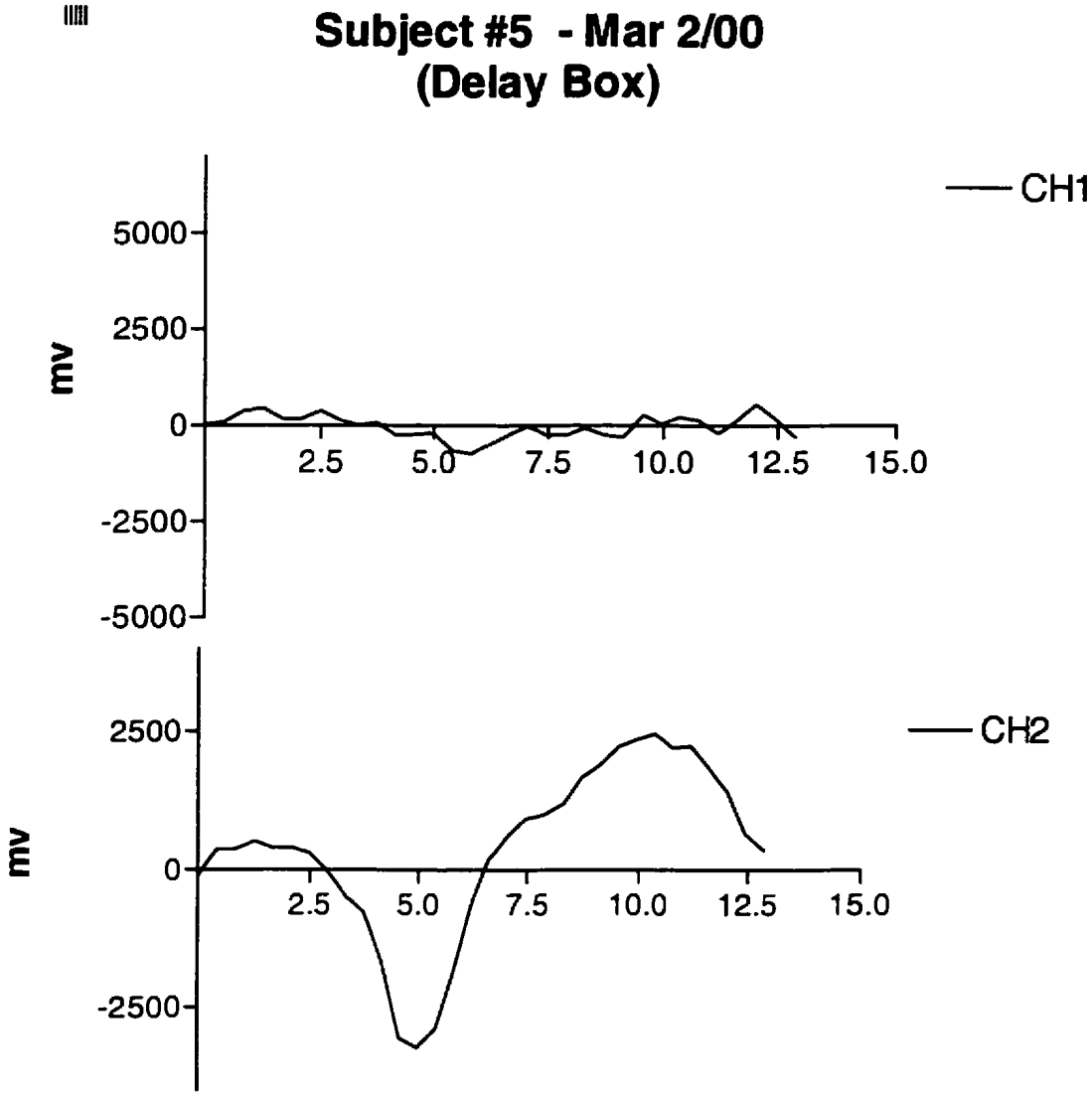
Subject #29 - Aug 27/00
(Delay Box)

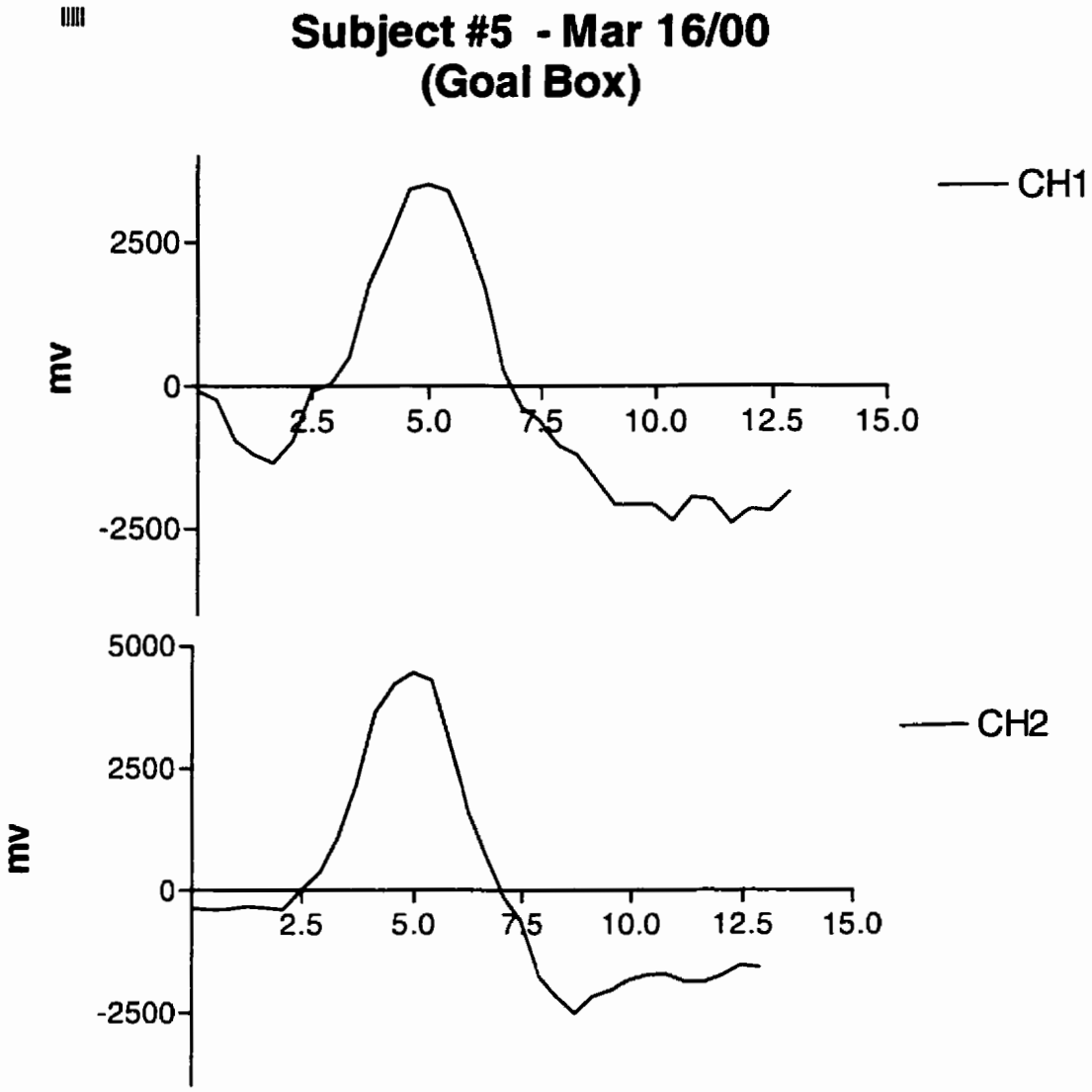


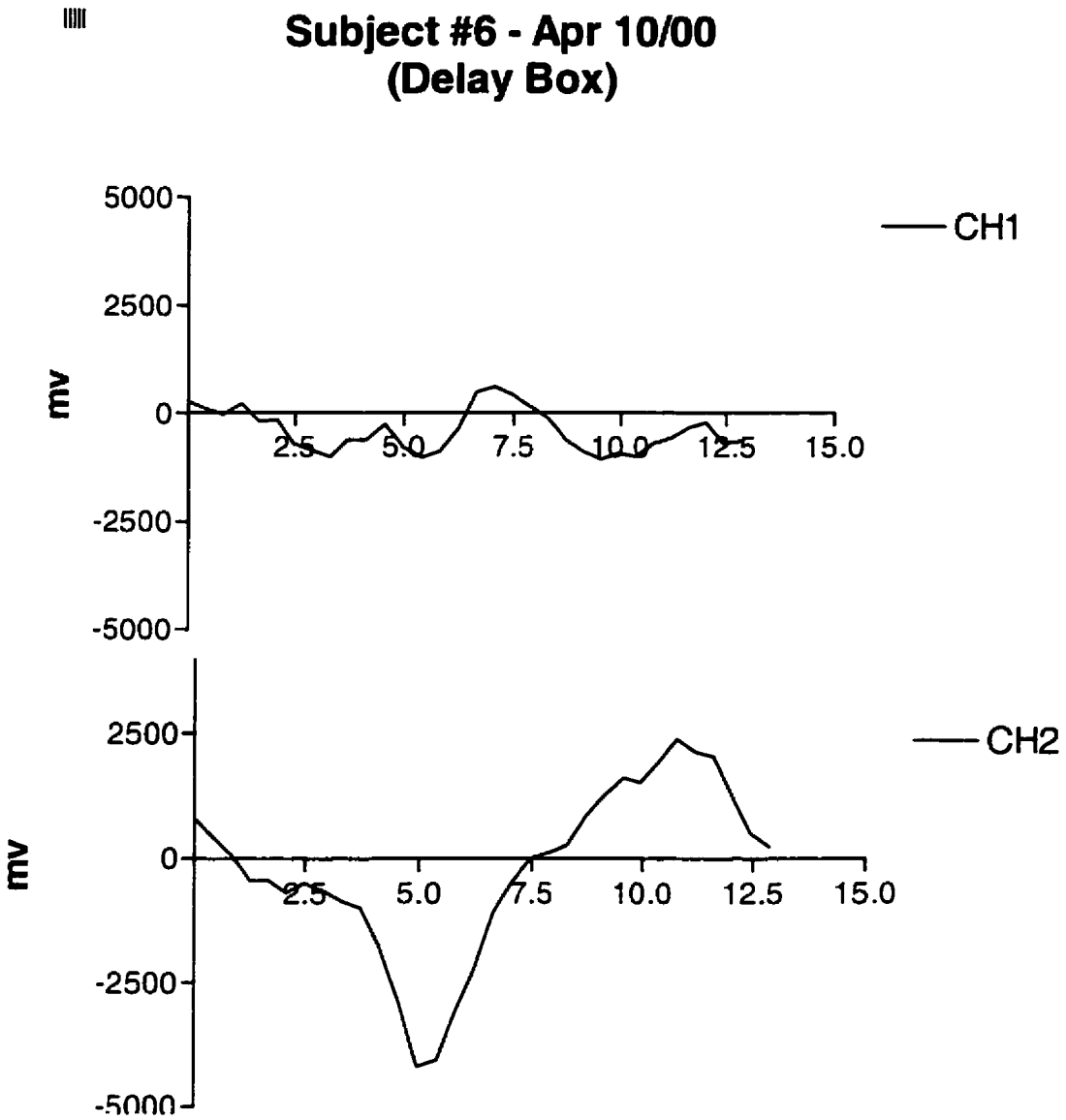

Subject #31 - July 3/00
(Delay Box)
CH1
5000
2500
0
-2500
-5000
-7500
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0

Subject #33 - Sept 5/00
(Goal Box)



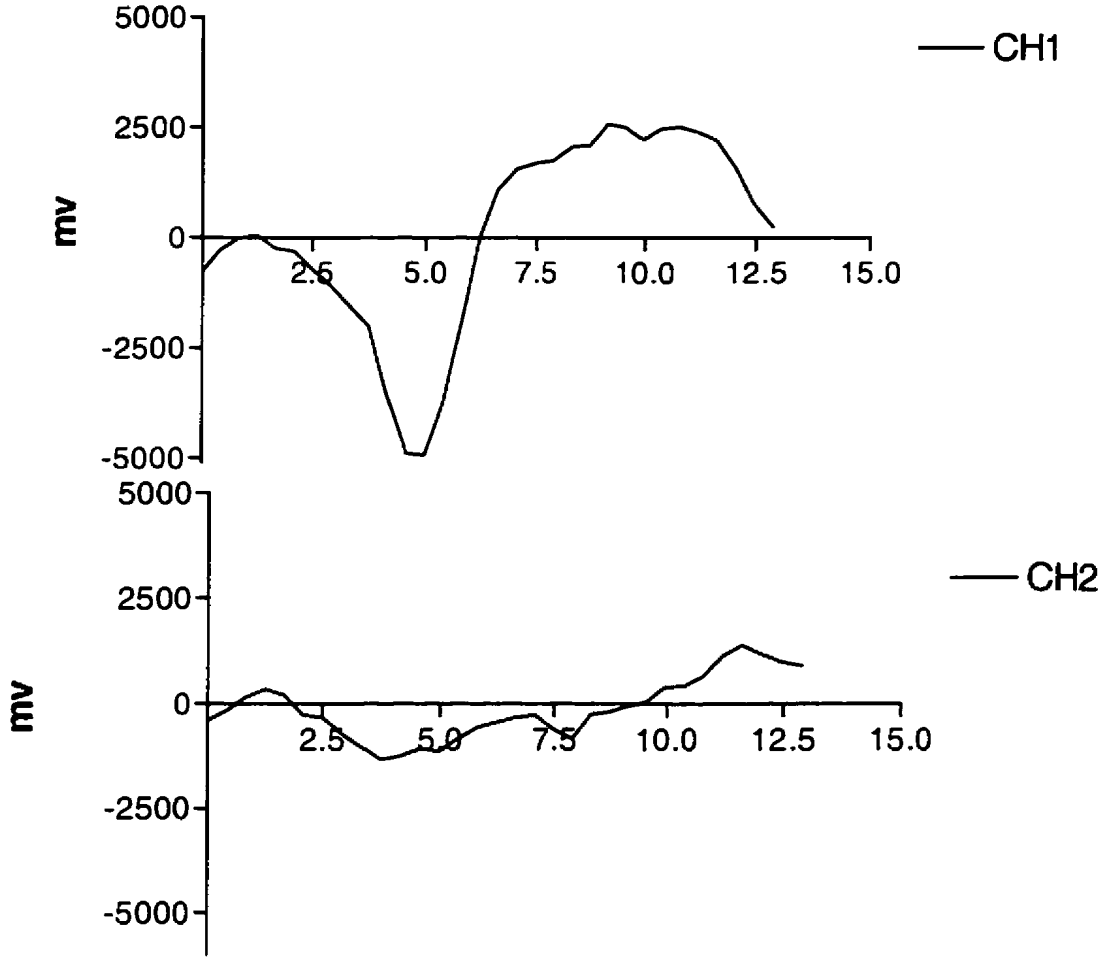

Subject #5 - Mar 2/00
(Delay Box)
CH1
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0


Subject #5 - Mar 16/00
(Goal Box)
CH1
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2
5000
2500
0
-2500
mv
2.5
5.0
7.5
10.0
12.5
15.0


Subject #6 - Apr 10/00
(Delay Box)
5000
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH1
2500
0
-2500
-5000
mv
2.5
5.0
7.5
10.0
12.5
15.0
CH2

IIIII

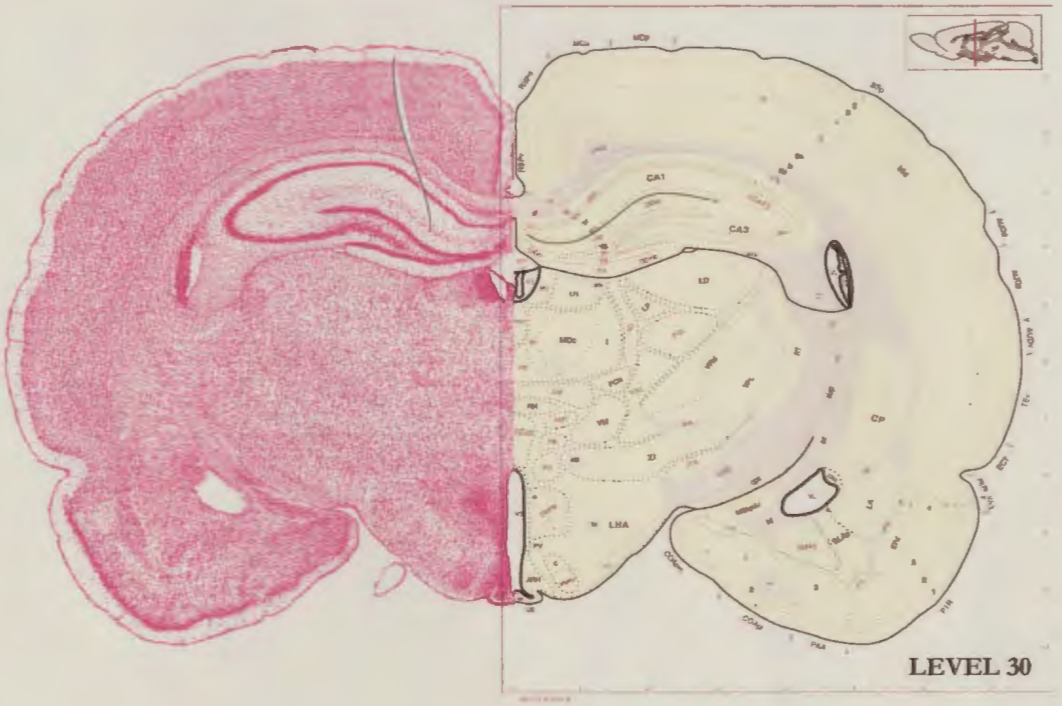
Subject #6 (Cell 2) - Apr 10/00 (Delay Box)



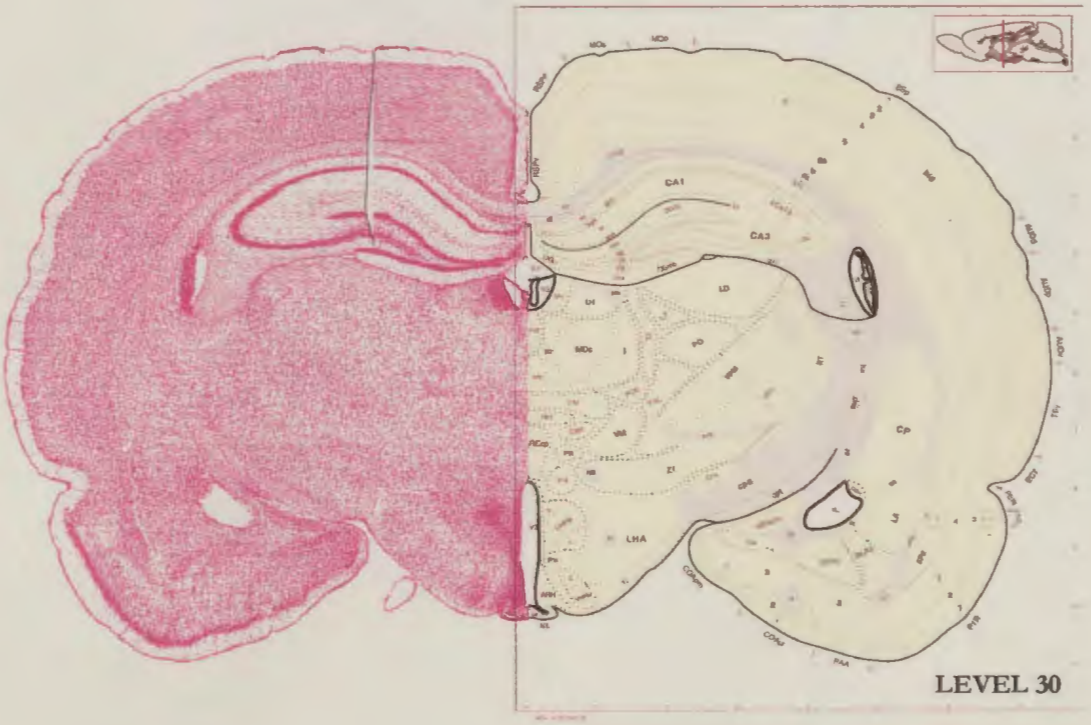
Appendix C

Histology

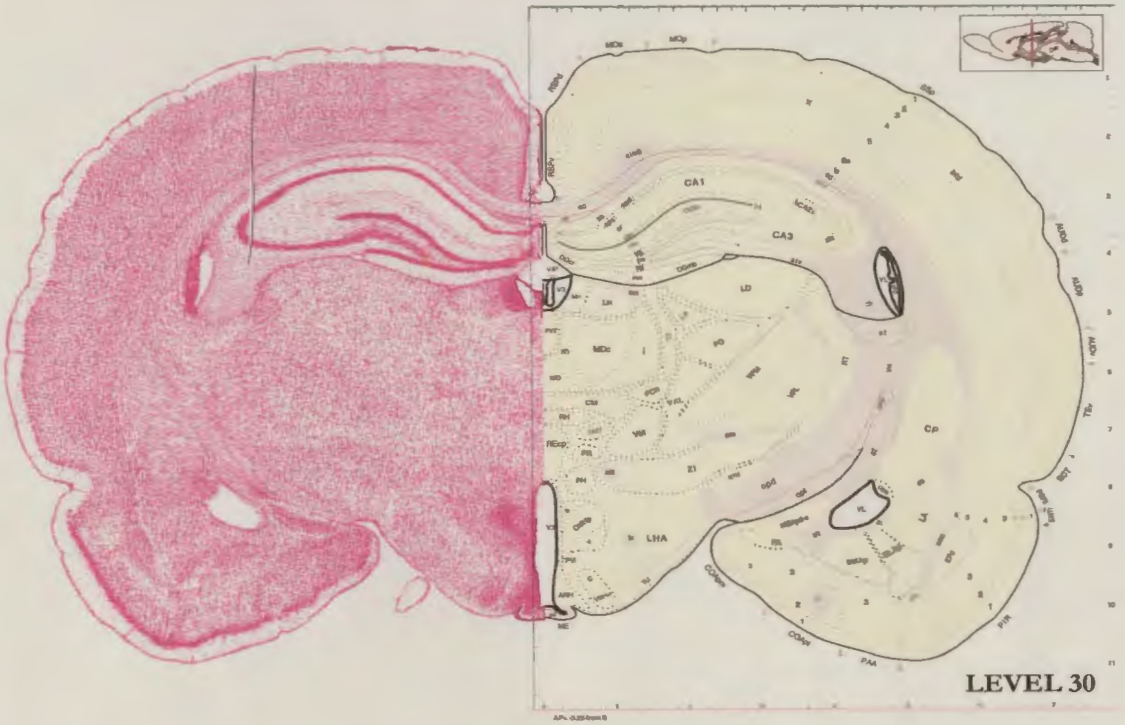
Subject 5



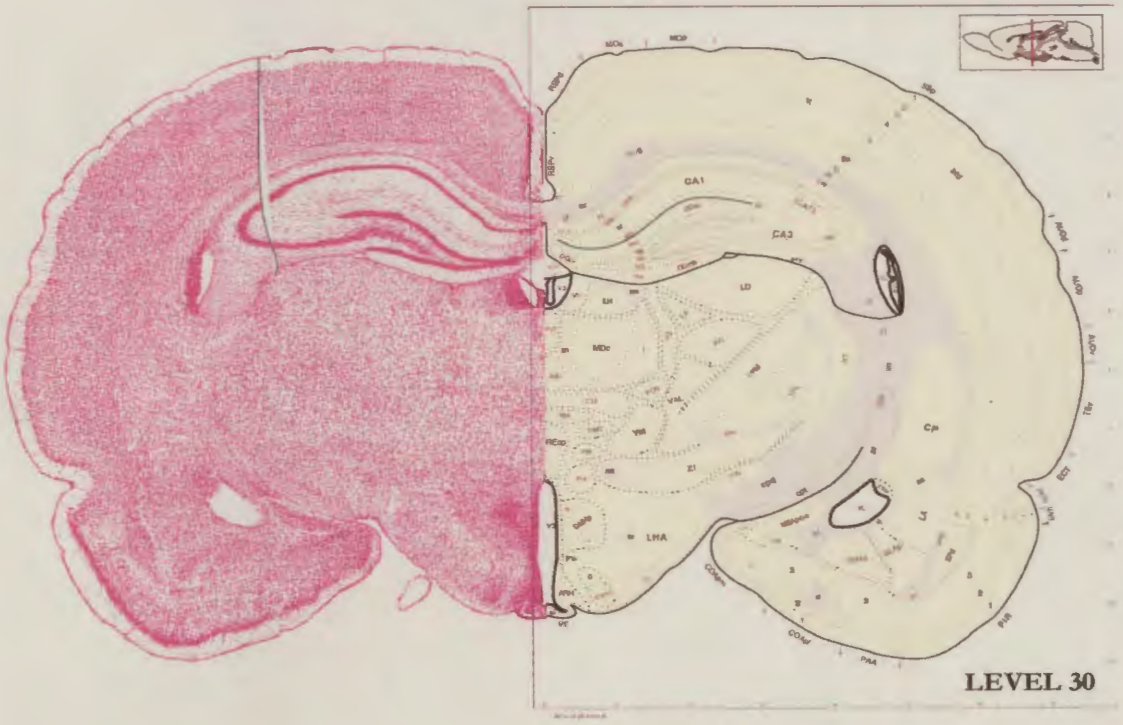
Subject 6



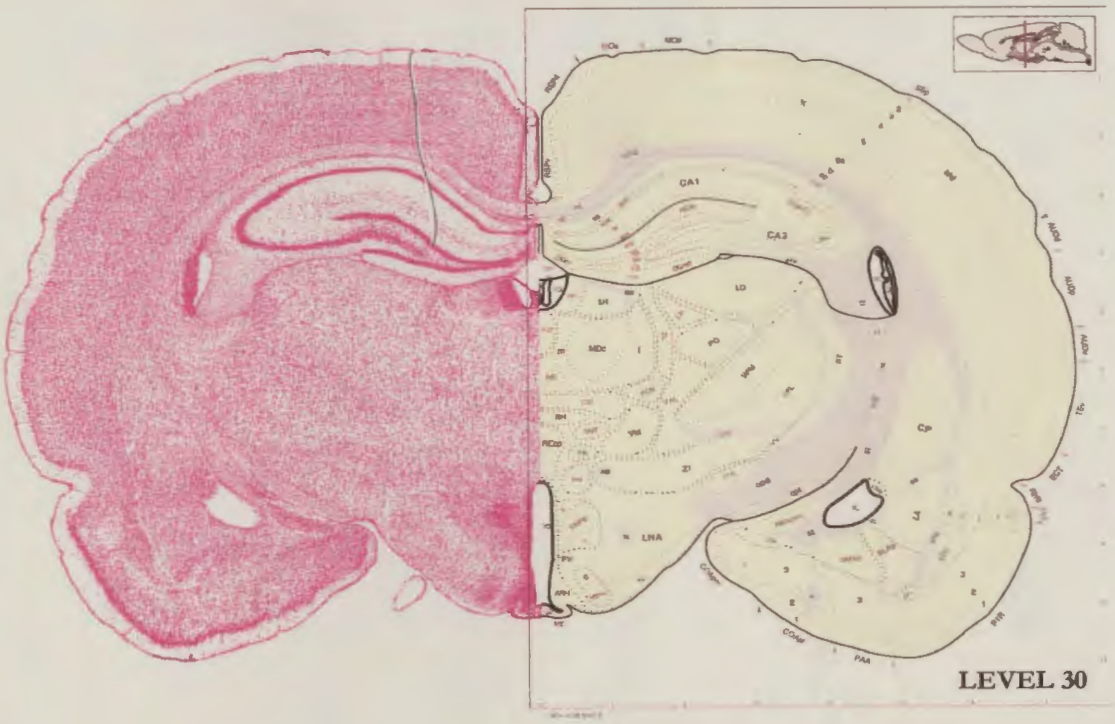
Subject 14



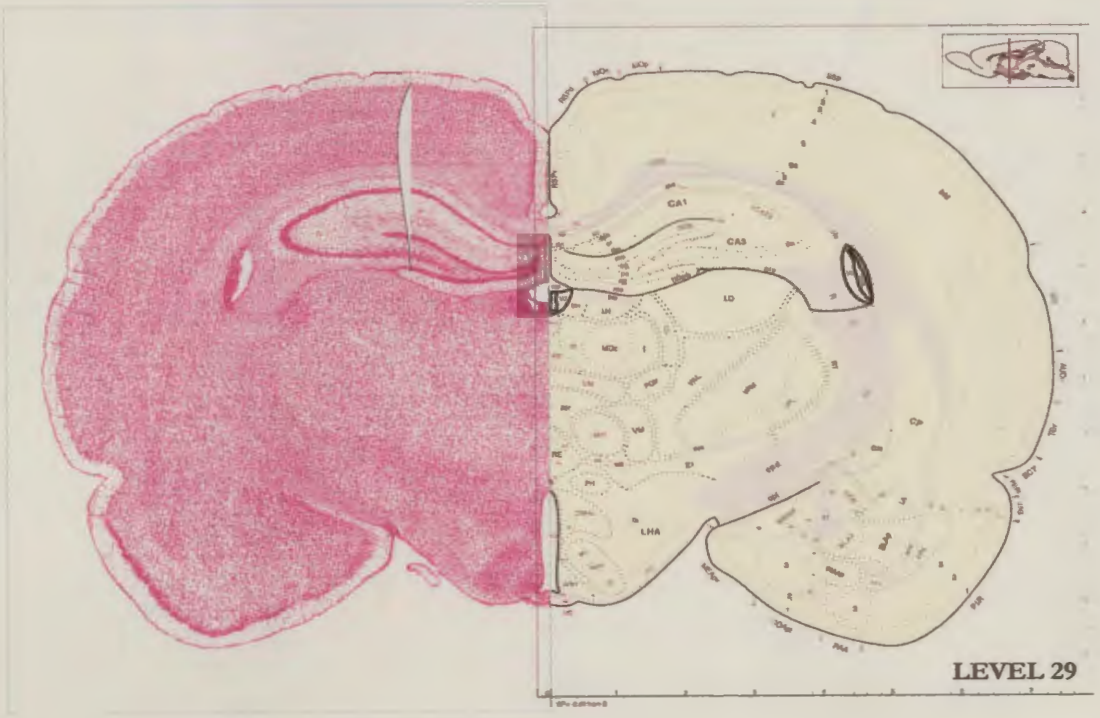
Subject 15



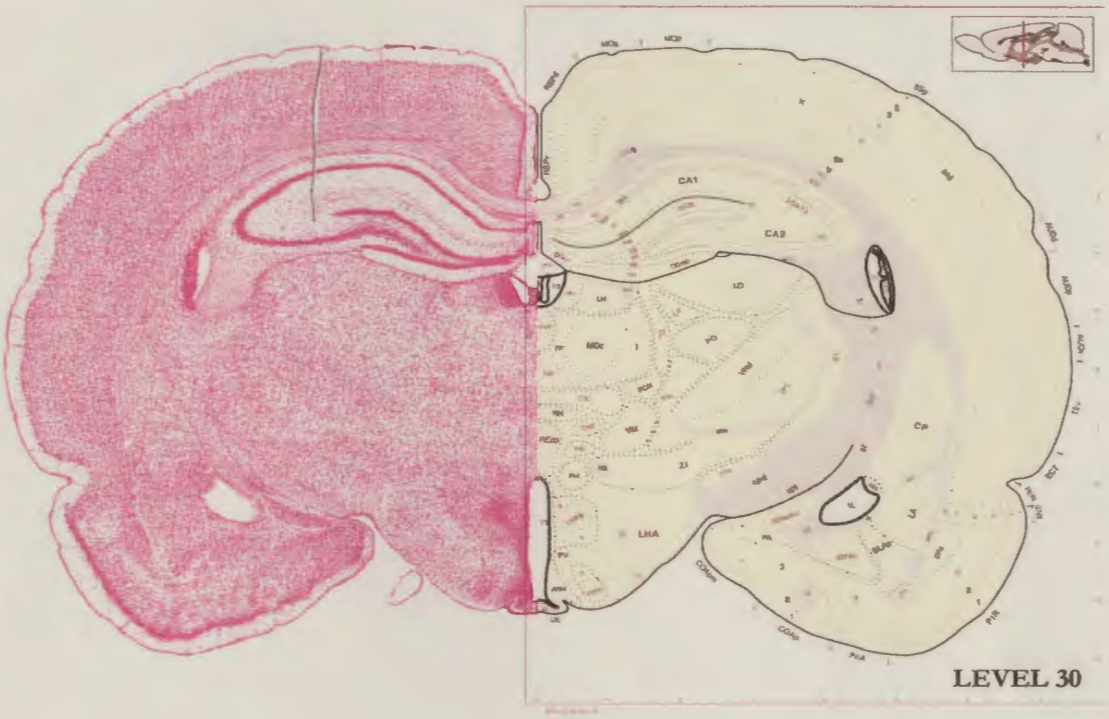
Subject 17



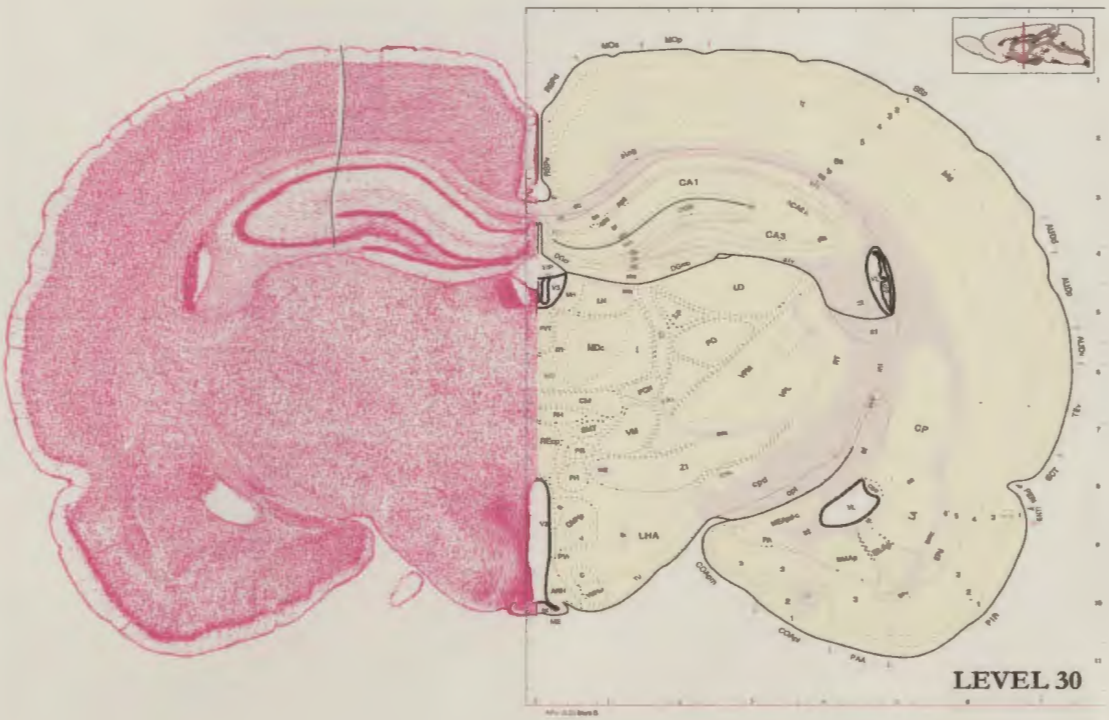
Subject 18



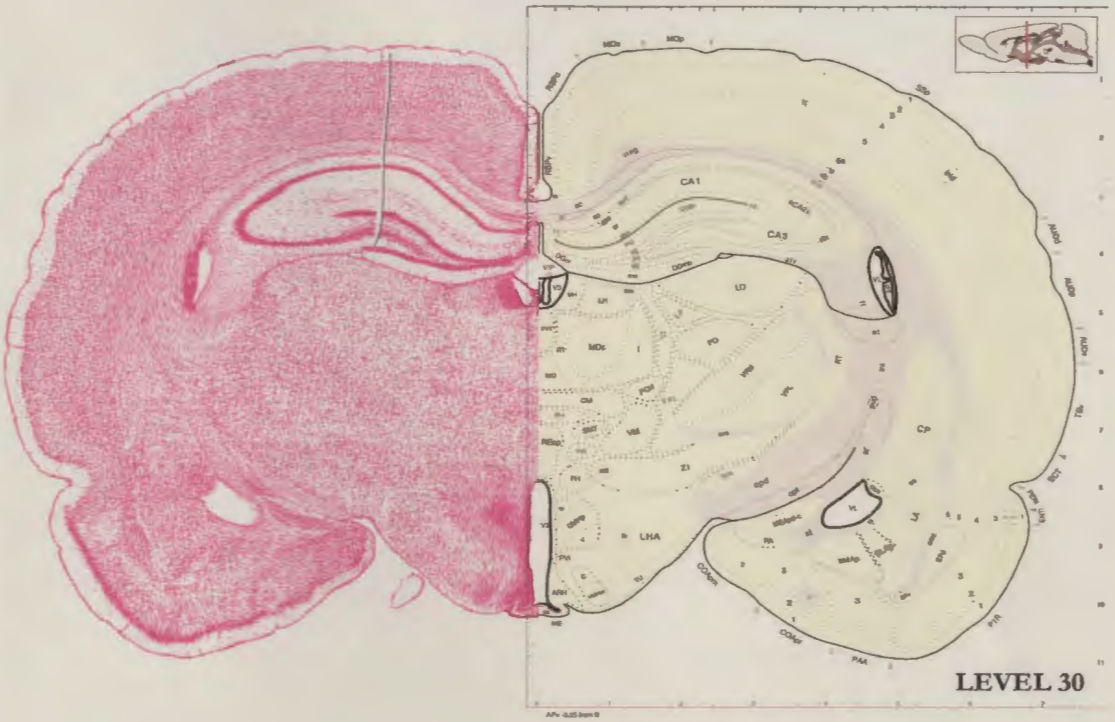
Subject 23



Subject 26



Subject 29



Subject 31

Subject 33